Sensory ecology and correlates of sociality in common mole-rats (*Fukomys* spp.) and other subterranean rodents

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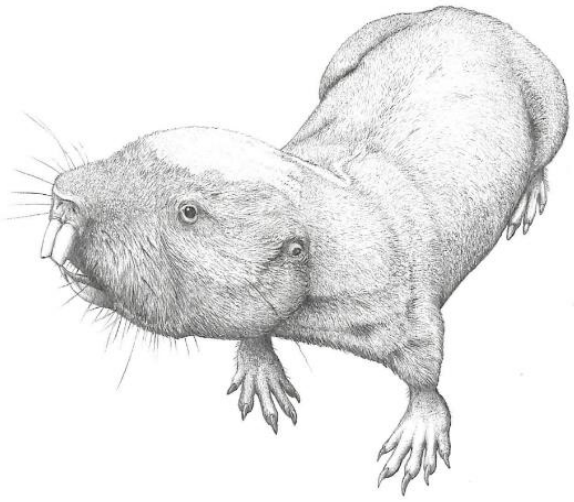

You merely adopted the dark; I was born in it, molded by it.

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Zusammenfassung

Das unterirdische Ökotop stellt Tiere vor einzigartige Herausforderungen hinsichtlich räumlicher Orientierung und Kommunikation. Es ist strukturell gleichförmig, dunkel und unterirdische Gangsysteme weisen eine ungewöhnliche Tunnel-Akustik auf. Diverse Linien grabender Säugetiere haben sich an diese Extrembedingungen angepasst, aber entscheidende Aspekte der Adaptation ihrer Sinnesorgane an das Leben unter der Erde bleiben rätselhaft. Am Beispiel von Graumullen der Gattung *Fukomys*, sozialen afrikanischen Sandgräbern, beschäftigte ich mich in einer Reihe von Laborstudien mit verschiedenen Aspekten der Sinnesökologie, Kommunikation und Sozialität unterirdisch lebender Nagetiere, um diese Thematik eingehender zu beleuchten.

Zunächst untersuchte ich den Magnetsinn dieser Tiere, eine sensorische Modalität, die für die Navigation unter der Erde von entscheidender Bedeutung sein könnte. In zwei Serien von Verhaltensversuchen an enukleierten und unbehandelten Graumullen wurde untersucht, ob sich die bisher nicht lokalisierten Magnetrezeptoren, die auf das Magnetfeld der Erde reagieren, in den Augen befinden. Die Ergebnisse zeigten, dass enukleierte Graumulle nicht in der Lage sind, sich anhand von magnetischen Stimuli zu orientieren, was auf einen augenbasierten Mechanismus der Magnetfeld-wahrnehmung hindeutet und es somit erlaubt, Forschungsansätze zur Identifikation der rätselhaften Magnetrezeptorzellen im Säugetierkörper zu präzisieren.

Zweitens studierte ich vergleichend die Hörempfindlichkeit von afrikanischen Sandgräbern, die eine entwicklungsgeschichtlich alte Gruppe unterirdisch lebender Nagetiere darstellen, und von Coruros, einer entscheidend jüngeren Linie von subterranen Nagern, durch die Messung von akustisch evozierten Hirnstammpotentialen. Während die auditorische Sensitivität bei ersteren deutlich eingeschränkt war, vermittelte das Gehör der Coruros zwischen dem abgeleiteten Zustand der Sandgräber und der hohen Empfindlichkeit epigäischer Nagetiere. Ein Vergleich dieser verschiedenen Gruppen deutet darauf hin, dass sowohl adaptive als auch degenerative Entwicklungen die Evolution des Gehörs von unterirdisch lebenden Nagetieren geprägt haben.

Drittens charakterisierte ich die perioralen Talgdrüsen von Graumullen, einen bei subterranen Nagetieren bisher nicht untersuchten chemischen Kommunikationskanal. Unter Anwendung verschiedenster Methoden, darunter morphologische Beobachtungen, Verhaltensversuche und Gaschromatographie-gekoppelte Massenspektrometrie, konnte gezeigt werden, dass periorale Drüsen geschlechtsspezifische olfaktorische Signale erzeugen, die eine Reihe von sozialen Verhaltensweisen modulieren könnten. Dies unterstreicht die Bedeutung von Gerüchen für die Kommunikation im unterirdischen Ökotop.

Viertens habe ich geschlechtsdimorphe Merkmale bei afrikanischen Sandgräbern, schwerpunktmäßig bei Ansell-Graumullen, mittels geometrischer Morphometrie und phylogenetischen Hauptachsen-Regressionsmodellen quantifiziert. Die Ergebnisse dieser Analysen

lassen auf eine wichtige Rolle der intrasexuellen Konkurrenz zwischen Männchen bei diesen Nagetieren schließen, auch innerhalb monogamer Paarungssysteme.

Darüber hinaus fasse ich das biologische Wissen über zwei Modellarten in der Forschung an subterranen Säugetieren, den Ansell-Graumull und den Riesengraumull, in je einem eigenen Übersichtsartikel zusammen.

Abschließend lässt sich festhalten, dass diese Ergebnisse unser Wissen darüber, wie Graumulle und andere subterrane Nagetiere unter der Erde kommunizieren, wie ihre sensorischen Systeme funktionieren und welche Faktoren ihren sozialen Dynamiken zugrunde liegen, erheblich erweitern.

Summary

The subterranean realm poses unique challenges to animal orientation and communication. It is structurally uniform, deprived of light, and underground tunnels exhibit peculiar acoustic features. Diverse lineages of burrowing mammals evolved to thrive under these extreme conditions but essential aspects of how their sensory systems adapt to life underground remain enigmatic. To further elucidate this topic and by focusing on the example of social African mole-rats of the genus *Fukomys*, I explored diverse aspects of subterranean mammal sensory ecology, communication, and sociality in a series of laboratory studies.

First, I studied magnetoreception, a sensory modality that might crucially aid navigation underground. Two series of behavioral assays in enucleated and untreated mole-rats should clarify whether the eyes house the so far unidentified receptors that respond to the Earth's magnetic field. Results revealed that enucleated mole-rats lack the ability to orient based on magnetic cues, which suggests an eye-based mechanism of magnetic field perception and thus narrows down the search space for the enigmatic magnetoreceptor cells in a mammal. Second, I comparatively examined hearing sensitivity in African mole-rats, a geologically old subterranean clade, and coruros, a younger lineage of rodent burrowers, by measuring auditory brainstem responses. While auditory sensitivity in the former was markedly restricted, hearing patterns in coruros mediated between the derived condition in mole-rats and the acute hearing of epigeic rodents. A comparison of these different groups suggests that both adaptive and degenerative trajectories shaped the evolution of hearing in subterranean rodents. Third, I studied mole-rats' perioral sebaceous glands, a so far neglected communicative channel in burrowing rodents. A range of methods, including morphological observations, behavioral assays, and gas chromatography-mass spectrometry revealed that perioral glands generate sex-specific olfactory signals which may modulate a range of social behaviors in these rodents, emphasizing the significance of odors for communication underground. Fourth, I quantified patterns of sexual dimorphism in African mole-rats, focusing on Ansell's mole-rat, by means of geometric morphometrics and phylogenetic major axis regression models, which suggest an important role of male-male intrasexual competition in these rodents' communities, even within monogamous mating systems. In addition to that, I summarize the biological knowledge on two model species in subterranean mammal research, Ansell's mole-rat and the giant mole-rat, in dedicated review articles.

In conclusion, these results significantly expand our knowledge on how African mole-rats communicate underground, how their sensory systems function, and which drivers underlie their social dynamics.

General introduction

Mostly overlooked by the public eye, subterranean rodents lead inconspicuous lives in their underground burrow systems. Despite of that, these curious animals comprise some of the most striking and extreme examples of ecological adaptation among mammals. Although some species, in particular the famed naked mole-rat, have become laboratory models and received notable research attention in recent years, many facets of the biology of these remarkable rodents remain enigmatic. This includes how their senses and strategies to communicate adapted to the peculiar demands of a life in subterranean tunnel systems and how their sometimes highly complex social groups are structured and maintained in the underground realm. This thesis aims to elucidate different aspects of the sensory biology and sociality of group-living subterranean rodents, with a focus on African mole-rats of the genus *Fukomys*. For selected comparisons, the naked mole-rat as well as the coruro, a South American burrowing rodent, have been examined as well.

Initially, I review the biology of two model species in subterranean rodent research, the giant mole-rat and Ansell's mole-rat (Chapter 2.1). That is followed by empirical studies that cover four distinct aspects of these animals' biology:

- The seat and functionality of receptors enabling magnetoreception (Chapter 2.2)
- Hearing sensitivity and its evolutionary trajectory in subterranean rodents (Chapter 2.3)
- Olfactory communication based on facial glandular secretions (Chapter 2.4)
- Sexual dimorphism and its implications for social dynamics (Chapter 2.5)

Specific research questions guiding the presented work are prefaced in the introduction of the thesis (Chapter 1). Individual manuscripts are followed by a discussion section in which I aim to include additional relevant aspects on the topic in focus that either had to be omitted from the respective manuscripts for reasons of conciseness or which were brought to my attention by colleagues after the publication of these papers. In several cases, I have also retrospectively discovered methodological errors in the studies that I want to discuss and make transparent here. Finally, these sections will also include discussions of new literature published over the course of the last two years that has either supported or challenged contents presented in the published papers. Chapter 2.4 was finalized immediately prior to the submission of this thesis and thus will not feature a dedicated discussion section. The thesis closes with a general synopsis of the findings and an outlook for future research on social subterranean rodents (Chapter 3).

Chapter 1 – Introduction

1.1 – A breviary of the biology of subterranean rodents

The adoption of a subterranean lifestyle has been a recurrent phenomenon in vertebrate evolution. Especially among reptiles and mammals, species from diverse lineages adapted to live, forage and reproduce underground, only seldomly leaving their self-excavated burrow-systems (Gans, 1978; Nevo, 1979). In mammalian evolution, subterranean forms evolved early (Luo et al., 2015) and repeatedly (Nevo, 1979) and are today found among almost all major phylogenetic lineages, including for instance marsupial moles (Notoryctemorphia – Marsupialia), fairy armadillos (Chlamyphorinae – Xenarthra), golden moles (Chrysochloridae – Afrotheria) and true moles (Talpidae – Laurasiatheria). However, most burrowing mammals are rodents (Rodentia – Euarchontoglires), comprising about 250 species within numerous clades which independently adapted to underground life (Begall et al., 2007), such as the African mole-rats (Bathyergidae), the Eurasian blind mole-rats (Spalacidae) and the South American tuco-tucos (Ctenomyidae). Different from all other subterranean mammals, burrowing rodents are primarily herbivorous and largely subsist on plant underground storage organs. Due to their frugal dietary and housing requirements, they are well suited for laboratory research, so that a wealth of biological data has been accumulated for representatives of various lineages. Thus underground-dwelling rodents today include the best characterized model species to study mammalian adaptations to subterranean life.

Many burrowing rodents are highly derived specialists. The underground ecotope poses numerous adaptive challenges, especially affecting locomotion (Stein et al., 2000), respiration (Malik et al., 2012), thermoregulation (Vejmělka et al., 2021), metabolism (Moshkin et al., 2007), orientation, and communication (see below). Comparative studies on different groups of burrowing mammals have a long tradition and convergent as well as divergent evolutionary trends are well characterized (Nevo, 1979). In recent years, molecular 'omics' approaches have gained a significant impact in the field and deepened our understanding of subterranean mammal evolution (Malik et al., 2012; Fang et al., 2014; Davies et al., 2018; Emerling, 2018). At the anatomical level, peculiar adaptations that emerged convergently in diverse underground-dwelling rodents include a cylindrical body shape, shortened and functionally specialized body appendages and integumental traits that aid in reducing friction (Klauer et al., 1997; Stein et al., 2000). The majority of burrowing rodents are tooth diggers and exhibit procumbent extrabuccal incisors to aid them in excavating their tunnel systems (Stein et al., 2000). Distinct physiological adaptations allow these animals to survive under hypoxic and hypercapnic conditions (Malik et al., 2012; Fang et al., 2014; Ivy et al., 2020), and many lineages of subterranean rodents display pronounced cancer resistance and longevity (Seluanov et al., 2018; Weigl, 2005).

Besides these specializations, they also evolved a suite of similarly striking unusual sensory adaptations and social behaviors in the context of their burrowing lifestyle. Both of these aspects are covered extensively in this thesis.

1.1.1 – Sensory ecology

Research on the sensory biology of subterranean rodents has so far focused on African mole-rats (Bathyergidae) and the blind mole-rats (Spalacidae) of the Mediterranean region (Burda et al., 1990; Burda, 2021). Sensory adaptations of the former also constitute the main focus of this thesis. So far, especially vision, hearing, and magnetoreception have received considerable, though not exhaustive, scientific attention in these animals, while less data is available on olfaction and the tactile sense. Furthermore, since the most intensively studied groups represent highly adapted, geologically old lineages, uncertainties remain regarding the mode and sequence of initial sensory adaptation to the subterranean realm. Geologically young and less specialized underground dwellers, such as the long-clawed mole-mice (*Geoxus* spp.) or the coruro (*Spalacopus cyanus*), might offer clues to retrace this process.

The subterranean ecotope must be characterized as an extreme environment in respect to orientation and communication. Compared to above ground habitats, it is deprived of many sensory cues or provides them in altered form. Obviously, light levels in underground tunnel systems are usually unperceivably low, leading to noticeable reduction of the visual system in the vast majority of specialized burrowers (Kott et al., 2014; Emerling, 2018). Besides that, substrate properties underground are comparatively uniform and structurally less complex than those encountered above ground, hindering orientation. However, the Earth's magnetic field provides a stable reference for navigation underground and there is evidence that various subterranean rodents can utilize geomagnetic cues to orient underground, compensating for a lack of visual landmarks (Němec et al., 2001; Kimchi & Terkel, 2001; Malewski et al., 2018). Furthermore, the substrate's conformation and density create specific demands for certain sensory modalities: Underground tunnels exhibit a unique acoustic environment, amplifying low-frequency sounds while strongly attenuating high frequencies (Lange et al., 2007). Also, communication between and within burrow systems can be facilitated via seismic signaling through ground vibrations, a strategy found among several subterranean rodent clades (Heth et al., 1987; Narins et al., 1992). However, it remains to be clarified how far these signals carry underground and to which extent respective species rely on them (compare Park et al., 2007). The relevance of olfactory cues to burrowers is not well understood, but multiple lines of evidence point to an important role of this sensory modality in several subterranean rodent lineages (Nevo et al., 1976; Stathopoulos et al., 2014; Dollas et al., 2019).

Learning about how the sense organs evolved to operate under these extreme conditions could not only uncover unique adaptations but could as well improve our general understanding of functionality and limitations of the mammalian sensory apparatus. The sensory modalities studied and prominently discussed hereafter in Chapter 2 are magnetoreception, hearing, and olfaction, which I will briefly introduce in some more detail here.

Magnetoreception remains to be the most puzzling of all vertebrate sensory modalities but it has nevertheless attracted significant research attention in subterranean rodents over the last three decades (Burda et al., 1990; Kimchi & Terkel, 2001; Malewski et al., 2018). In fact, a burrowing rodent, the Zambian Ansell's mole-rat (*Fukomys anselli*) was the first mammal in which a magnetic sense was experimentally demonstrated under controlled laboratory conditions (Burda et al., 1990 – note that the species was at that time still subsumed under *Cryptomys hottentotus*). Still, the cellular mechanisms and identity of receptors enabling the perception of magnetic fields in this species or in fact any vertebrate remain unidentified and render the physiology of magnetoreception one of the greatest enigmas of sensory biology (Begall et al., 2014).

The geomagnetic field can provide different types of sensory cues that may be exploited for navigation via different physiological pathways (Thalau et al., 2006). On the one hand, animals may sense the polarity of the Earth's magnetic field, which would enable the differentiation between magnetic North and South. On the other, they might respond to the inclination of the geomagnetic field, i.e. the angle between the magnetic field lines and the horizontal of the Earth's surface. The inclination angle steepens towards the poles, so that an inclination compass would allow an animal to differentiate between the position of the nearest magnetic pole relative to the equator. Furthermore, the field intensity of the geomagnetic field, which generally increases towards either magnetic pole, could represent a navigation cue (Fransson et al., 2001).

Two main hypotheses have been put forward to explain how mammals might sense magnetic fields. The first one proposes a magnetite-based polarity compass. Such a system would work based on tissue-embedded magnetite (Fe_3O_4) concretions, which, like microscopic bar magnets, would change their orientation dependent on field polarity and intensity (differentiation: North vs. South). If coupled to sensory neurons expressing mechanosensitive ion channels, the magnetite particles could enable magnetoreception (Begall et al., 2014). Among mammals, behavioral evidence for such a magnetite compass is, for instance, available for the Chinese noctule (*Nyctalus plancyi*), a species of bat (Wang et al., 2007).

Second, a radical pair mechanism might be at play, which would work based on a complex photochemical reaction (formulated by Ritz et al., 2000; reviewed in e.g., Hore & Mouritsen, 2016). In brief, a photo-excitable donor molecule (most often hypothesized to be a protein of the cryptochrome family) is stimulated by a photon and transfers an electron to an acceptor molecule

(likely flavin adenine dinucleotide, a cryptochrome cofactor). That results in both the donor and the acceptor becoming radicals presenting unpaired electrons. These electrons can now adopt different spin states, so called singlet or triplet states. The interconversion of spin states is influenced by the intensity and inclination of the ambient magnetic field. Spin states again affect the decay characteristics of the respective radical pairs, so that different concentrations of singlet and triplet state decay products are yielded in response to changes in the aforementioned magnetic parameters. Hypothetically, this process could enable responsiveness to geomagnetic field inclination (differentiation: poleward vs. equatorward) and is hypothesized to take place in the photoreceptor cells of the retina (Kishkinev & Chernetsov, 2015, Hore & Mouritsen, 2016). Different from the magnetite-based mechanism, photochemical magnetoreception can be disturbed by radio frequency electromagnetic radiation (Thalau et al., 2006). A mammalian species that might utilize a radical pair mechanism to sense magnetic fields is the wood mouse (*Apodemus sylvaticus*), an epigeic rodent (Malkemper et al., 2015).

Different from birds (Hore & Mouritsen, 2016), there is no general hypothesis concerning the molecular and cellular processes underlying photochemical magnetoreception in mammals (compare Nießner et al., 2016). Not considering behavioral evidence (e.g., Malkemper et al., 2015), some authors even conclude that the available molecular and physiological data indicate that mammals lack photochemical magnetoreception altogether and cannot respond to field inclination (Kavet & Brain, 2021). Finally, it should be pointed out that the two mechanisms laid out above are not mutually exclusive and animals might leverage both of them simultaneously to exploit geomagnetic stimuli more effectively. Robust evidence indicates that this is the case in birds (Kishkinev & Chernetsov, 2015) but there are also indications for the co-occurrence of both magnetoreception pathways in mammals, such as dwarf hamsters of the genus *Phodopus* (Malewski, 2018).

Which of these alternatives do most likely underlie magnetoreception in subterranean rodents such as Ansell's mole-rat? This species exhibits a population-level bias to spontaneously construct nests in the south-eastern sector of a circular arena (Burda et al., 1990). Based on experimental manipulation of that behavior, researchers were able to robustly deduce important functional aspects of magnetic sensing in these rodents. The mole-rat's magnetic compass works independent of light, responds to changes in field polarity but not field inclination and it can be impaired by strong magnetic pulses but not by radiofrequency magnetic fields (Marhold et al., 1997; Thalau et al., 2006). All of these features are compatible with a magnetite-based mechanism of magnetoreception and contradict a photochemical pathway (Begall et al., 2014). Nevertheless, magnetite-based receptors could, hypothetically, be located anywhere in the body, complicating their identification and further study. Yet, there are some clues as to where they might be located in African mole-rats. Experiments with lidocaine-mediated corneal anesthesia in Ansell's mole-

rats have hinted at the eye as an organ of vital importance for sensing magnetic fields: Treated mole-rats showed randomized instead of directional nest-building, while light perception remained unaffected (Wegner et al., 2006). This suggests a crucial involvement of the cornea, rather than, for instance, the retina, in mole-rat magnetoreception. However, the use of lidocaine as an anesthetic in behavioral testing has been criticized recently and it was proposed that nerve ablation or similar procedures should yield more reliable results (Engels et al. 2018). Chapter 2.2 will present results on tests with surgically manipulated mole-rats, which aim to clarify whether the eyes indeed play a role in magnetoreception in mole-rats, and potentially in other mammals.

Hearing is probably the most intensively studied sense in burrowing rodents and highly divergent when compared to epigeic forms. Subterranean species so far examined have comparatively high hearing thresholds, are most sensitive to low-frequency sounds (0.5 – 2 kHz), and are unable to respond to frequencies > 6 kHz (Heffner & Heffner, 1993; Gerhardt et al., 2017). Thus, both their hearing range and sensitivity are severely restricted. Above that, their ability to localize sound sources appears to be strongly impaired (Heffner & Heffner, 1993). These observations were originally explained as resulting from degeneration of the inner ear in subterranean groups due to a lack of selective pressures to maintain its acuity (Heffner & Heffner, 1993). Other authors interpreted these patterns as adaptive (Burda et al., 1990), pointing out that the peculiar acoustic properties of the tunnel environment could explain their recurrent evolution. Arguments for either side of the debate continue to be formulated and defended (Mason et al., 2016; Burda, 2021) and so the evolutionary trajectories shaping hearing in burrowing mammals remain contested.

Recently, Pyott et al. (2020) presented a comprehensive study on hearing physiology in African mole-rats. They leveraged data from electrophysiology, otoacoustic emissions, receptor morphology, and mutations in genes relevant to audition to describe and explain poor hearing in this group. The main conclusion of the study is that African mole-rats would uniquely lack cochlear amplification mechanisms, explaining their low hearing sensitivity. The cochlear amplifier is an important component of mammalian hearing physiology. Within the organ of Corti, hair cells, the mechanoreceptors of the ear, are arranged in two distinct parallel bands, one row of inner and typically three rows of outer hair cells (Fig. 1). These hair cell bands are covered by an acellular fibrous sheet, the tectorial membrane. The inner hair cells are the principal mechanoreceptors of the ear and transduce auditory information to the brain via the vestibulocochlear nerve (Raphael & Altschuler, 2003). In contrast, the outer hair cells contribute little to stimulus transduction (Ashmore, 2008). Different from the inner hair cells, the tips of the outer hair cells' stereocilial hair bundles are embedded in the tectorial membrane (Fig. 1) and they respond to incoming sound by contractions of their soma. This active process is critically enabled by the membrane-bound motor protein prestin (reviewed extensively in Ashmore, 2008).

Due to their mechanic coupling, contracting outer hair cells set the respective portion of the tectorial membrane and thus the cochlear endolymph into motion, which amplifies the sound stimulus for the inner hair cells and therefore increases hearing sensitivity (Raphael & Altschuler, 2003; Ashmore, 2008).

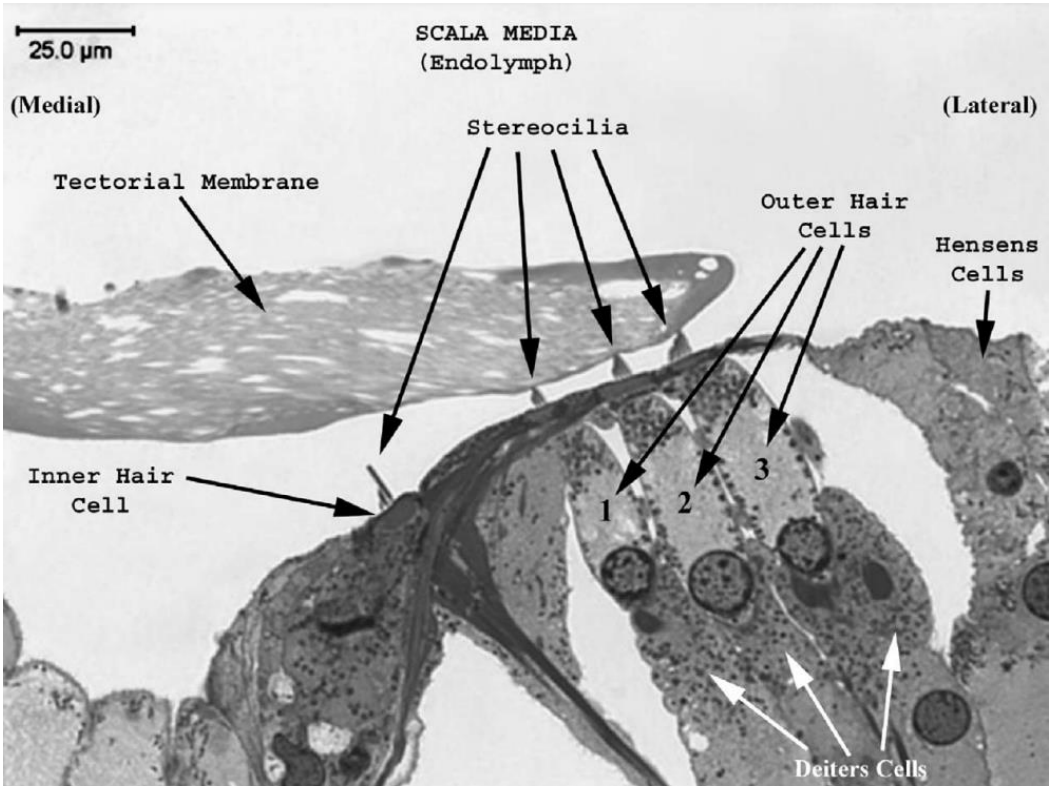

Figure 1: Micrographical cross section through the organ of Corti of a blind mole-rat (*Nannospalax* sp.) from Raphael & Altschuler (2003) which represents the general mammalian condition. The organ of Corti is situated in the cochlear scala media and rests on the basilar membrane (not shown). Inner hair cells constitute the principal mechanoreceptors of the ear (note conspicuous afferent myelinated fibers), while outer hair cells amplify signals by contracting and displacing the acellular tectorial membrane in response to incoming sound. A single row of inner hair cells is opposed to typically three distinct rows of outer hair cells. Note that only the stereocilial bundle tips of the outer hair cells are embedded in the tectorial membrane while those of the inner hair cells move freely in the endolymph. Hensen's cells and Deiter's cells provide structural support for the hair cells.

For bathyergids, however, Pyott et al. (2020) suggest that cochlear amplification is dysfunctional because the stereocilial bundles of the outer hair cells are structurally distorted or even absent and hence cannot set the tectorial membrane into motion (Pyott et al., 2020). To bolster this interpretation, they present recordings of distortion product otoacoustic emissions (DPOAE; evoked emissions from the cochlea that derive from sound stimulus-induced hair cell motility) and of auditory brainstem responses (ABR; acoustically-evoked potentials of the brainstem) that align with their predictions: DPOAE were not recovered in bathyergids and ABR

indicated extremely elevated hearing thresholds. Yet, these findings contradict results from earlier auditory and behavioral studies on bathyergids which suggest both lower hearing thresholds than communicated by Pyott et al. (2020) and the presence of DPOAE (Kössl et al., 1996; Gerhardt et al., 2017). Chapter 2.3 presents new electrophysiological data on hearing sensitivity in African mole-rats and coruros. There were two aims for this study: First, it should contribute to the ongoing debate about whether hearing alterations in burrowing rodents are driven by adaptive or degenerative processes by comparing representatives of geologically young and old lineages that differ in their degree of subterranean specialization. Second, it attempts to reconcile the findings of Pyott et al. (2020) with results from the previous literature by presenting new ABR data on bathyergids and reflecting on potential methodological biases.

Olfaction constitutes an understudied sensory modality in burrowing rodents. Yet, there is evidence that it serves important functions for these animals, both in foraging and in social contexts. Laboratory studies have shown that various subterranean rodents can use substrate-born odors to identify and navigate towards food plants in forced choice assays (Heth et al., 2002; Lange et al., 2005). This suggests that foraging in the wild is at least partially dependent on odor cues, but field experiments remain to be conducted. Additionally, in both social and solitary species, olfactory cues have been demonstrated to aid in conspecific recognition (Nevo et al., 1976; Hagemeyer et al., 2004). Indeed, it is assumed that the social system in cooperatively-breeding African mole-rats is chiefly maintained by olfactory cues, which enable the unambiguous identification of relatives and inhibit incestuous mating (Heth et al., 2004). Given the apparently crucial significance of the olfactory sense it comes as no surprise that both neuroanatomical and genetic evidence supports the assumption that at least African mole-rats possess exceptionally acute olfactory capabilities (Stathopoulos et al., 2014; Dollas et al., 2019). Although odor cues modulating social behaviors are typically processed by the vomeronasal organ in rodents, this structure appears to be regressed in the African mole-rat family (Dennis et al., 2020, compare Jastrow et al., 1998). Interestingly, both solitary and social subterranean rodents show a reduced diversity of intact vomeronasal receptor genes, indicating a subordinate role of pheromonal communication (Jiao et al., 2019). It remains to be clarified which body odors carry specific social information in subterranean rodents. Different from many epigeic forms, their skin is largely devoid of apocrine or holocrine glands, the secretions of which might convey such cues (Sokolov, 1982; Hesselmann, 2010) and their urine lacks major urinary proteins, which are crucially involved in social communication of murid rodents (Hagemeyer et al., 2011). In Ansell's mole-rats, anogenital odor has been demonstrated to allow individual-level recognition of conspecifics (Hagemeyer et al., 2004; Heth et al., 2004) and specialized sebaceous glands that might produce such informative secretions have indeed been described in the perineal area of the naked mole-rat and Southern common mole-rats (Tullberg, 1899; Kimani, 2013).

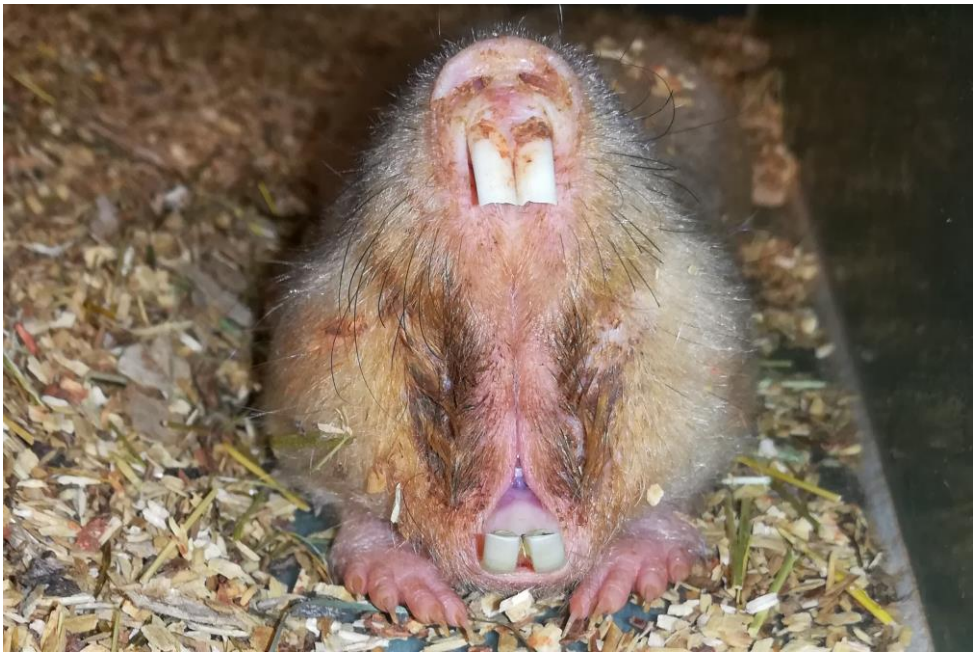

Figure 2: Well-visible rusty-brown perioral secretions in a yawning male giant mole-rat (*Fukomys mechowii*). Despite their conspicuousness, these secretions have so far received little scientific attention. Photo: Kristin Katschak.

Perioral glands could be another so far neglected source of socially relevant secretions in subterranean rodents. Although these structures are commonly found in the mouth corners of diverse epigeic as well as burrowing rodents (Sokolov, 1982; Kimani, 2013), they have remained virtually unstudied. In common mole-rats (genera *Cryptomys* and *Fukomys*), such perioral glands produce a waxy secretion that results in a conspicuous staining of the mouth corners (Fig. 2). Chapter 2.4 characterizes the sex-specific expression and composition of perioral gland secretions in Northern common mole-rats and discusses their potential role in social communication.

1.1.2 – Sociality

Although the lifestyle and foraging ecology of subterranean rodents is rather uniform, they evolved a striking diversity of social systems, ranging in complexity from solitariness, over various degrees of gregariousness, to cooperatively breeding family groups (Burda et al., 2000; Smorkatcheva & Lukhtanov, 2014). Many subterranean rodents are strictly solitary with single individuals occupying a respective burrow system. These include blind mole-rats, zokors, pocket gophers, and most tuco-tuco species. Social burrowing rodents, in which several individuals permanently share a burrow system, comprise species such as the coruro, the common mole-rats of the genera *Cryptomys* and *Fukomys*, and the eponymous social tuco-tuco (*Ctenomys sociabilis*). The highest degree of social organization is found among the monogynous, cooperatively breeding Northern common mole-rats (*Fukomys* spp. - Patzenhauerová et al., 2013, Chapter 1.2.1) and naked mole-rats (*Heterocephalus glaber* - Szafranski et al., 2022, Chapter 2.5), a well as in the less

popular mole-voles (*Ellobius* spp. - Smorkatcheva & Kumaitova, 2014; Smorkatcheva, 2021). While both naked and common mole-rats are bathyergids (Chapter 1.2.1), they evolved monogyny and cooperative breeding independently (see e.g., Jarvis & Bennett, 1993). In all these species, the dispersal of offspring is typically delayed, so that the non-reproductive young may stay with their parents well into adulthood. This gives rise to large family groups with high reproductive skew. Cooperatively-breeding African mole-rats are even championed by various authors as examples for mammalian eusociality (Jarvis & Bennett, 1993; Burda et al., 2000; but see below). Late dispersing non-breeders are hypothesized to aid group survival and the fitness of the breeders by maintaining the tunnel system, locating food sources, and practicing alloparental care (Zöttl et al., 2016).

It remains challenging to explain why social patterns among in parts closely related species of subterranean rodents can be so variable and also why extreme forms of cooperative-breeding emerged multiple times among these animals. Nowak et al. (2010) have devised an influential model to retrace the evolution of cooperative breeding communities, in which some members (temporarily) sacrifice their own reproductive potential. They emphasize that important exaptations for cooperative breeding and eventually eusociality are defendable nests and the provisioning of offspring, since these factors would chiefly enable non-dispersing offspring to significantly contribute to the survival of their parents and siblings. Alleles that control late (or deferred) dispersal can then spread in the population, because the inclusive fitness that non-breeders gain for supporting the reproductive female (i.e. their mother) exceeds the fitness of non-cooperatively breeding conspecifics. The lifestyle of subterranean rodents therefore likely predisposed them to form cooperatively breeding family groups. Interestingly, entomologist R. D. Alexander predicted the discovery of eusocial burrowing rodents in 1975 based on similar arguments (Braude, 1997), years before such a social organization was indeed discovered (Jarvis, 1981).

Mathematical models also indicate that cooperatively breeding communities have a fitness benefit over egalitarian social groups or solitary breeders when resources are costly to acquire or scarce (Fronhofer et al., 2018), as it the case with the underground storage organs of plants, on which most subterranean rodents are dependent on. This might suggest that the relative availability of food resources might be a decisive factor underlying the evolution of social complexity and its disparity in burrowing rodents. For the African mole-rats in particular, the so-called aridity-food-distribution-hypothesis has prominently made the case that social species evolved on several occasions from solitary ancestors over the course of adaptation to more arid, food deprived, environments (Jarvis & Bennett, 1991, 1993; Burda et al., 2000). However, food plant abundance and soil hardness, as approximated by precipitation data, does not correlate with sociality in African mole-rats and current phylogenetic considerations favor the assumption that

the common ancestor of bathyergids was a social, not a solitary animal (Burda, 2001). In fact, there is evidence that sociality is an exaptation for subterranean adaptation in rodents in general (Smorkatcheva & Lukhtanov, 2014). Thus, both cooperatively breeding and solitary species acquired their peculiar life histories secondarily and derived them from already gregarious ancestors. Conformingly, solitary burrowing species are almost exclusively found among geologically old lineages such as the Spalacidae, Geomyidae, and Bathyergidae and are absent from younger clades (Smorkatcheva & Lukhtanov, 2014). Given all the aforementioned evidence, it might appear even more difficult to explain the emergence of solitary habits rather than cooperative breeding in burrowing rodents, but little research has so far focused on this issue.

What is the justification to classify the social African mole-rat genera *Heterocephalus* and *Fukomys* as eusocial (Jarvis, 1981; Jarvis & Bennett, 1993) rather than as ordinary cooperatively breeding mammals? Burda et al. (2000) defined mammalian eusociality based on the following criteria: Reproductive division of labor with non-breeding animals of a group performing alloparental care; overlap of adult 'generations' (litters) in one family; permanent philopatry of the majority of offspring. However, because the term eusociality was originally coined to describe the highly distinct community structure of social insects such as ants and termites (Wilson, 1971) and was later on specified to more precisely fit these invertebrate societies (Crespi & Yanega, 1995), its applicability to subterranean rodents remains contested (Zöttl et al., 2016; Thorley et al., 2021).

Different from typical workers in eusocial insects, non-breeding mole-rats are not permanently sterile, but are ready to copulate and reproduce once an opportunity presents itself (e.g., Burda, 1995). It is the high mortality among non-breeders, often related to failed dispersal attempts, rather than permanent physiological constraints that maintains a high reproductive skew and apparent philopatry at the population level in the wild (Jarvis & Bennett, 1993; Braude, 2000). To demonstrate that late-dispersing offspring do effectively assist in raising their siblings, a positive correlation between reproductive female fecundity and family size should be evident. Yet, in Damaraland mole-rats, a widely agreed on example of mammalian eusociality (Jarvis & Bennett, 1993; Burda et al., 2000), the evidence for that is mixed. One study evaluated field data from a 14-year period collect at Dordabis, Namibia, and found that worker recruitment rates but not pup survival were indeed positively correlated with group size (Young et al., 2015). Similar findings were communicated by a study on captive Damaraland mole-rats (Houslay et al., 2020). However, these results could not be replicated by a 7-year field project conducted in the South African portion of the Kalahari in which only a marginal increase of reproductive female fitness with group size was recovered (Thorley et al., 2021). Thus, additional data is needed to discern to which extent non-dispersing offspring is contributing to the survival of their siblings and discharge of their parents and also whether the conclusions drawn from aridophile Damaraland

mole-rats can be generalized. The current evidence at least suggests that *Fukomys* parents do not need to rely on their late-dispersing offspring to effectively care for younger litters (Thorley et al., 2021), a striking contrast to what is observed in eusocial insects (Wilson, 1971).

Finally, there is no differentiation into morphologically and ethologically fixed worker types (soldiers, nurses etc.) as known from social insects among any subterranean rodents. Although a common misconception (Braude et al., 2021), social mole-rats therefore do not form castes, which are defined as "groups of individuals that become irreversibly behaviorally distinct at some point prior to reproductive maturity" (Crespi & Yanega, 1995) in the ethological literature. Instead, there is a moderate age specific polyethism in the frequencies of specific tasks that non-breeders engage in, though the overlap in working profiles between age groups remains substantial in both naked and common mole-rats (Mooney et al., 2015; Zöttl et al., 2016; Gilbert et al., 2020; Siegmann et al., 2021). All in all, these results suggest a continuity between the social systems of mole-rats and cooperatively breeding epigeic mammals such as meerkats and social canids and refute a resemblance to eusocial insect societies (Zöttl et al., 2016; Thorley et al., 2021). As I feel compelled by the arguments against the classification of mole-rats as eusocial, I will not adopt the term in this thesis and instead denote them simply as cooperative breeders. At the same time, it might also be worthwhile to point out the possibility that not all species of the "eusocial" mole-rat genus *Fukomys*, on which this thesis is centered, do in fact breed cooperatively. In the understudied Ghana mole-rat (*Fukomys zechi*) mean family sizes of just 4.2 animals (maximum: 7) have been reported (Yeboah & Dakwa, 2002). This indicates a timing of births and offspring dispersal that leaves only little opportunity for young from different litters to interact.

The cryptic lifestyle of subterranean rodents complicates the monitoring of social behaviors and how family groups develop over time in the wild. Yet, both genetic methods and long-term capture recapture studies have generated valuable insights in the social systems of African mole-rats in particular (Chapter 1.2). An analysis of morphological correlates of behavior may help to elucidate the social dynamics of these rodents even further. For instance, reproductive competition among males is an important component of most mammalian communities, but we know comparatively little about its significance in underground-dwelling mammals. On a morphological level, it is usually positively correlated with male-biased sexual dimorphism in body size and weaponry. Particularly in species with little paternal investment in offspring, these traits enable males to physically monopolize females against same-sex rivals and thus to markedly increase their fitness (Lindenfors et al., 2007). In African mole-rats, patterns of sexual dimorphism have received little attention so far, yet appear paradoxical: Both highly dimorphic and monomorphic species co-occur among solitary as well as social genera, seemingly irrespective of phylogenetic affiliation (Burda, 1990).

Figure 3: Sexual dimorphism in Ansell's mole-rat (*Fukomys anselli*). The male (top) is markedly larger than the female and exhibits a more massive head. Both animals shown are fully adult. Photo: Kai R. Caspar.

The primarily monogamous mating system of naked mole-rats and Northern common mole-rats is typically associated with monomorphism among mammals (Kleimann, 1977). However, many species of the latter genus display a strikingly dimorphic anatomy (Fig. 3). Chapter 2.5 analyses patterns of sexual size dimorphism in African mole-rats as well as sexually dimorphic skull traits in the cooperatively-breeding Ansell's mole-rat to infer the role of male-male competition in social subterranean rodents.

1.2 – Model species

1.2.1 – African mole-rats (family Bathyergidae)

The African mole-rats constitute the primary research models of this theses. Two social genera of these strictly subterranean rodents have been experimentally studied here, the Northern common mole-rats of the genus *Fukomys* and the naked mole-rat of the monotypic genus *Heterocephalus*. The remaining bathyergid genera, the social *Cryptomys* as well as the solitary *Bathyergus*, *Georychus*, and *Heliophobius*, are discussed in passing (Chapter 2.5) but do not feature prominently here.

The family Bathyergidae occurs across the semi-deserts, grasslands, savannahs and mesic woodlands of sub-Saharan Africa (Burda, 2001). Interrelations of the six genera comprised by the clade are depicted in Figure 4. African mole-rats are part of the hystricomorph rodent radiation that also encompasses porcupines, cavies, and their kin (Lacher et al., 2016). Within this speciose clade, they arguably represent the lineage most strongly adapted to a life underground: All

bathyergids are strictly subterranean and dwell in self-excavated burrow systems. The characteristic extrabuccal incisors are the most procumbent among rodents and are employed for chisel-tooth digging (Landry, 1957; but note that one genus, *Bathyergus*, is primarily a scratch-digger – Montoya-Sanhueza et al., 2019). Surface activity is essentially restricted to dispersal from the natal burrow (e.g., Braude, 2000; but see Kawalika & Burda, 2007), although even for this process, it is debated to which degree the animals might rely on underground rather than surface routes (Patzenhauerová et al., 2013). All bathyergids primarily subsidize on tubers, roots, and rhizomes, although some species may supplement their diet with animal protein (Kawalika & Burda, 2007).

Bathyergids have become a model group to study mammalian adaptation to life underground (e.g., Gomes Rodrigues et al., 2016; Burda, 2021) and their peculiar morphology and physiology have been the subject of countless studies. The extensive literature on bathyergid subterranean adaptations and life history, exemplified by two species of *Fukomys*, is reviewed in Chapter 2.1 and will not be repeated here.

Patterson & Upham (2014) suggested that the naked mole-rat, which is traditionally comprised as the basal-most branch within the Bathyergidae, should constitute its own family, the Heterocephalidae. This idea has been highly influential and was adopted by authoritative textbooks (Lacher et al., 2016) as well as by Wikipedia (see "naked mole-rat," 2022). Curiously, the proposal was largely ignored by the African mole-rat research community, which has since pointed out various flaws in the argumentation of Patterson & Upham (Braude et al., 2021). In this thesis, I will adopt the traditional view of the naked mole-rat as a bathyergid, although I want to point out that I consider both positions justifiable, given the evidence available at the moment.

Bathyergids have long been championed as an ancient family of rodents and were thought to have emerged in the early Eocene (ca. 45 million years ago – Faulkes et al., 2004; Ingram et al., 2004; compare Thenius, 1979). The most basal lineage, *Heterocephalus*, has even been described as a living fossil (Bredberg & Schmitz, 2019). Yet, current evidence for the antiquity of bathyergids is mixed and more recent analyses incorporating both molecular and fossil evidence instead point to a diversification of the group in the early Miocene (ca. 21 million years ago – Bryja et al., 2018). A major issue with dating the origin and diversification of the group is the scarcity of fossil material (Winkler et al., 2010), making the claim that naked mole-rats in particular are "phenotypically largely unchanged since 30-50 million years ago" (Bredberg & Schmitz, 2019) a rather imaginative one. The oldest bathyergid fossils are not older than the early Miocene and their affinities to the different crown group lineages are largely unknown (Winkler et al., 2010). Despite this lack of fossil evidence, the phylogenetic relationships among crown-group bathyergids have been robustly resolved by genetic analyses, which all arrive at the same topology for the African mole-rat family tree (Faulkes et al., 2004; Ingram et al., 2004; Bryja et al., 2018;

Visser et al., 2019; illustrated in Fig. 4). The biogeography of the group suggests an origin and early split of *Heterocephalus* in Eastern Africa, followed by a southward migration and subsequent diversification of the clade in the African cape region (Van Daele et al., 2007a). The most speciose genus *Fukomys* presumably split from its sister genus *Cryptomys* in Southern Africa as well, before migrating northwards, eventually colonizing vast portions of sub-Saharan Africa over the course of the Pliocene and Pleistocene (Van Daele et al., 2007a; Bryja et al., 2018).

The Northern common mole-rats, constituting the genus *Fukomys* (Kock et al., 2006), represent the main focus of this thesis. Over the last three decades, these animals became the subject of both extensive field and laboratory research and emerged as models to study cooperative breeding in small mammals (Jarvis & Bennett, 1993; Burda et al., 2000; Zöttl et al., 2016) and physiological adaptation to the underground realm in rodents, particularly in regard to their senses (reviewed in Burda, 2021 and Chapter 2.1). Thus, a remarkably diverse literature on *Fukomys* now exists, second in volume perhaps only to the naked mole-rat among all African rodents. *Fukomys* represents the most diverse bathyergid genus, encompassing species ranging from just 65 g (*F. darlingi*) to over 700 g (*F. mechowii*) in adult (male) body mass (Chapters 2.1 and 2.5). All species display varying degrees of male-biased sexual dimorphism (Lacher et al., 2016).

Two clades are found within *Fukomys*, a northern one from the savannahs and woodlands adjacent to the Sahel zone and a much more diversified southern hemispheric lineage (Lacher et al., 2016; Visser et al., 2019; see Fig. 4). Both clades are in dire need of taxonomic revision and species numbers and diagnoses are expected to change significantly over the course of the coming years. At the time of writing, 16 *Fukomys* species are commonly recognized (Mammal Diversity Database, 2022), several of them being of questionable validity (Van Daele et al., 2007b; Chapter 2.1). So far, research has almost exclusively focused on species of the southern clade, leaving the distribution, ecology and behavior of northern *Fukomys* species enigmatic. Based on current data, the southern clade comprises six lineages, the unambiguous delineation of which is only possible based on molecular genetics (Visser et al., 2019). Within the southern clade, research has focused on three species, the Damaraland mole-rat (*Fukomys damarensis*) of the Kalahari Desert, Ansell's mole-rat (*Fukomys anselli*) from the woodlands of Central Zambia, and the giant mole-rat (*Fukomys mechowii*) which occurs across a variety of habitats in Zambia, Angola and the Democratic Republic of Congo. The latter two species, which are representative for the genus in many respects, will also feature prominently in this thesis and are introduced in detail in Chapter 2.1. Accordingly, I want to refer to these Chapters regarding details on *Fukomys* biology and only sketch selected elements of their life history here.

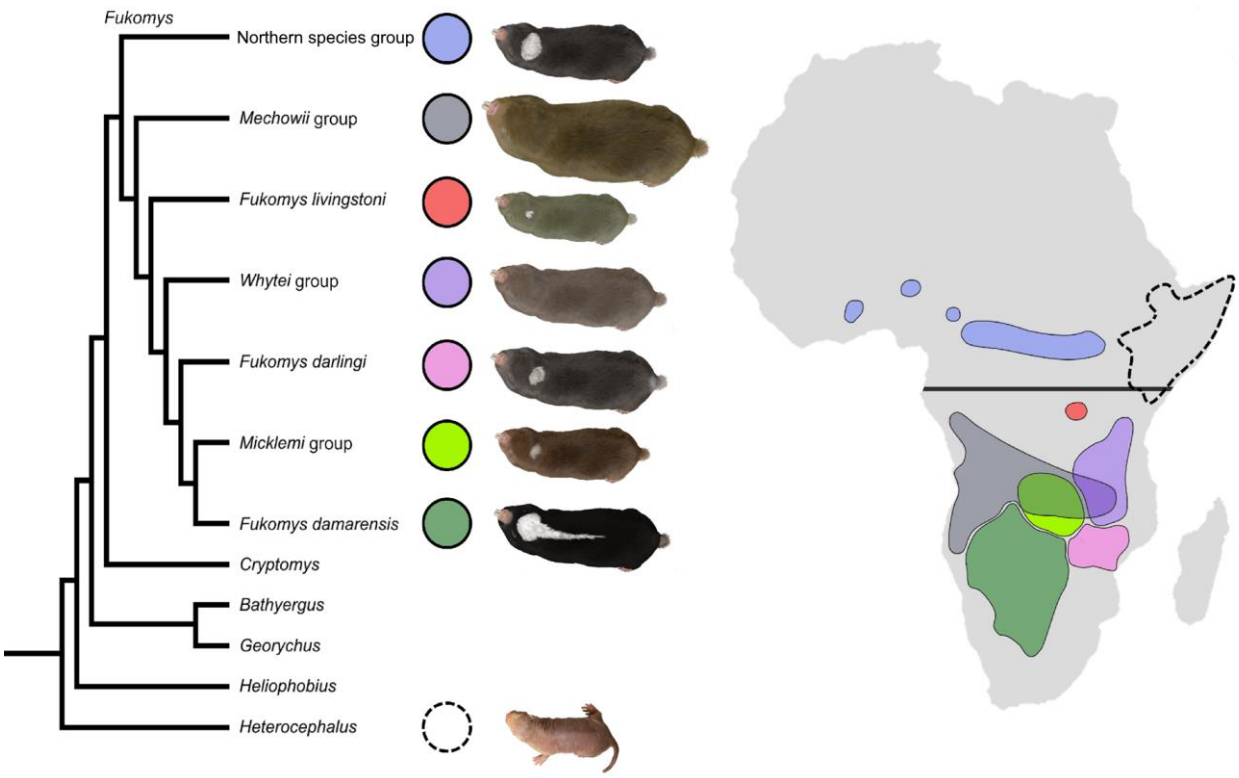

Figure 4: Phylogeny of African mole-rats (Bathyergidae) according to Visser et al. (2019), with an emphasis on evolutionary lineages within the Northern common mole-rat genus *Fukomys*. The geographical distribution and general habitus (to scale, only females shown) of *Fukomys* clades and the naked mole-rat are shown and color-coded (following Lacher et al., 2016 and Visser et al., 2019). The position of the equator is visualized by the solid black line. Note that many ranges shown here are highly conjectural. Depicted *Fukomys* clades contain the following conventionally accepted species: Northern species group – *F. foxi, F. ochraceocinereus, F. zechi; Mechowii* group – *F. bocagei, F. mechowii, F. vandewoestijneae; Whytei* group – *F. amatus, F. hanangensis, F. whytei; Micklemi* group – *F. anselli, F. kafuensis, F. micklemi*. The Mammal Diversity Database (2022) additionally lists the extremely dubious species *F. ilariae,* which is ignored here. Figure by Kai R. Caspar.

Based on available evidence from the field, all *Fukomys* species live in family groups organized around a single reproductive pair that share a burrow system (Yeboah & Dakwa, 2002; Kawalika & Burda, 2007; Patzenhauerová et al., 2013). *Fukomys* are thus monogamous (but see below) and they are aseasonal breeders, which contrasts with polygynandry and seasonal breeding observed in their sister genus *Cryptomys*, the Southern common mole-rats from the African Cape region (Bishop, 2004). Dependent on the species, average family size in *Fukomys* may vary between 4.2 (*Fukomys zechi* - Yeboah & Dakwa, 2002) to 12 (*Fukomys damarensis* – Bennett & Jarvis, 2004) members, although up to 41 animals may comprise a group (again in *F. damarensis* – Bennett & Jarvis, 2004). Greater family sizes are occasionally reported from locals, but these numbers remain unconfirmed and may result from the unintentional pooling of neighboring groups (see Chapter 2.1).

As in all African mole-rats, the ontogeny of *Fukomys* is extremely slow-paced (e.g., Kawalika & Burda, 2007). Litters are small, typically comprising two to four neonates which eventually reach sexual maturity at an age of approximately 12 months (Lacher et al., 2016; Chapter 2.1). The offspring shows delayed dispersal from the natal group, and may stay with its parents and siblings well into adulthood (Burda et al., 2000). However, differences in average group size suggest that patterns of philopatry differ markedly between *Fukomys* species (Yeboah & Dakwa, 2002). The reasons for this intrageneric variation remain unexplored. While staying in the natal group, the offspring does not reproduce and avoids incest through odor-based recognition of siblings and parents (Burda, 1995; Heth et al., 2004).

Thus, reproductive skew within a group is high, and cases of multiple breeders of either sex being simultaneously present in a burrow system appear to be rare (e.g., Patzenhauerová et al., 2013). For Damaraland mole-rats, there is evidence that males disperse slightly earlier than females and do so more frequently (Torrents-Ticó et al., 2018). Interestingly, males are also more likely to invade established families than females after leaving their natal group (Young & Bennett, 2013; Torrents-Ticó et al., 2018; Mynhardt et al., 2021). In line with this, both long-term field studies and paternity analyses of free-living families from different species indicate that breeding males have a markedly shorter tenure than breeding females, resulting in patchwork families in which litters are sequentially sired by different fathers (Šumbera et al., 2012; Young & Bennett, 2013; Patzenhauerová et al., 2013). The social system of wild *Fukomys* may therefore best be described as a form of serial monogamy.

Capture-recapture studies indicate a very high mortality rate for wild *Fukomys* non-breeders, which is hypothesized to largely account to failed dispersal (Thorley et al., 2021). Only about 11% of Damaraland mole-rats that disappear from their natal group are eventually found to have successfully dispersed (Jarvis & Bennett, 1993). The mean life expectancy of non-breeding females in this species is just 1.3 years in the wild, compared to 6.2 years in breeding females (Schmidt et al., 2013). However, once successfully dispersed, the odds of survival starkly increase and females may indeed live alone for several years until they are eventually found by a male and form a family (Thorley et al., 2021). In captivity, different *Fukomys* species have been shown to reach an age of over 20 years (detailed in Chapter 2.1).

The naked mole-rat (*Heterocephalus glaber*) is, without question, the most unusual bathyergid, and one of the most recognizable of all extant mammals. Over the last two decades, naked mole-rats have become a laboratory model species for cancer resistance and various other medical research topics (Buffenstein, 2005; Braude et al., 2021). Although zoologists have been fascinated by these rodents since their discovery in the mid-19th century (Thomas, 1885; de Beaux, 1934; Hill et al., 1957; Starck, 1957), wide scientific and public interest in naked mole-rats first sparked in the early 1980s, after Jennifer Jarvis described the social organization of captive

naked mole-rats in the journal *Science*, famously arguing for them to be eusocial mammals (Jarvis, 1981; but see Chapter 1.1.2). Naked mole-rats occur in the sandy soils of the Horn of Africa across Ethiopia, Djibouti, Somalia and Kenia (Lacher et al., 2016; Fig. 4). Traditionally, naked mole-rats are viewed as a single species but recent genetic results suggest that patterns of cryptic diversification might warrant the designation of two parapatric species in the future (Zemlemerova et al., 2021). Morphologically, the naked mole-rat most notably derives from other bathyergids in lacking a pelage, retaining a well-developed tail, showing a distinct anatomy of the digestive tract and in exhibiting various autapomorphic skeletal traits, some of them being indicative of neoteny (Patterson & Upham, 2014; Montoya-Sanhueza, 2020). Many other characteristics that have at times been proposed to separate the naked mole-rat from other bathyergids such as pronounced cancer resistance (Patterson & Upham, 2014) have never been studied systematically in the latter, making claims about their uniqueness premature (Braude et al., 2021). With a mean adult body mass of just 35 g, it is the smallest of all subterranean rodents (Brett, 1991).

A decisive difference between naked mole-rats and all its bathyergid relatives is the size of their social groups. These typically comprise between 70 and 80 animals, but may in exceptional cases grow to comprise up to approximately 300 individuals (Brett, 1991). Despite that, the social organization and life history of naked mole-rats bears great similarities to that of Northern common mole-rats of the genus *Fukomys*: Groups occupying a shared burrow system are typically organized around a single breeding female which appears to preferably mate with just one partner (Szafranski et al., 2022; but note the lack of dedicated parentage analyses from the wild) and with all remaining individuals being non-reproductive helpers (Brett, 1991). Reproductive female naked mole-rats develop an exceptional elongation of the lumbar portion of the vertebral column (in *Fukomys*, an elongation of respective vertebrae also happens but is less extreme – Thorley et al., 2018) to accommodate the large litters of on average 12 neonates typical for this species (Jarvis, 1991). As in other African mole-rats, the juveniles develop slowly and attain sexual maturity at an age of approximately 12 months (Jarvis, 1991 – but note the great plasticity in the developmental timing of this species). When excluding reproductive females, there is no discernable sexual dimorphism found in naked mole-rats (Brett, 1991; Jarvis, 1991), which is unusual for subterranean rodents (Chapter 2.5).

Only a tiny fraction of animals in a naked mole-rat population will eventually succeed to breed (Jarvis & Bennett, 1993; Braude, 2000), but exact figures still need to be determined. In combination with large group sizes, this creates a pronounced reproductive skew that is unparalleled among mammals. The proximate reason for this pattern, similar to what is hypothesized for common mole-rats, is a high dispersal-related mortality in non-breeding individuals. On average, wild naked mole-rat non-breeders live between one and two years, while

reproductive animals have been reported to reach an age of over 16 years (Hochberg et al., 2017). The predation risk that naked mole-rats face is likely significantly larger than in other burrowing rodents. For instance, different from all other bathyergids, naked mole-rats do not plug the entrances to their tunnel systems and are frequently observed to openly discard spoil dirt (Brett, 1991). In combination with the small size of these animals, this opens ample opportunities for predators to attack. Snake predation in particular is commonly observed in the wild (Braude, 2000). In captivity, both breeding and non-breeding naked mole-rats famously show great longevity, attaining ages of up to around 30 years (Buffenstein, 2008; Dammann et al., 2019). The maximum recorded age is 37 years (Can et al., 2022), making the naked mole-rat the longest-lived rodent species (compare Weigl, 2005).

1.2.2 – Coruros (*Spalacopus cyanus*)

Coruros (*Spalacopus cyanus* – Fig. 5) are part of the rodent family Octodontidae, which is distributed across the Andean region of South America and most prominently includes the common degu (*Octodon degus*). The coruro is the only subterranean representative of its family and occurs in Central Chile, ranging from the coastal areas to high alpine regions at 3500 m altitude (Torres-Mura & Contreras, 1998; Ojeda et al., 2013). Interestingly, its sister genus *Aconaemys* is also reported to have burrowing habits but the few observations available make it hard to determine the degree of fossoriality exhibited by these animals (Gallardo & Reise, 1992; Frugone et al., 2019). Although no fossils of stem-coruros have been recovered so far (Torres-Mura & Contreras, 1998), molecular data unambiguously indicate that the *Spalacopus-Aconaemys* clade represents a geologically young lineage of burrowing rodents, presumably originating in the Pliocene (3.5 million years ago – Upham & Patterson, 2012). As such, coruros provide interesting models for the initial evolution of subterranean adaptations in rodents. While certain aspects of their morphology represent specializations for life underground, such as procumbent incisors that aid in tooth digging and a shortened tail (Torres-Mura & Contreras, 1998), others appear plesiomorphic. For instance, coruros retain acute vision (Kott et al., 2016) and are occasionally observed to actively watch out from their tunnel entrances (Begall & Gallardo, 2000). The pinnae are not as strongly reduced as in other burrowing rodents and the sensitivity of their hearing resembles that of epigeic rodents more than that of species from geologically old subterranean lineages (Begall et al., 2004; Begall & Burda, 2006; discussed in depth in Chapter 2.3). In fact, coruros appear to be ecologically flexible, with some populations relying heavily on surface foraging while in others activity is almost totally restricted to their underground burrow systems (Begall & Gallardo, 2000).

Similar to naked mole-rats and common mole-rats, coruros are a social subterranean rodent species. Yet, different from social bathyergids, they live in polygynous colonies, typically containing multiple adult females and one to several adult males sharing a burrow system (Begall

et al., 1999; Lacey et al., 2019). Paternity analyses performed on wild colonies suggest that even if several adult males are present in a group, just a single one is monopolizing reproduction (Begall, 1999). Despite that, coruros are only weakly sexually dimorphic (mean male mass: 93 g, mean female mass: 90 g – Begall, 1999, data from free-living animals), suggesting that physical intrasexual competition between males is not of great significance in this species.

Figure 5: A breeding pair of coruros (*Spalacopus cyanus*). Photo: Kai Caspar.

Unfortunately, little is known about the social dynamics and temporal structure of wild coruro groups. The reproductive biology of wild and captive coruros has been described by Begall et al. (1999): Mating in the wild appears to take place from June to January but is aseasonal in captivity. Gestation lasts about 77 days and neonates, as is typical for caviomorphs, are precocious. Eyes and fur are fully developed at birth and locomotor independence is acquired within days. Coruros reach adulthood at an age of approximately 190 days. Little data is available on longevity in this species. Captive coruros may reach an age of 13 years (S. Begall, pers. com.).

References

Ashmore, J. (2008). Cochlear outer hair cell motility. *Physiological Reviews, 88*(1), 173–210.

Begall, S. (1999). Verhaltensökologische und genetische Analysen der Sozial- und Populationsstruktur von Coruros (*Spalacopus cyanus*, Octodontidae, Rodentia) aus Chile. Ph.D. dissertation, Universität-GH Essen, Essen, Germany.

Begall, S., & Burda, H. (2006). Acoustic communication and burrow acoustics are reflected in the ear morphology of the coruro (*Spalacopus cyanus*, Octodontidae), a social fossorial rodent. *Journal of Morphology*, *267*(3), 382–390.

Begall, S., & Gallardo, M. H. (2000). *Spalacopus cyanus* (Rodentia: Octodontidae): an extremist in tunnel constructing and food storing among subterranean mammals. *Journal of Zoology, 251*(1), 53–60.

Begall, S., Burda, H., & Gallardo, M. H. (1999). Reproduction, postnatal development, and growth of social coruros, *Spalacopus cyanus* (Rodentia: Octodontidae), from Chile. *Journal of Mammalogy*, *80*(1), 210–217.

Begall, S., Burda, H., & Malkemper, E. P. (2014). Magnetoreception in mammals. In M. Naguib (Ed.), *Advances in the Study of Behavior, Vol. 46* (pp. 45-88). Elsevier.

Begall, S., Burda, H., & Schleich, C. E. (2007). Subterranean rodents: news from under-ground. In S. Begall, H. Burda, & C. E. Schleich (Eds.), *Subterranean Rodents: News from Underground* (pp. 3–9). Springer.

Begall, S., Burda, H., & Schneider, B. (2004). Hearing in coruros (*Spalacopus cyanus*): special audiogram features of a subterranean rodent. *Journal of Comparative Physiology A*, *190*(11), 963–969.

Begall, S., Malkemper, E. P., Červený, J., Němec, P., & Burda, H. (2013). Magnetic alignment in mammals and other animals. *Mammalian Biology*, *78*(1), 10–20.

Bennett, N. C., & Jarvis, J. U. (2004). *Cryptomys damarensis*. *Mammalian Species, 2004*(756), 1–5.

Bishop, J. M., Jarvis, J. U. M., Spinks, A. C., Bennett, N. C., & O'Ryan, C. (2004). Molecular insight into patterns of colony composition and paternity in the common mole-rat *Cryptomys hottentotus hottentotus*. *Molecular Ecology, 13*(5), 1217-1229.

Braude, S. (1997). The predictive power of evolutionary biology and the discovery of eusociality in the naked mole-rat. *Reports of the National Center for Science Education*, *17*(4), 12–15.

Braude, S. (2000). Dispersal and new colony formation in wild naked mole-rats: evidence against inbreeding as the system of mating. *Behavioral Ecology*, *11*(1), 7–12.

Braude, S., Holtze, S., Begall, S., Brenmoehl, J., Burda, H., Dammann, P., ... & Hildebrandt, T. B. (2021). Surprisingly long survival of premature conclusions about naked mole-rat biology. *Biological Reviews*, *96*(2), 376–393.

Bredberg, A., & Schmitz, B. (2019). Human cancer, the naked mole rat and faunal turnovers. *Cancer Medicine*, *8*(4), 1652–1654.

Brett, R. A. (1991). The population structure of naked mole-rat colonies. In P. W. Sherman, J. U. M. Jarvis, & R. D. Alexander (Eds.), *The Biology of the Naked Mole-Rat* (pp. 97–136). Princeton University Press.

Bryja, J., Konvičková, H., Bryjová, A., Mikula, O., Makundi, R., Chitaukali, W.N., & Šumbera, R. (2018). Differentiation underground: range-wide multilocus genetic structure of the silvery mole-rat does not support current taxonomy based on mitochondrial sequences. *Mammalian Biology, 93*(1), 82–92.

Buffenstein, R. (2005). The naked mole-rat: a new long-living model for human aging research. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*(11), 1369–1377.

Buffenstein, R. (2008). Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. *Journal of Comparative Physiology B*, *178*(4), 439–445.

Burda, H. (1990). Constraints of pregnancy and evolution of sociality in mole-rats with special reference to reproductive and social patterns in *Cryptomys hottentotus* (Bathyergidae, Rodentia). *Journal of Zoological Systematics and Evolutionary Research, 28*(1), 26–39.

Burda, H. (1995). Individual recognition and incest avoidance in eusocial common mole-rats rather than reproductive suppression by parents. *Experientia, 51*(4), 411–413.

Burda, H. (2001). Determinants of the distribution and radiation of African mole-rats (Bathyergidae, Rodentia). In C. Denys, L. Granjon, & A. Poulet (Eds.), *African Small Mammals* (pp. 261–277). IRD Editions.

Burda, H. (2021). Sensory perception of mole-rats and mole rats: assessment of a complex natural global evolutionary "experiment". In S. P. Wasser & M. Frenkel-Morgenstern (Eds.), *New Horizons in Evolution* (pp. 161–191). Academic Press.

Burda, H., Bruns, V., & Müller, M. (1990). Sensory adaptations in subterranean mammals. *Progress in Clinical and Biological Research*, *335*, 269–293.

Burda, H., Honeycutt, R. L., Begall, S., Locker-Grütjen, O., & Scharff, A. (2000). Are naked and common mole-rats eusocial and if so, why? *Behavioral Ecology and Sociobiology*, *47*(5), 293–303.

Burda, H., Marhold, S., Westenberger, T., Wiltschko, R., & Wiltschko, W. (1990). Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). *Experientia, 46*(5), 528–530.

Can, E., Smith, M., Boukens, B. J., Coronel, R., Buffenstein, R., & Riegler, J. (2022). Naked mole-rats maintain cardiac function and body composition well into their fourth decade of life. *GeroScience*, https://doi.org/10.1007/s11357-022-00522-6.

Crespi, B. J., & Yanega, D. (1995). The definition of eusociality. *Behavioral Ecology, 6*(1), 109–115.

Dammann, P., Scherag, A., Zak, N., Szafranski, K., Holtze, S., Begall, S., ... & Platzer, M. (2019). Comment on 'Naked mole-rat mortality rates defy Gompertzian laws by not increasing with age'. *Elife*, *8*, e45415.

Davies, K. T., Bennett, N. C., Faulkes, C. G., & Rossiter, S. J. (2018). Limited evidence for parallel molecular adaptations associated with the subterranean niche in mammals: a comparative study of three superorders. *Molecular Biology and Evolution, 35*(10), 2544–2559.

de Beaux, O. (1934). Mammiferi raccolti dal prof. G. Scortecci nella Somalia italiana centrale e settentrionale nel 1931 con l'aggiunta di alcuni mammiferi della Somalia italiana meridionale. *Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale in Milano, 73*(3), 261–300.

Dennis, J. C., Stilwell, N. K., Smith, T. D., Park, T. J., Bhatnagar, K. P., & Morrison, E. E. (2020). Is the mole rat vomeronasal organ functional? *The Anatomical Record*, *303*(2), 318–329.

Dollas, A., Oelschläger, H. H., Begall, S., Burda, H., & Malkemper, E. P. (2019). Brain atlas of the African mole-rat *Fukomys anselli*. *Journal of Comparative Neurology*, *527*(11), 1885–1900.

Emerling, C. A. (2018). Regressed but not gone: patterns of vision gene loss and retention in subterranean mammals. *Integrative and Comparative Biology, 58*(3), 441–451.

Engels, S., Treiber, C. D., Salzer, M. C., Michalik, A., Ushakova, L., Keays, D. A., ... & Heyers, D. (2018). Lidocaine is a nocebo treatment for trigeminally mediated magnetic orientation in birds. *Journal of The Royal Society Interface*, *15*(145), 20180124.

Fang, X., Seim, I., Huang, Z., Gerashchenko, M. V., Xiong, Z., Turanov, A. A., ... & Yao, X. (2014). Adaptations to a subterranean environment and longevity revealed by the analysis of mole rat genomes. *Cell Reports, 8*(5), 1354–1364.

Faulkes, C. G., Verheyen, E., Verheyen, W., Jarvis, J. U. M., & Bennett, N. C. (2004). Phylogeographical patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae). *Molecular Ecology, 13*(3), 613–629.

Fransson, T., Jakobsson, S., Johansson, P., Kullberg, C., Lind, J., & Vallin, A. (2001). Magnetic cues trigger extensive refuelling. *Nature*, *414*(6859), 35–36.

Fronhofer, E. A., Liebig, J., Mitesser, O., & Poethke, H. J. (2018). Eusociality outcompetes egalitarian and solitary strategies when resources are limited and reproduction is costly. *Ecology and Evolution*, *8*(24), 12953–12964.

Frugone, M. J., Correa, L. A., & Sobrero, R. (2019). Activity and group-living in the Porter's rock rats, *Aconaemys porteri*. *Mastozoología Neotropical*, *26*(2), 487–492.

Gallardo, M. H., & Reise, D. (1992). Systematics of *Aconaemys* (Rodentia, Octodontidae). *Journal of Mammalogy, 73*(4), 779–788.

Gans, C. (1978). The characteristics and affinities of the Amphisbaenia. *Transactions of the Zoological Society of London, 34*(4), 347–416.

Gerhardt, P., Henning, Y., Begall, S., & Malkemper, E. P. (2017). Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency cut-offs. *Journal of Experimental Biology*, *220*(23), 4377–4382.

Gilbert, J. D., Rossiter, S. J., & Faulkes, C. G. (2020). The relationship between individual phenotype and the division of labour in naked mole-rats: it's complicated. *PeerJ*, *8*, e9891.

Gomes Rodrigues, H., Šumbera, R., & Hautier, L. (2016). Life in burrows channelled the morphological evolution of the skull in rodents: the case of African mole-rats (Bathyergidae, Rodentia). *Journal of Mammalian Evolution*, *23*(2), 175–189.

Hagemeyer, P., Begall, S., Janotova, K., Todrank, J., Heth, G., Jedelsky, P. L., ... & Stopka, P. (2011). Searching for major urinary proteins (MUPs) as chemosignals in urine of subterranean rodents. *Journal of Chemical Ecology*, *37*(7), 687–694.

Hagemeyer, P., Peuckmann, S., Papenfuhs, N., & Burda, H. (2004). Chemical communication in blind, subterranean eusocial mole-rats (genus *Coetomys*): which odour source codes information for individual recognition? *Advances in Ethology Supplement, 38*, 56.

Heffner, R. S., & Heffner, H. E. (1993). Degenerate hearing and sound localization in naked mole rats (*Heterocephalus glaber*), with an overview of central auditory structures. *Journal of Comparative Neurology*, *331*(3), 418–433.

Hesselmann, F. (2010). Vergleich der mikroskopischen Anatomie der Haut und Hautanhangsorgane des Hausmeerschweinchens (*Cavia aperea* f. *porcellus*) und des Ansells Kleingraumulls (*Fukomys anselli*) unter Berücksichtigung ihrer Anpassung an unterschiedliche Lebensräume. Ph.D. dissertation, Tierärztliche Hochschule Hannover, Hannover, Germany.

Heth, G., Frankenberg, E., Raz, A., & Nevo, E. (1987). Vibrational communication in subterranean mole rats (*Spalax ehrenbergi*). *Behavioral Ecology and Sociobiology*, *21*(1), 31–33.

Heth, G., Todrank, J., Begall, S., Koch, R., Zilbiger, Y., Nevo, E., ... & Burda, H. (2002). Odours underground: subterranean rodents may not forage "blindly". *Behavioral Ecology and Sociobiology*, *52*(1), 53–58.

Heth, G., Todrank, J., Begall, S., Wegner, R. E., & Burda, H. (2004). Genetic relatedness discrimination in eusocial *Cryptomys anselli* mole-rats, Bathyergidae, Rodentia. *Folia Zoologica*, *53*(3), 269–278.

Hill, W. C. O, Porter, A., Bloom, R. T., Seago, J., & Southwick, M. D. (1957). Field and laboratory studies on the naked mole rat, *Heterocephalus glaber*. *Proceedings of the Zoological Society of London*, *128*(4), 455–514.

Hochberg, H., Noble, R. J., & Braude, S. (2016). A hypothesis to explain cancers in confined colonies of naked mole-rats. bioRxiv 079012; doi: https://doi.org/10.1101/079012

Hore, P. J., & Mouritsen, H. (2016). The radical-pair mechanism of magnetoreception. *Annual Review of Biophysics, 45*, 299–344.

Houslay, T. M., Vullioud, P., Zöttl, M., & Clutton-Brock, T. H. (2020). Benefits of cooperation in captive Damaraland mole-rats. *Behavioral Ecology, 31*(3), 711–718.

Ingram, C. M., Burda, H., & Honeycutt, R.L. (2004). Molecular phylogenetics and taxonomy of the African mole-rats, genus *Cryptomys* and the new genus *Coetomys* Gray, 1864. *Molecular Phylogenetics and Evolution, 31*(3), 997–1014.

Ivy, C. M., Sprenger, R. J., Bennett, N. C., Van Jaarsveld, B., Hart, D. W., Kirby, A. M., ... & Pamenter, M. E. (2020). The hypoxia tolerance of eight related African mole-rat species rivals that of naked mole-rats, despite divergent ventilatory and metabolic strategies in severe hypoxia. *Acta Physiologica, 228*(4), e13436.

Jarvis, J. U. M. (1981). Eusociality in a mammal: cooperative breeding in naked mole-rat colonies. *Science*, *212*(4494), 571–573.

Jarvis, J. U. M. (1991). Reproduction of naked mole-rats. In P. W. Sherman, J. U. M. Jarvis, & R. D. Alexander (Eds.), *The Biology of the Naked Mole-rat* (pp. 384–425). Princeton University Press.

Jarvis, J. U. M., & Bennett, N. C. (1991). Ecology and behavior of the family Bathyergidae. In P. W. Sherman, J. U. M. Jarvis, & R. D. Alexander (Eds.), *The Biology of the Naked Mole-rat* (pp. 66–96). Princeton University Press.

Jarvis, J. U. M., & Bennett, N. C. (1993). Eusociality has evolved independently in two genera of bathyergid mole-rats—but occurs in no other subterranean mammal. *Behavioral Ecology and Sociobiology*, *33*(4), 253–260.

Jastrow, H., Burda, H., & Oelschläger, H. H. (1998). Unilateral absence of the terminal nerve and distribution of gonadotropin-releasing hormone immunoreactive neurons in the brain of the common mole-rat (*Cryptomys*, Rodentia). *Brain Research, 813*(2), 229–240.

Jiao, H., Hong, W., Nevo, E., Li, K., & Zhao, H. (2019). Convergent reduction of V1R genes in subterranean rodents. *BMC Evolutionary Biology, 19*(1), 1–9.

Kavet, R., & Brain, J. (2021). Cryptochromes in mammals and birds: clock or magnetic compass? *Physiology, 36*(3), 183–194.

Kawalika, M., & Burda, H. (2007). Giant mole-rats, *Fukomys mechowii*, 13 years on the stage. In S. Begall, H. Burda, & C. E. Schleich (Eds.), *Subterranean Rodents: News from Underground* (pp. 205–219). Springer.

Kimani, J. M. (2013). Comparative skin morphology and wound healing in Kenyan African mole rat (*Tachyoryctes ibeanus*) and naked mole rat (*Heterocephalus glaber*). Master thesis. Department of Veterinary Anatomy and Physiology, University of Nairobi, Kenia.

Kimchi, T., & Terkel, J. (2001). Magnetic compass orientation in the blind mole rat *Spalax ehrenbergi*. *Journal of Experimental Biology*, *204*(4), 751–758.

Kishkinev, D. A., & Chernetsov, N. S. (2015). Magnetoreception systems in birds: a review of current research. *Biology Bulletin Reviews, 5*(1), 46–62.

Klauer, G., Burda, H., & Nevo, E. (1997). Adaptive differentiations of the skin of the head in a subterranean rodent, *Spalax ehrenbergi*. *Journal of Morphology, 233*(1), 53–66.

Kleiman, D. G. (1977). Monogamy in mammals. *The Quarterly Review of Biology*, *52*(1), 39–69.

Kock, D., Ingram, C. M., Frabotta, L. J., Honeycutt, R. L., & Burda, H. (2006). On the nomenclature of Bathyergidae and *Fukomys* n. gen. (Mammalia: Rodentia). *Zootaxa, 1142*(1), 51–55.

Kössl, M., Frank, G., Burda, H., & Müller, M. (1996). Acoustic distortion products from the cochlea of the blind African mole rat, *Cryptomys* spec. *Journal of Comparative Physiology A, 178*(3), 427–434.

Kott, O., Moritz, R. E., Šumbera, R., Burda, H., & Němec, P. (2014). Light propagation in burrows of subterranean rodents: tunnel system architecture but not photoreceptor sensitivity limits light sensation range. *Journal of Zoology, 294*(1), 67–75.

Kott, O., Němec, P., Fremlová, A., Mazoch, V., & Šumbera, R. (2016). Behavioural tests reveal severe visual deficits in the strictly subterranean African mole-rats (Bathyergidae) but efficient vision in the fossorial rodent coruro (*Spalacopus cyanus*, Octodontidae). *Ethology*, *122*(8), 682–694.

Lacey, E. A., O'Brien, S. L., Sobrero, R., & Ebensperger, L. A. (2019). Spatial relationships among free-living cururos (*Spalacopus cyanus*) demonstrate burrow sharing and communal nesting. *Journal of Mammalogy*, *100*(6), 1918–1927.

Lacher, T. E., Mittermeier, R. A., & Wilson, D. E. (Eds.). (2016). *Handbook of the Mammals of the World: Lagomorphs and Rodents I*. Lynx Edicions.

Landry, S. O. (1957). Factors affecting the procumbency of rodent upper incisors. *Journal of Mammalogy, 38*(2), 223–234.

Lange, S., Burda, H., Wegner, R. E., Dammann, P., Begall, S., & Kawalika, M. (2007). Living in a "stethoscope": burrow-acoustics promote auditory specializations in subterranean rodents. *Naturwissenschaften, 94*(2), 134–138.

Lange, S., Neumann, B., Hagemeyer, P., & Burda, H. (2005). The smell of carrots: kairomone-guided food location in subterranean Zambian mole-rats (*Cryptomys* spp., Bathyergidae). *Folia Zoologica, 54*(3), 263–268.

Luo, Z. X., Meng, Q. J., Ji, Q., Liu, D., Zhang, Y. G., & Neander, A. I. (2015). Evolutionary development in basal mammaliaforms as revealed by a docodontan. *Science*, *347*(6223), 760–764.

Malewski, S. (2018). Magnetoreception in rodents. Ph.D. dissertation, Universität Duisburg-Essen, Essen, Germany.

Malewski, S., Begall, S., Schleich, C. E., Antenucci, C. D., & Burda, H. (2018). Do subterranean mammals use the Earth's magnetic field as a heading indicator to dig straight tunnels? *PeerJ, 6*, e5819.

Malik, A., Korol, A., Weber, M., Hankeln, T., Avivi, A., & Band, M. (2012). Transcriptome analysis of the *Spalax* hypoxia survival response includes suppression of apoptosis and tight control of angiogenesis. *BMC Genomics, 13*(1), 615.

Malkemper, E. P., Eder, S. H., Begall, S., Phillips, J. B., Winklhofer, M., Hart, V., & Burda, H. (2015). Magnetoreception in the wood mouse (*Apodemus sylvaticus*): influence of weak frequency-modulated radio frequency fields. *Scientific Reports, 5*, 9917.

Mammal Diversity Database. (2022). Mammal Diversity Database (Version 1.9). http://doi.org/10.5281/zenodo.4139818

Marhold, S., Wiltschko, W., & Burda, H. (1997). A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften 84*(9), 421–423.

Mason, M. J., Cornwall, H. L., & Smith, E. S. J. (2016). Ear structures of the naked mole-rat, *Heterocephalus glaber*, and its relatives (Rodentia: Bathyergidae). *PLoS One*, *11*(12), e0167079.

Montoya-Sanhueza, G. A. (2020). Functional anatomy, osteogenesis and bone microstructure of the appendicular system of African mole-rats (Rodentia: Ctenohystrica: Bathyergidae). Ph.D. dissertation, University of Cape Town, Cape Town, South Africa.

Montoya-Sanhueza, G. A., Wilson, L. A., & Chinsamy, A. (2019). Postnatal development of the largest subterranean mammal (*Bathyergus suillus*): Morphology, osteogenesis, and modularity of the appendicular skeleton. *Developmental Dynamics*, *248*(11), 1101–1128.

Mooney, S. J., Filice, D. C., Douglas, N. R., & Holmes, M. M. (2015). Task specialization and task switching in eusocial mammals. *Animal Behaviour*, *109*, 227–233.

Moshkin, M., Novikov, E., & Petrovski, D. (2007). Skimping as an adaptive strategy in social fossorial rodents: the mole vole (*Ellobius talpinus*) as an example. In S. Begall, H. Burda, & C. E. Schleich (Eds.), *Subterranean Rodents: News from Underground* (pp. 49-60). Springer.

Mynhardt, S., Harris-Barnes, L., Bloomer, P., & Bennett, N. C. (2021). Spatial population genetic structure and colony dynamics in Damaraland mole-rats (*Fukomys damarensis*) from the southern Kalahari. *BMC Ecology and Evolution, 21*(1), 1–17.

Naked mole-rat. (2022, April 13). In Wikipedia. https://en.wikipedia.org/wiki/Naked_mole-rat

Narins, P. M., Reichman, O. J., Jarvis, J. U., & Lewis, E. R. (1992). Seismic signal transmission between burrows of the Cape mole-rat, *Georychus capensis*. *Journal of Comparative Physiology A*, *170*(1), 13–21.

Němec, P., Altmann, J., Marhold, S., Burda, H., & Oelschlager, H. H. (2001). Neuroanatomy of magnetoreception: the superior colliculus involved in magnetic orientation in a mammal. *Science*, *294*(5541), 366–368.

Nevo, E. (1979). Adaptive convergence and divergence of subterranean mammals. *Annual Review of Ecology and Systematics, 10*(1), 269–308.

Nevo, E., Bodmer, M., & Heth, G. (1976). Olfactory discrimination as an isolating mechanism in speciating mole rats. *Experientia*, *32*(12), 1511–1512.

Nießner, C., Denzau, S., Malkemper, E. P., Gross, J. C., Burda, H., Winklhofer, M., & Peichl, L. (2016). Cryptochrome 1 in retinal cone photoreceptors suggests a novel functional role in mammals. *Scientific Reports, 6*, 21848.

Nowak, M. A., Tarnita, C. E., & Wilson, E. O. (2010). The evolution of eusociality. *Nature*, *466*(7310), 1057–1062.

Ojeda, A. A., Novillo, A., Ojeda, R. A., & Roig-Juñent, S. (2013). Geographical distribution and ecological diversification of South American octodontid rodents. *Journal of Zoology*, *289*(4), 285–293.

Park, T. J., Catania, K. C., Samaan, D., & Comer, C. M. (2007). Adaptive neural organization of naked mole-rat somatosensation (and those similarly challenged). In S. Begall, H. Burda, & C. E. Schleich (Eds.), *Subterranean Rodents: News from Underground* (pp. 175–193). Springer.

Patterson, B. D., & Upham, N. S. (2014). A newly recognized family from the Horn of Africa, the Heterocephalidae (Rodentia: Ctenohystrica). *Zoological Journal of the Linnean Society*, *172*(4), 942–963.

Patzenhauerová, H., Šklíba, J., Bryja, J., & Šumbera, R. (2013). Parentage analysis of Ansell's mole-rat family groups indicates a high reproductive skew despite relatively relaxed ecological constraints on dispersal. *Molecular Ecology*, *22*(19), 4988–5000.

Pyott, S. J., van Tuinen, M., Screven, L. A., Schrode, K. M., Bai, J. P., Barone, C. M., ... & Park, T. J. (2020). Functional, morphological, and evolutionary characterization of hearing in subterranean, eusocial African mole-rats. *Current Biology, 30*(22), 4329–4341.

Raphael, Y., & Altschuler, R. A. (2003). Structure and innervation of the cochlea. *Brain Research Bulletin, 60*(5-6), 397–422.

Ritz, T., Adem, S., & Schulten, K. (2000). A model for photoreceptor-based magnetoreception in birds. *Biophysical Journal, 78*(2), 707–718.

Schmidt, C. M., Jarvis, J. U., & Bennett, N. C. (2013). The long-lived queen: reproduction and longevity in female eusocial Damaraland mole-rats (*Fukomys damarensis*). *African Zoology, 48*(1), 193–196.

Seluanov, A., Gladyshev, V. N., Vijg, J., & Gorbunova, V. (2018). Mechanisms of cancer resistance in long-lived mammals. *Nature Reviews Cancer, 18*(7), 433–441.

Siegmann, S., Feitsch, R., Hart, D. W., Bennett, N. C., Penn, D. J., & Zöttl, M. (2021). Naked mole-rats (*Heterocephalus glaber*) do not specialise in cooperative tasks. *Ethology*, *127*(10), 850–864.

Smorkatcheva, A. V. (2021). Weakened inbreeding avoidance in a monogamous subterranean vole, *Ellobius tancrei*. *Mammalian Biology*, *101*(5), 601–607.

Smorkatcheva, A. V., & Kumaitova, A. R. (2014). Delayed dispersal in the Zaisan mole vole (*Ellobius tancrei*): helping or extended parental investment? *Journal of Ethology, 32*(1), 53–61

Smorkatcheva, A. V., & Lukhtanov, V. A. (2014). Evolutionary association between subterranean lifestyle and female sociality in rodents. *Mammalian Biology*, *79*(2), 101–109.

Sokolov, V. E. (1982). *Mammal Skin*. University of California Press.

Starck, D. (1957). Beobachtungen an *Heterocephalus glaber* Rüppel, 1842 (Rodentia, Bathyergidae) in der Provinz Harar. *Zeitschrift für Säugetierkunde, 22*, 50–56.

Stathopoulos, S., Bishop, J. M., & O'Ryan, C. (2014). Genetic signatures for enhanced olfaction in the African mole-rats*. PLoS One, 9*(4), e93336.

Stein, B. R., Lacey, E. A., Patton, J. L., & Cameron, G. N. (2000). Morphology of subterranean rodents. In E. A. Lacey, J. L. Patton, & G. N. Cameron (Eds.), *Life Underground: The Biology of Subterranean Rodents* (pp. 19–61). University of Chicago Press.

Šumbera, R., Mazoch, V., Patzenhauerová, H., Lövy, M., Šklíba, J., Bryja, J., & Burda, H. (2012). Burrow architecture, family composition and habitat characteristics of the largest social African mole-rat: the giant mole-rat constructs really giant burrow systems. *Acta Theriologica, 57*(2), 121-130.

Szafranski, K., Wetzel, M., Holtze, S., Büntjen, I., Lieckfeldt, D., Ludwig, A., Huse, K., Platzer, M., & Hildebrandt, T. (2022). The mating pattern of captive naked mole-rats is best described by a monogamy model. *Frontiers in Ecology and Evolution, 10*, 855688.

Thalau, P., Ritz, T., Burda, H., Wegner, R. E., & Wiltschko, R. (2006). The magnetic compass mechanisms of birds and rodents are based on different physical principles. *Journal of the Royal Society Interface*, *3*(9), 583–587.

Thenius, E. (1979). *Die Evolution der Säugetiere: Eine Übersicht über Ergebnisse und Probleme*. Fischer.

Thomas, O. (1885). Notes on the rodent genus *Heterocephalus*. *Proceedings of the Zoological Society of London, 53*(4), 845–849.

Thorley, J., Bensch, H., Finn, K., Clutton-Brock, T., & Zöttl, M. (2021). Damaraland mole-rats are not obligate cooperative breeders. *bioRxiv,* 2021.12.08.471794; doi: https://doi.org/10.1101/2021.12.08.471794

Thorley, J., Katlein, N., Goddard, K., Zöttl, M., & Clutton-Brock, T. (2018). Reproduction triggers adaptive increases in body size in female mole-rats. *Proceedings of the Royal Society B*, *285*(1880), 20180897.

Torrents-Ticó, M., Bennett, N. C., Jarvis, J. U., & Zöttl, M. (2018). Sex differences in timing and context of dispersal in Damaraland mole-rats (*Fukomys damarensis*). *Journal of Zoology, 306*(4), 252–257.

Torres-Mura, J. C., & Contreras, L. C. (1998). *Spalacopus cyanus*. *Mammalian Species, 1998*(594), 1–5.

Tullberg, T. (1899). Ueber das System der Nagethiere: Eine phylogenetische Studie. Akademische Buchdruckerei, Upsala.

Upham, N. S., & Patterson, B. D. (2012). Diversification and biogeography of the Neotropical caviomorph lineage Octodontoidea (Rodentia: Hystricognathi). *Molecular Phylogenetics and Evolution, 63*(2), 417–429.

Van Daele, P. A. A. G., Faulkes, C. G., Verheyen, E., & Adriaens, D. (2007a). African mole-rats (Bathyergidae): a complex radiation in tropical soils. In S. Begall, H. Burda, & C. E. Schleich (Eds.), *Subterranean Rodents: News from Underground* (pp. 357–373). Springer.

Van Daele, P. A. A. G., Verheyen, E., Brunain, M., & Adriaens, D. (2007b). Cytochrome b sequence analysis reveals differential molecular evolution in African mole-rats of the chromosomally hyperdiverse genus *Fukomys* (Bathyergidae, Rodentia) from the Zambezian region. *Molecular Phylogenetics and Evolution*, *45*(1), 142–157.

Vejmělka, F., Okrouhlík, J., Lövy, M., Šaffa, G., Nevo, E., Bennett, N. C., & Šumbera, R. (2021). Heat dissipation in subterranean rodents: the role of body region and social organisation. *Scientific Reports, 11*(1), 1–17.

Visser J.H., Bennett, N.C., & Jansen van Vuuren, B. (2019). Phylogeny and biogeography of the African Bathyergidae: a review of patterns and processes. *PeerJ*, *7*, 7730.

Wang, Y., Pan, Y., Parsons, S., Walker, M., & Zhang, S. (2007). Bats respond to polarity of a magnetic field. *Proceedings of the Royal Society B: Biological Sciences, 274*(1627), 2901–2905.

Wegner, R. E., Begall, S., & Burda, H. (2006). Magnetic compass in the cornea: local anaesthesia impairs orientation in a mammal. *Journal of Experimental Biology*, *209*(23), 4747–4750.

Weigl, R. (2005). *Longevity of Mammals in Captivity; from the Living Collections of the World*. Kleine Senckenberg-Reihe.

Wilson, E. O. (1971). *The Insect Societies*. Harvard University Press.

Winkler, A. J., Denys, C., & Avery, D. M. (2010). Rodentia. In L. Werdelin & W. J. Sanders (Eds.), *Cenozoic Mammals of Africa* (pp. 263–304). University of California Press.

Yeboah, S., & Dakwa, K. B. (2002). Colony and social structure of the Ghana mole-rat (*Cryptomys zechi*, Matchie) (Rodentia: Bathyergidae). *Journal of Zoology, 256*(1), 85–91.

Young, A. J., & Bennett, N. C. (2013). Intra-sexual selection in cooperative mammals and birds: why are females not bigger and better armed? *Philosophical Transactions of the Royal Society B: Biological Sciences, 368*(1631), 20130075.

Young, A. J., Jarvis, J. U., Barnaville, J., & Bennett, N. C. (2015). Workforce effects and the evolution of complex sociality in wild Damaraland mole rats. *The American Naturalist*, *186*(2), 302–311.

Zemlemerova, E. D., Kostin, D. S., Lebedev, V. S., Martynov, A. A., Gromov, A. R., Alexandrov, D. Y., & Lavrenchenko, L. A. (2021). Genetic diversity of the naked mole-rat (*Heterocephalus glaber*). *Journal of Zoological Systematics and Evolutionary Research*, *59*(1), 323–340.

Zöttl, M., Vullioud, P., Mendonça, R., Ticó, M. T., Gaynor, D., Mitchell, A., & Clutton-Brock, T. (2016). Differences in cooperative behavior among Damaraland mole rats are consequences of an age-related polyethism. *Proceedings of the National Academy of Sciences*, *113*(37), 10382–10387.

Chapter 2 – Manuscripts

2.1 – Species Accounts – *Fukomys mechowii* & *Fukomys anselli*

Fukomys mechowii (Rodentia: Bathyergidae)

Caspar, K. R., Burda, H., & Begall, S.

Mammalian Species, 2021, 53(1011), 145-159, doi.org/10.1093/mspecies/seab014

URL: https://academic.oup.com/mspecies/article/53/1011/145/6447566

Fukomys anselli (Rodentia: Bathyergidae)

Begall, S., Burda, H., & **Caspar, K. R.**

Mammalian Species, 2021, 53(1012), 160-173, doi.org/10.1093/mspecies/seab015

URL: https://academic.oup.com/mspecies/article/53/1012/160/6447567

Contributions (applies to both Mammalian Species manuscripts):

- **Conception** – 100 %: I proposed the idea to write the two manuscripts to SB and HB.

- **Literature research and writing** – 60 %: I researched the following topics to include into the reviews and wrote the respective sections: Synonymy and etymology, diagnosis and general characteristics, distribution, fossil record, form and function (with input from SB), genetics, conservation, and the ecology subsection on diet. I reviewed and edited the remaining sections drafted by SB and HB and prepared the figures.

- **Revising the manuscript** – 60 %: I revised the manuscripts following the reviewer's comments together with significant input from SB and HB.

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Signature of Ph.D. student Signature of supervisor

MAMMALIAN SPECIES 53(1011):145–159

Fukomys mechowii (Rodentia: Bathyergidae)

KAI R. CASPAR, HYNEK BURDA, AND SABINE BEGALL

www.mammalogy.org

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Abstract: *Fukomys mechowii* (Peters, 1881), the giant mole-rat or Mechow's mole-rat, is a cooperatively breeding, tooth-digging, subterranean rodent. It is the largest representative of the genus *Fukomys*, which is part of the African mole-rat family Bathyergidae. It is found in mesic shrubland, savannah habitats, and agricultural lands in central Africa, its range extending through Angola, the Democratic Republic of Congo, and Zambia. *Fukomys mechowii* is hunted for its meat in rural areas but its population is considered to be stable. It is rarely housed in captivity and is listed as "Least Concern" (LC) on the International Union for Conservation of Nature Red List of Threatened Species.

Key words: eusociality, mole-rat, subterranean rodent, tooth-digger

Synonymies completed 1 July 2021
DOI: 10.1093/mspecies/seab014
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Nomenclatural statement.—A life science identifier (LSID) number was obtained for this publication: urn:lsid:zoobank.org:pub:CC743E24-1EB4-4753-93AB-6F68932B22B5

Fukomys Kock, Ingram, Frabotta, Honeycutt, and Burda, 2006

Bathyergus: Ogilby, 1838:5. Part not *Bathyergus* Illiger, 1811:86.
Georychus: Gray, 1843:149. Part not *Georychus* Illiger, 1811:87.
Georhychus: Wagner, 1843:47. In Part. Incorrect subsequent spelling of *Georychus* Illiger, 1811:87.
Cryptomys Gray, 1864:124. Type species *Georychus holosericeus* Wagner, 1843 (= *Cryptomys hottentotus* (Lesson, 1826)). Designated as a subgenus of *Georychus*, elevated to generic rank by Thomas 1917:442 to encompass all species grouped as either *Cryptomys* or *Coetomys* by Gray 1864.
Coetomys Gray, 1864:125. Type species *Bathyergus caecutiens* Brants, 1827 (= *Cryptomys hottentotus* (Lesson, 1826)) by subsequent designation (Ellerman, Morrison-Scott, and Hayman 1953). Designated as a subgenus of *Georychus*, elevated erroneously to generic rank by Ingram, Burda, and Honeycutt 2004:1008 to encompass species currently classified as *Fukomys* (Kock et al. 2006).
Typhloryctes Fitzinger, 1867:502. In Part. Type species *Bathyergus caecutiens* Brants, 1827 (= *Cryptomys hottentotus* (Lesson, 1826)) by subsequent designation (Ellerman, Morrison-Scott, and Hayman 1953).
Typhlorychus Trouessart, 1881:160. Incorrect subsequent spelling of *Typhloryctes* Fitzinger, 1867.

Fig. 1.—Group of *Fukomys mechowii* photographed in captivity at the Department of General Zoology, University of Duisburg-Essen, Essen, Germany. The animals display the typical huddling behavior that aids in conserving body heat. The animal in the upper center is an adult male, the one below an adult female. Note the cylindrical body shape, microphthalmy, and procumbent incisors. The male is paler than the female. At least in captive *F. mechowii*, this dimorphic color pattern has been repeatedly observed. Photograph used with permission of the photographer, Ulrich Hellinger.

Caetomys Thomas, 1917:442. Incorrect subsequent spelling of *Coetomys* Gray, 1864.

Cryptomis Hoesch, and von Lehmann, 1956:34. Incorrect subsequent spelling of *Cryptomys* Gray, 1864.

Fukomys Kock, Ingram, Frabotta, Honeycutt, and Burda, 2006. Type species *Bathyergus damarensis* Ogilby, 1838 (= *Fukomys damarensis* (Ogilby, 1838)).

CONTEXT AND CONTENT. Order Rodentia, suborder Hystricomorpha, infraorder Hystricognathi, family Bathyergidae, subfamily Bathyerginae, genus *Fukomys*. *Fukomys* is polytypic but the number of species is debatable. The ASM Mammal Diversity Database (2020) and Burgin et al. (2020) recognize 16 extant species, *F. amatus*, *F. anselli*, *F. bocagei*, *F. damarensis*, *F. darlingi*, *F. foxi*, *F. hanangensis*, *F. ilariae*, *F. kafuensis*, *F. livingstoni*, *F. mechowii*, *F. micklemi*, *F. ochraceocinereus*, *F. vandewoestijneae*, *F. whytei*, and *F. zechi*. However, caveats must be provided for the validity of *F. ilariae* Gippoliti and Amori, 2011 (Burgin et al. 2020). Its assignment to *Fukomys* is based on a single damaged study skin (no skull or molecular data are available) of uncertain origins, and it displays various traits atypical of the genus (Gippoliti and Amori 2011).

It should be emphasized that it is currently not possible to convincingly determine most small-bodied *Fukomys* species based on morphological characters alone. Morphometric data sets are only available for a few species and their diagnostic value has not been adequately assessed. Fur color is intraspecifically highly variable and frequently age-dependent. The taxonomic significance of other, traditionally valued anatomical characters, such as the shape of the infraorbital foramen, remains to be tested. It is strongly recommended to incorporate both karyological information and sequence data to securely identify *Fukomys* species. The genus is in dire need of a taxonomic revision and a significant portion of cryptic diversity needs to be taxonomically explored (Van Daele et al. 2004, 2007). For these reasons, we do not attempt to provide a morphology-based key to the species of *Fukomys*.

Fukomys mechowii (Peters, 1881)

Giant Mole-rat

Georychus Mechowii Peters, 1881:133. Type locality "Malange," Angola.

G[*eorychus*]. *Mechowi*: Bocage, 1890:271. Incorrect subsequent spelling of *Georychus Mechowii* Peters, 1881.

Georychus Ansorgei Thomas, and Wroughton, 1905:175. Type locality "Kukema R., Bihé. Alt. 5900 ft.," Angola.

Georychus Mellandi Thomas, 1906:178. Type locality "Mpika, N.E. Rhodesia. Alt. 5000 ft.," Zambia.

C[*ryptomys*]. *mechowi*: Thomas, 1917:443. Name combination.

Cryptomys blainei Hinton, 1921:372. Type locality "Chisongwe, Luando River, (altitude 4000')," Angola. Incorrectly spelled "*C. blaenei*" by Van Daele et al. (2013:185).

Cryptomys mechowii: Allen, 1939:429. Name combination.

Cryptomys mechowi mechowi: Ellerman, Morrison-Scott, and Hayman, 1953:236. Name combination.

Cryptomys mechowi mellandi: Ellerman, Morrison-Scott, and Hayman, 1953:236. Name combination.

Georhychus mechowi: Ansell, 1978:67. Incorrect subsequent spelling of *Georychus* Illiger, 1811:87.

Georhychus mellandi: Ansell, 1978:67. Incorrect subsequent spelling of *Georychus* Illiger, 1811:87.

Coetomys mechowi: Ingram, Burda, and Honeycutt, 2004:1008. Name combination. Incorrect subsequent spelling of epitheton derived from *Georychus Mechowii* Peters, 1881.

Fukomys mechowii: Kock, Ingram, Frabotta, Honeycutt, and Burda, 2006:53. First use of current name combination.

F[*ukomys*]. *mechowi*: Deuve et al., 2006:2. Incorrect subsequent spelling of *Fukomys mechowii* Kock, Ingram, Frabotta, Honeycutt, and Burda 2006.

CONTEXT AND CONTENT. Context as for genus. No subspecies are currently recognized. *Georychus ansorgei*, *Georychus mellandi*, and *Cryptomys blainei* are synonyms (but see "Nomenclatural Notes").

NOMENCLATURAL NOTES. Various authors have incorrectly adopted the spelling *mechowi* for the epitheton of *Fukomys mechowii*, leading to the frequent occurrence of this term in the literature. The genus name comprises the term "fuko" which derives from Mfuko, a vernacular name for mole-rat in certain Zambian Bantu languages such as Bemba and Kaonde (Ansell 1978; Kock et al. 2006), as well as *mys* which translates to "mouse" from ancient Greek. The latter is a common suffix of scientific names given to rodents. The epitheton *mechowii* honors the German explorer Friedrich Wilhelm Alexander von Mechow (1831–1904) who is especially known for his expeditions to Angola, where he collected the holotype of *F. mechowii* (Peters 1881; Beolens et al. 2009). Common names are giant mole-rat, giant Zambian mole-rat, or Mechow's mole-rat. It is not to be confused with the East African spalacid *Tachyoryctes macrocephalus* that is also frequently referred to as the giant mole-rat.

Molecular evidence suggests that populations of giant mole-rats that have traditionally been comprised under *F. mechowii* are actually paraphyletic. There is an "Eastern" (Zambia and presumably eastern Democratic Republic of Congo) and a "Western" (Angola, western Democratic Republic of Congo) clade of morphologically similar giant mole-rats (see "Genetics"). However, the Eastern lineage is more closely related to the small-bodied *F. vandewoestijneae* (Caroline's mole-rat; previously: Salujinga haplotype and cytotype) from the Ikelenge region of Zambia and adjacent regions (Van Daele et al. 2007; Visser et al. 2019; J. Krásová et al., in litt., University of South Bohemia, České Budějovice, Czech Republic, 24 May 2021). The name *F. mechowii* (Peters, 1881) is attached to the Western group, as the holotype derives from Northern Angola (Peters 1881).

The oldest available name for the Eastern lineage is *Georychus mellandi* Thomas, 1906. The name applied to these animals should therefore be *Fukomys mellandi* (Thomas, 1906).

Although we are aware of this issue, we choose to apply the name *F. mechowii* in a traditional sense to both Eastern and Western giant mole-rats here to not preempt a proper taxonomic revision of the group. The vast majority of data available on giant mole-rats corresponds to the Eastern lineage found in Zambia. To discern information referring to either the Western or the Eastern clade, we attempted to include the geographic provenance of the populations described whenever feasible. If not done so, data refer to the Eastern giant mole-rat clade from Zambia.

DIAGNOSIS

Fukomys mechowii is easily distinguished from sympatric mole-rat species based on its body size (females: 200–355 g; males: 250–995 g), which is by far the largest in the genus (Kawalika and Burda 2007; Van Daele et al. 2013; see Caspar et al. 2021 for body size variation within *Fukomys*). However, subadult individuals might be mistaken for the closely related *F. bocagei* (Bocage's mole-rat) and *F. vandewoestijneae* that share a similar fur coloration and which may occur in sympatry with *F. mechowii* in parts of its range. *Fukomys mechowii* and *F. vandewoestijneae* typically lack the white head spot characteristic for many species of the smaller *Fukomys* mole-rats. Definite species assignment of immature specimens can therefore only be achieved based on karyotyping (*F. mechowii*: 2n = 40—Macholán et al. 1993; all other species: 2n ≥ 42—Van Daele et al. 2004) and DNA sequence markers (Van Daele et al. 2013).

GENERAL CHARACTERS

Fukomys mechowii is a large, robustly built African mole-rat. Adults display concolorous ochre-brown to golden pelage (Fig. 1). Fur color changes markedly during ontogeny. The pelage of pups is typically dark gray, but slowly turns brown after weaning and eventually takes on an ochre hue when attaining sexual maturity; it may get paler with increasing age (Kawalika and Burda 2007; Bennett and Burda 2013). A white head spot, typical for most other *Fukomys* mole-rats, is usually lacking in *F. mechowii*. Individuals (of both sexes) show only very rarely a small white spot on the forehead (Kawalika and Burda 2007). The body hair is uniformly short (hair length: 7 mm at the trunk, 7.5 mm at the ventrum—Šumbera 2019). There can be prominent patches of dark rusty-brown coloration in the corners of the mouth in adult males (Fig. 1; Peters 1881, only rarely distinctly expressed in females). These markings appear to be stained by wax-like glandular secretions and have been linked to reproductive status by some authors (e.g., Sichilima et al. 2008a), an association we have not observed in captivity. Apart from that, no sexual dichromatism is observable. Local mole-rat hunters in the Zambian Kasama region anecdotally describe exceptionally large, white-bellied or completely white giant mole-rats (Kawalika 2004). So far, these reports remain unconfirmed.

Hands and feet lack fur and are instead dorsally covered and fringed by sparse pale hairs. Similarly, the short tail is naked except for white caudal bristles. Claws on the hind feet are dorsoventrally flattened and appear nail-like (Kingdon 1974). The rhinarium, eyelids, and the immediate area surrounding the external ear opening are naked. As all bathyergids, *F. mechowii* lack pinnae. Females exhibit two pairs of pectoral and one pair of inguinal nipples (Scharff et al. 1999).

Fukomys mechowii is the largest species of the genus *Fukomys* and shows pronounced sexual size dimorphism (Burda and Kawalika 1993) which develops shortly after the onset of sexual maturity (Scharff et al. 1999; Chimimba et al. 2010). The degree of body size dimorphism is among the most extreme among the Bathyergidae and rodents in general (Caspar et al. 2021). Average body size as approximated by body mass varies substantially between populations. In a sample of individuals from several Zambian localities in the Copperbelt Province, the mass (g; range and sample size *n* presented in parentheses) of adult males was found to be 345.3 ± 95.4 *SD* (250–560; *n* = 15), whereas that of adult females was 252 ± 34 *SD* (200–295; *n* = 18—Scharff et al. 2001). Higher average body masses were found in specimens from the Chingola area in the Zambian Copperbelt province (males: 570.7 ± 20.7 *SE* (220–995; *n* = 79); females: 391.3 ± 11.7 *SE* (240–650; *n* = 76—Sichilima et al. 2008b)). *Fukomys mechowii* from Chibale and Ndola-Chichele (Zambia) is reported to be smaller than specimens from other areas (Kawalika and Burda 2007), with a body mass of typically less than 300 g in males (Scharff et al. 2001). However, this population was found not to be distinct from other *F. mechowii* based on sequence data derived from the mitochondrial cytochrome-*b* gene (Van Daele et al. 2007). Individuals from Mount Moko in Angola have been noted to be the largest representatives of the species (Bennett and Burda 2013), but no data on body mass have been published and no molecular or karyological data on this population are available. In general, exceptionally large males of *F. mechowii* can reach weights of 700–800 g (Kawalika 2004; Kawalika and Burda 2007; but see Sichilima et al. 2008b for reporting on wild males weighing up to 995 g).

Further standard body measurements (mm; mean with *SD* and range presented in parentheses) are available for 10 male and 10 female specimens of *F. mechowii*, respectively, deriving from the Copperbelt Province in Zambia (Bennett and Burda 2013): head–body length—190 (22, 156–262), 165 (18, 135–205); tail length—27.3 (2.3, 23–31), 27.8 (3.8, 23–33.7); length of hind foot—35.3 (2.0, 30.6–37.8), 32.2 (1.0, 31–34); pinna length—not applicable.

The skull of *F. mechowii* (Fig. 2), apart from its size, shows the morphology typical of the genus and resembles that of other tooth-digging bathyergids (e.g., McIntosh and Cox 2016; see "Form and Function"). It displays reduced bony orbits, hystricognathy (Gomes Rodrigues et al. 2016) and

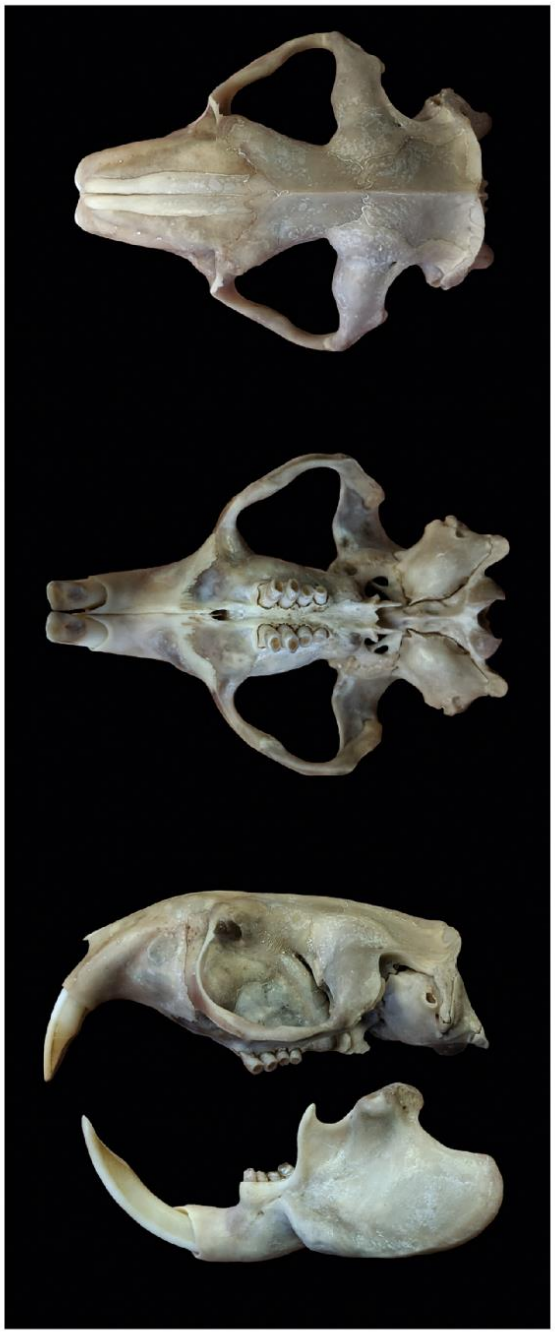

Fig. 2.—Dorsal, ventral, and lateral views of skull and lateral view mandible of an adult male *Fukomys mechowii* from the research collection of the Department of General Zoology, University of Duisburg-Essen, Essen, German. Greatest length of skull is 51.8 mm. Photographs by KRC.

hystricomorphy. However, the small infraorbital foramen, as in all mole-rats of the *Cryptomys* and *Fukomys* genera, is only penetrated by a rudimentary extension of the zygomaticus muscle (Van Daele et al. 2008). The infraorbital foramen is elliptical and thin-walled (Kawalika and Burda 2007). Sexual dimorphism in skull morphology is pronounced (Chimimba et al. 2010).

Mean skull measurements (mm; ranges presented in parentheses) of five males and five females from the Copperbelt Province in Zambia (Bennett and Burda 2013), respectively, were: greatest skull length—52.0 (45.6–59.2), 42.2 (34.0–49.7); greatest skull width—46.7 (40.3–53.2), 33.2 (28.6–37.0); length of toothrow (P4–M3)—9.1 (7.9–10.2), 7.8 (6.9–9.2). More detailed cranial measurements (mm, approximated to 0.1 mm; mean with *SD* and range presented in parentheses), some selectively presented below, are available for a large sample of *F. mechowii* (n = 47; sexes and ages combined) deriving from various locations throughout its range in Angola, Democratic Republic of Congo, and Zambia (Van Daele et al. 2013): greatest skull length—47.75 (5.1, 38.6–61.4); skull width at processus mastoideus—24.48 (3.4, 20.1–30.6); diastema length—17.0 (2.4, 12.3–23.7); palatilar length—31.4 (3.7, 12.3–23.7); length of bulla—12.48 (1.0, 10.3–14.7); width of bulla—9.2 (0.9, 7.4–11.0); width of zygomatic plate—11.7 (2.1, 7.8–18.3); maximum zygomatic width—37.6 (4.9, 28.7–50.2); length of infraorbital foramen—5.8 (1.2, 3.6–9.1); rostral width—11.9 (1.8, 8.7–16.1); length of mandible—38.1 (5.0, 29.4–52.0); distance between coronoid and processus angularis—23.8 (3.6, 17.9–32.0). Further craniological measurements from 265 specimens from Chingola, Zambia, were provided by Chimimba et al. (2010) and grouped in respect to ontogeny.

The dentition is typical for the genus *Fukomys*. Each jaw quadrant carries one incisor, one premolar, and three molars (e.g., Van Daele et al. 2013). Incisors are ungrooved with roots extending posteriorly beyond the molar alveoli (Honeycutt et al. 1991). The single premolar is larger than the molars, which decrease in size from anterior to posterior (Honeycutt et al. 1991). Molars are highly simplified and appear as enamel cylinders enclosing a dentine core. Traces of reentrant folds may be observable on the labial molar surface (Honeycutt et al. 1991).

DISTRIBUTION

Fukomys mechowii is restricted to inland central Africa south of the equator. Its distribution extends from northern Angola and the southern Democratic Republic of Congo into northern Zambia (Fig. 3; Maree and Faulkes 2016). However, because detailed surveys are lacking, it is unknown whether the range is continuous (Kawalika and Burda 2007). Profound genetic differences between Eastern (Zambia, presumably eastern Democratic Republic of Congo) and Western (Angola, western Democratic Republic of Congo) lineages of giant mole-rats (see "Nomenclatural Notes" and "Genetics") could indicate a disjunct range. Additional records from Malawi and Tanzania are doubtful (Ansell and Dowsett 1988; Maree and Faulkes 2016). In the southeast, the Zambian Muchinga Escarpment, Mulungushi River, Lukanga swamps, and Kafue River have been suggested to restrict its distribution, whereas in the southwest the range

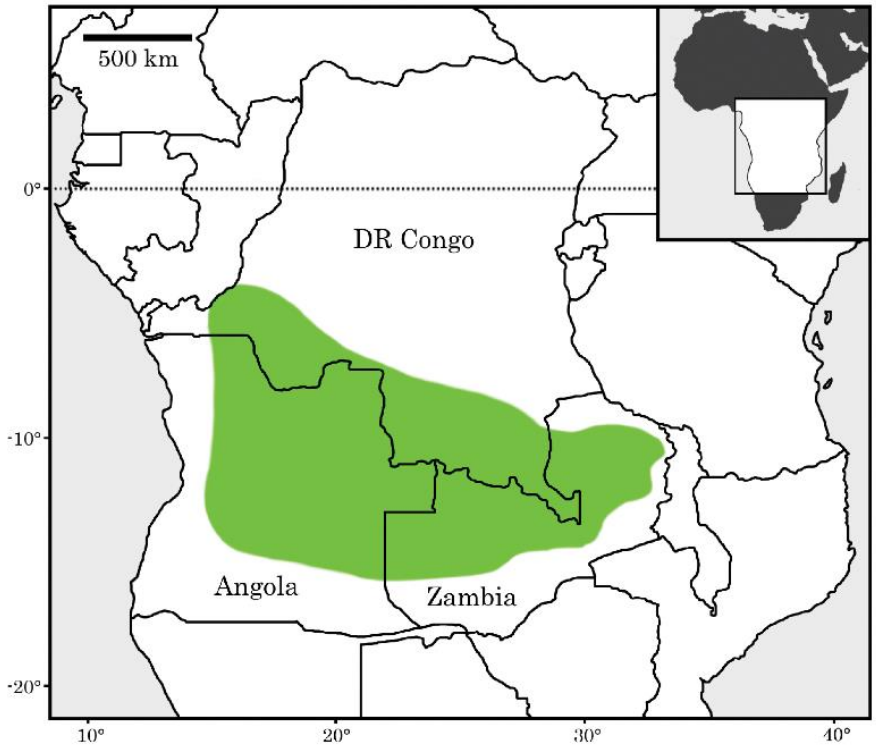

Fig. 3.—Geographic distribution of *Fukomys mechowii*. Names of countries from which the species has been reported are annotated. The range boundaries are largely conjectural and modified after Van Daele et al. (2013) and Maree and Faulkes (2016).

borders at the arid Kalahari basin (Ansell 1978; Kawalika and Burda 2007). The Congo River and the equatorial rainforest belt in the northwest can be assumed to act as dispersal barriers as well. Its northernmost confirmed record is on the Batéké Plateau, close to Kinshasa in the Democratic Republic of Congo (Palata-Kabudi et al. 2005). The range of *F. mechowii* extends into western Angola but does not reach the coastal areas (Van Daele et al. 2013; Maree and Faulkes 2016).

As suggested by its wide distribution, *F. mechowii* is adaptable and tolerates a broad range of habitats and varying seasonality. It is typically found in bushland and mesic savannahs but also occurs in forested areas, marshes (dambo), and in diverse cultivated lands (plantations, fields, orchards—Kawalika and Burda 2007). *Fukomys mechowii* has been recovered from various soil types, ranging from ones with a stony, lateritic consistency to pure sand or clay soils (Ansell and Dowsett 1988; Bennett and Burda 2013).

FOSSIL RECORD

African mole-rats have long been considered to be an ancient group of hystricomorph rodents. Initial molecular studies supported this view by placing their emergence into the early Eocene, about 45 Ma (Faulkes et al. 2004; Ingram et al. 2004). However, lately a more recent time of origin for the clade in the early Miocene, about 21 Ma, has been suggested (Bryja et al. 2018). The earliest fossils of bathyergids also date to the early Miocene and are assigned to genera which cannot be securely placed in respect to crown-group taxa (Winkler et al. 2010). Current fossil evidence suggests that the family was restricted to sub-Saharan Africa since its emergence. The social, sister genera, *Cryptomys* and *Fukomys* (common mole-rats), represent the most recent and most successful radiation of bathyergids. Given the topology of the bathyergid family tree and distribution patterns of extant genera, the *Cryptomys–Fukomys* clade was suggested to have originated in Africa south to the Kalahari Desert (Faulkes et al. 2004). The palaeo-Zambezi River could have acted as a barrier separating ancestral populations of these genera (Ingram et al. 2004). Conflicting molecular data suggest the clade originated either in the middle Miocene, 11–17 Ma (Faulkes et al. 2004; Ingram et al. 2004), or in the latest Miocene, 6 Ma (Bryja et al. 2018). The oldest known fossils of small bathyergids akin to modern *Cryptomys* and *Fukomys* date to the early Pliocene of South Africa (Winkler et al. 2010). Problematically, an assignment of fossils to either genus is not possible, because both are currently distinguished only by means of ethology, and on the karyological and molecular level (Kock et al. 2006; Monadjem et al. 2015). Although extant species of *Cryptomys* are restricted to South Africa and southern Zimbabwe, *Fukomys* colonized diverse habitats of varying

humidity, primarily west to the African Rift Valley, as far north as into the northern tropical savannahs (Faulkes et al. 2010a). However, fossil material that could potentially be assigned to *Fukomys* (but was originally classified as *Cryptomys*) is sparse (Monadjem et al. 2015), not unambiguously referable to extant species, and does not elucidate phylogeographic patterns within the genus.

FORM AND FUNCTION

Like all African mole-rats, *Fukomys mechowii* displays a strictly subterranean lifestyle and shows striking anatomical adaptations to life underground. Similarly, its physiology is adapted to the peculiar conditions of its underground habitat which poses specific demands on respiration, thermoregulation, and sensory systems (Bennett et al. 1994; Begall et al. 2018). The body of all *Fukomys* mole-rats is cylindrical, their extremities and tail are short, testes lay abdominal, pinnae are missing, and their hearing range is restricted to comparatively low frequencies (see below). Combined, these traits represent an adaptive syndrome to subterranean life, though they might separately occur in epigeic mammals as well. The skin of bathyergids is only loosely attached to the underlying musculature by connective tissue. Thus, it can be widely shifted along the rump, reducing friction on the body when moving in narrow underground tunnels. The velvety, evenly short fur brushes in either direction and facilitates locomotion in the burrow system (e.g., Kingdon 1974). Well-innervated guard hairs, probably enhancing tactile sensing, are found all over the body (Krehbiehl 2010). The density of guard hairs is especially high at the tail allowing the animal tactile control when moving backwards.

When burrowing, soil is removed from the surrounding substrate by the incisors. Accordingly, *F. mechowii*, as most bathyergids, employs a chisel-tooth-mediated mode of digging (McIntosh and Cox 2016). As typical for the family, hairy lip folds close medially behind the procumbent incisors, even at full gape. Thereby, dirt is prevented from being ingested during the digging process (Kingdon 1974). To avoid obstruction of the airways, the nostrils can be sealed as well (compare Banke et al. 2001). Loosened soil is pushed below the rump by the forelimbs and is dispensed posteriorly by the hindlimbs (Kingdon 1974; Morlok 1983). To allow for efficient shoveling, both fore- and hind feet are laterally widened by a sesamoid bone (prepollex and prehallux, respectively—Schmitt et al. 2009; Prochel et al. 2013). Furthermore, the os lunatum and os scaphoideum of the carpus are fused, which might increase the stability of the palm when digging (Prochel et al. 2013). Whereas the forefeet display pointed claws, the ones of the hind feet are dorsoventrally flattened and appear nail-like, assisting in effectively dispensing loosened soil (Kingdon 1974). The shoulder girdle is translocated cranially, compared to epigeic rodents, and exhibits ossified mesoscapular segments, situated between the shoulder blades and clavicles. Both characters are associated with a burrowing lifestyle and are frequently observed in different subterranean mammal clades (Morlok 1983). The angulus caudalis of the shoulder blade is elongated to enlarge the attachment site for the teres major muscle, a forelimb retractor that facilitates digging (Morlok 1983). However, most important for burrowing are the adaptations found in the skull and dentition.

Compared to its body size, the skull of *F. mechowii* is notably large and its jaws are elongated, permitting a wide gape (McIntosh and Cox 2016). Similar to other chisel-tooth-digging rodents, the skull is deep and broad with widened zygomatic arches for supporting the substantially hypertrophied jaw musculature (Gomes Rodrigues et al. 2016; Cox et al. 2020). Especially the relative size of the temporalis muscle is increased, as it allows to sustain high bite forces at the wide gapes (approaching 70°) which typically occur during tooth-digging (McIntosh and Cox 2016; Van Wassenbergh et al. 2017). Relative to body mass, *Fukomys* mole-rats display the second most massive jaw musculature of all subterranean rodents, being surpassed only by the naked mole-rat (*Heterocephalus glaber*—Morlok 1983). *Fukomys mechowii* shows the strongest relative bite force of all mammals studied so far (P. A. A. G. Van Daele, in litt., Ghent University, Ghent, Belgium, 1 May 2010). Adding to this, evidence from finite element analyses of virtual skull models point to a high mechanical efficiency of biting in *F. mechowii* which is comparatively well maintained at wide gapes (Cox et al. 2020). The mandible is highly mobile due to an unfused mandibular symphysis and enlarged, flattened glenoid fossae (Gomes Rodrigues et al. 2016). The size of the brain (approximately 2.15 g) and the number of neurons in specific brain regions of *F. mechowii* conform to rodent scaling rules (Kverková et al. 2018).

As typical for tooth-digging rodents, *F. mechowii* shows pronounced upper incisor procumbency, which, besides its direct relevance for digging, reduces friction on the nasal region (Agrawal 1967). However, contrary to some claims (e.g., Landry 1957) procumbency in the *Fukomys* genus is not notably expressed in comparison to most other bathyergids (McIntosh and Cox 2016). The roots of both the upper and lower incisors in *F. mechowii* are remarkably deep and extend caudally beyond the respective molar rows, a character exclusively shared by tooth-digging bathyergids among rodents (Ellerman et al. 1953). The extreme depth of the tooth alveoli could serve to increase mechanical resilience and facilitate force dissipation in the skull (Landry 1957).

The anatomy of the gastrointestinal tract of *F. mechowii* shows no peculiarities and is very similar to the gastrointestinal tract of other bathyergids (Kotzé et al. 2010). The stomach is simple and it amounts to about 9.6% of the total gastrointestinal length. The length of the small intestine relative to the total alimentary tract (47.2%) is roughly as long as the combined length of colon and cecum (43%). The cecum (with two taenia, and 13–16 haustra) is spirally coiled and, as in most mammals, lacks an appendix (Kotzé et al. 2010). The ascending colon loops and is folded in the right abdominal cavity. The mean (± *SD*) total

length of the gastrointestinal tract amounts to 1,268 ± 162 mm with a mean (± *SD*) total surface area of 46,829 ± 9,914 mm^2 (Kotzé et al. 2010).

The hair density of the fur is much lower on the ventrum (2,800 hairs/mm^2) compared to the trunk (11,200 hairs/mm^2) and might help the animal dissipate heat effectively (Okrouhlík et al. 2015; Šumbera 2019). In line with this, the mean (± *SD*) conductance of the skin of *F. mechowii* (0.09 ± 0.01 cm^3 O_2 × g^{-1} × h^{-1} × °C) is higher compared to surface living rodents (Bennett et al. 1994). The thermoneutral zone of *F. mechowii* is reportedly between 29°C and 30°C (Bennett et al. 1994). The mean (± *SD*) body temperature is on average 34 ± 0.53°C in the ambient temperature range 29–34°C, and the resting metabolic rate (0.6 ± 0.08 cm^3 O_2 × g^{-1} × h^{-1}) at thermoneutrality is significantly lower than expected in respect to body mass (Bennett et al. 1994). Digging metabolic rate of *F. mechowii* is 4.5 times higher than resting metabolic rate and independent from soil type (hard versus soft soil) or sex (Zelová et al. 2010). Under laboratory testing conditions (temperature gradient: 16–37°C), *F. mechowii* prefers mean (± *SD*) ambient temperatures of 25.6 ± 1.2°C, thus much lower than its thermoneutral zone (Begall et al. 2015). This might be due to a favorable volume to surface ratio in this large bathyergid.

The eyes of *F. mechowii* are tiny (equatorial diameter: 2.4–2.6 mm) but possess all structures typical for a mammalian eye (e.g., stratified retina, iris, lens) and permit dichromatic color vision (Peichl et al. 2004). Apart from a well-developed musculus retractor bulbi, the extraocular musculature is reduced. The miniscule ocular dimensions likely impede the effective resolution of shapes or movement, while keeping sensitivity to changing light intensities. Avoidance of full-spectrum (white), blue, and green-yellow light but not UV or red light has been experimentally demonstrated (Kott et al. 2010). The photoreceptor layer is dominated by rods that are fewer in number than in (nocturnal) epigeic rodents, but individual rods are larger (Peichl et al. 2004). The proportion of cones (ca. 10%) among the total amount of photoreceptors is higher than in diurnal mammals. Most cones (ca. 90%) express S-opsins, which appears puzzling because blue light propagates much less efficiently in underground tunnels than light of longer wavelengths (Peichl et al. 2004; Kott et al. 2014). However, it can be assumed that the high proportion of S-cones relates to an unusually low level of serum thyroxine (T4) as is the case in Ansell's mole-rats (*F. anselli*—Henning et al. 2018). If so, this exceptional opsin expression pattern might be a neutrally selected side effect of keeping the basal metabolic rate low (Henning et al. 2018).

Hearing in *F. mechowii* is restricted to relatively low frequencies up to 4 kHz and high levels of sound pressure as has been shown by studying auditory brain-stem responses (Gerhardt et al. 2017). The species therefore displays one of the lowest high-frequency cutoffs among mammals. The highest hearing sensitivity is present at 1–1.5 kHz, where the hearing threshold is still located at about 30 dB sound pressure level (Gerhardt et al. 2017). It was shown that *F. mechowii*, similar to other bathyergids, is sensitive to magnetic stimuli under controlled laboratory conditions (Oliveriusová et al. 2012).

ONTOGENY AND REPRODUCTION

Ontogeny.—Newborn *Fukomys mechowii* weigh on average 19.6 g (range 12.6–27.7 g—Scharff et al. 1999) and are 5–6 cm long (Bennett and Aguilar 1995). Body mass of neonates is not correlated with litter size, but it is a good predictor of further survival (Burda and Kawalika 1993; Scharff et al. 1999). Neonates are colored dark pink and are sparsely covered by thin whitish hairs. Their eyes and auditory meatus are closed but their incisors already pierce the lips (Bennett and Aguilar 1995). Postpartal growth during the first 180 days after birth is slow with no significantly different mean growth rates between males (0.62 g/day) and females (0.61 g/day—Scharff et al. 1999). Eyes and ear meatus might be open by 6 days after birth (Bennett and Aguilar 1995) but can remain closed until the newborns are up to 3–4 weeks old (Burda and Kawalika 1993). Dark-gray pelage starts to develop within the first week, and at the age of 5–6 weeks pelage color changes from gray to brown (Bennett and Aguilar 1995; Scharff et al. 1999). Pups are typically nursed for at least 3 months (Burda and Kawalika 1993; Scharff et al. 1999); however, weaning might occur after as soon as 5–6 weeks (Bennett and Aguilar 1995). Suckling bouts typically extend over more than 20 min (Bennett and Aguilar 1995). Pups start to consume solids (including feces from family members) at the age of 2 weeks and commence to actively explore the environment at 7–10 days after birth (Scharff et al. 1999). At that time, they also begin to spar with each other. Sparring with adults occurs at 2 weeks after birth (Scharff et al. 1999).

Fukomys mechowii reaches sexual maturity at about 12 months, but pair formation in captivity is suggested to take place at 18 months of age or later (Begall et al. 2018). The maximum life span of the species in captivity is > 26 years (Begall et al. 2021; a female captured as an adult in June 1995 died in July 2020 at the University of Duisburg-Essen), data on free-living animals are not available. The median age reached by captive reproductive animals is 13.4 years (4,882 days) while that of nonreproductive ones is 8.3 years (3,012 days); a pattern that fits the bimodal aging pattern also found in congeneric species (Dammann et al. 2011; Begall et al. 2021). There is no significant difference in life expectancy between males and females although the age difference between the oldest documented female (> 26 years) and male (16.4 years) is striking (Begall et al. 2021).

Several genes related to the hypothalamic–pituitary–adrenal stress axis seem to be downregulated in breeding *F. mechowii* compared to age-matched nonreproductive siblings, and might provide an explanation for the bimodal aging pattern (Sahm et al. 2021). Indeed, hair cortisol levels are significantly higher in nonreproductive animals compared to breeders in captivity.

Captive nonbreeding *F. mechowii* that do not live in family groups together with their parents show markedly lower cortisol levels than individuals that remained in intact families and can approach reproductive animals in longevity (Begall et al. 2021). In general, stability of gene expression in *F. mechowii* is remarkably high across various tissues when compared to laboratory Norway rats (*Rattus norvegicus*) of similar body mass, providing a proximate mechanism for comparatively slow aging in this species (Sahm et al. 2018).

Reproduction.—*Fukomys mechowii* lives in monogamous families where typically only one pair (sometimes termed "king" and "queen") is reproductively active, resulting in a strong reproductive skew (Šumbera et al. 2012). Offspring remain with the parents into adulthood, in some cases, possibly lifelong, and support them in raising further young. Due to the reproductive division of labor, cooperative care for the young and strong philopatry of offspring, *F. mechowii* and all its congeners have been referred to as eusocial mammals (Burda and Kawalika 1993; Kock et al. 2006; Kverkovà et al. 2018; the definition and applicability of the term are discussed by Burda et al. 2000). This social system relies on individual recognition combined with incest avoidance (see "Behavior"). Breeding occurs throughout the year (Scharff et al. 2001; Sichilima et al. 2008b).

The proportion of juveniles (body mass less than 50 g) among the total number of trapped *F. mechowii* in the field is, at 8%, very low compared to other social rodents (Scharff et al. 2001; Kawalika and Burda 2007). Under laboratory conditions, the breeding pair shows lifelong sexual monogamy, and incestuous matings between siblings or parents and offspring are exceedingly rare (Kawalika and Burda 2007). During the 25-year breeding history of the species at the University of Duisburg-Essen, only one incestuous mating between father and daughter was observed and it occurred after the original queen had died (Begall et al. 2021). In most groups of *F. mechowii* captured in the field, there is also only one female found to be reproductively active (Wallace and Bennett 1998; Scharff et al. 2001; Sichilima et al. 2008b; Šumbera et al. 2012). Although they do not reproduce, nonbreeding *F. mechowii* of either sex are not sterile. Pituitary sexual hormone parameters do not differ between breeders and nonbreeders, suggesting behavioral instead of physiological regulations of reproduction (Bennett et al. 2000; see "Reproductive behavior"). Although observations on the reproductive physiology of nonbreeding male *F. mechowii* are sparse, its constitution might mirror the one of the better studied congeneric *F. anselli*. Testes of reproductive *F. anselli* males are significantly larger than those of nonreproductive males; however, nonreproductive individuals also produce viable sperm (Garcia Montero et al. 2016). Information on whether *F. mechowii* females are induced or spontaneous ovulators is ambiguous (Faulkes et al. 2010b). However, because *F. anselli* (Willingstorfer et al. 1998) displays induced ovulation, it can be considered likely for *F. mechowii* as well. Females possess a uterus duplex, as typical for most rodent taxa.

The male's penis shows no ornamentation (Faulkes et al. 2010b) and exhibits a baculum. Females display an elongate flattened os clitoridis (baubellum) that approaches the baculum in size (Thomas 1917).

Gestation lasts on average (mean ± *SD*) 112 ± 9 days with a minimum interbirth interval of 89 days indicating that the females experience postpartum estrus (Scharff et al. 1999). The mean litter size is 2.1 ± 1.1 (range 1–5) and increases with the number of births experienced by one female (Scharff et al. 1999). Very rarely, six pups are born in one litter (Matschei and Bäthe 2012).

ECOLOGY

Population characteristics.—Little is known about the population ecology of wild *Fukomys mechowii*. Like other subterranean mammals, *F. mechowii* is a comparatively long-lived K-strategist (see "Ontogeny and Reproduction" and "Grouping behavior"), and its populations seem to be locally stable. Average size of wild family groups is reported to range from 9 to 13 animals (Scharff et al. 2001; Sichilima et al. 2008b; Šumbera et al. 2012; see "Grouping behavior" for details). There is no aging marker to reliably estimate the age structure (i.e., the number of litters) of families (and the population).

A sex ratio strongly skewed toward females (1.46 females:1 males) was reported in a large sample of wild *F. mechowii* from Zambia, captured near Chingola (n = 317—Sichilima 2008b). A far smaller sample of animals from Ndola recovered male-biased ratios in juveniles (< 1 year; 1.22, n = 40) but among adults (> 1 year), the proportion of males decreased (0.96, n = 45—Scharff et al. 2001). Data on captive families revealed also a female-biased neonate sex ratio of 0.54 among laboratory-born animals (Scharff et al. 1999).

Space use.—Each family group occupies a single burrow system. The size (length, extension) of burrows reflects their stability in time (age), the number of inhabitants (family size), and availability of food resources. The smallest burrow systems (encompassing ca. 0.2 ha) occur in marshlands (dambo). Burrow systems in acacia forests are on average only slightly larger, whereas those in pine forests and open fields can cover about 2–3 ha. The number of mounds in areas occupied by *Fukomys* mole-rats ranges from 1–2 to 18 per 100 m^2 (up to 225 mounds in an area of 25 m by 50 m). Building of mounds (but not digging per se) seems to be a seasonal affair restricted to the dry season (Scharff et al. 2001; Kawalika and Burda 2007). Two burrow systems of *Fukomys mechowii* mapped in detail in a miombo woodland in Zambia were very large (total lengths, 2,245 and 743 m) and locally densely branched (Šumbera et al. 2012). In contrast, 32 burrows studied on Zambian farmland were rather short (mean ± *SD*: 249 ± 34 m; range 190–310) and structurally simple (Sichilima et al. 2008a). Unfortunately,

the latter study was carried out in habitats markedly influenced by human activity, thus shedding doubt on whether the results can be generalized. Because the two large burrow systems contained 14% and 7% backfilled segments of the total tunnel lengths (Šumbera et al. 2012), the complete absence of backfilled tunnels in the earlier study (Sichilima et al. 2008a) has been interpreted as an indication that smaller tunnels have been ignored and only main tunnels have been mapped (Šumbera et al. 2012).

The general structure of burrow systems of *F. mechowii* is similar to those described for other members of the genus and for other bathyergids, and consists of nests, food chambers, defecation chambers, superficial foraging burrows, and deeper connecting tunnels (Scharff et al. 2001; Šumbera et al. 2012). All burrow systems are sealed with mounds and additional plugs. The diameter of tunnels is on average 8 cm (range 6.5–11.5 cm). The main tunnel ("runway") in one examined burrow system was 200 m long, 30 cm deep, and mounds were 2–3 m apart. The level from the surface to the roof of the tunnel was at minimum 5 cm, at maximum about 200 cm. There was no correlation between the diameter, and the depth of the tunnels (15–60 cm) studied in two burrow systems, indicating that animals of all age classes are burrowing and foraging near the surface (Šumbera et al. 2012). Eight nests (diameter: 20–40 cm; height: 10–20 cm) from four family groups in Zambia were excavated at an average depth (mean ± *SD*) of 90 ± 40 cm (range 50–160 cm—Scharff et al. 2001; Šumbera et al. 2012). Consistent with that, a nest at a depth of 80 cm was reported from Democratic Republic of Congo (Kisasa et al. 2004). In some areas (Chichele, Zambia), nests frequently occur in termite hills, which may be a strategy to avoid the nest chambers being flooded during the rainy season (Kawalika and Burda 2007). Grass and root fibers constitute common litter material for nests. In general, food and defecation chambers are located near the nest (Kisasa et al. 2004). Burrow systems display areas with densely branched superficial tunnels along with more straight and unbranched tracts (Šumbera et al. 2012). This indicates usage of the area-restricted search strategy. Sites with highly branched superficial burrows were probably those with a high food density.

Data on the stability of burrow systems of *F. mechowii* are missing, yet judging from the family size and the extension of a burrow system, they appear to be stable for several years, consistent with findings on other social bathyergids, such as *F. damarensis* (Damaraland mole-rats—Bennett and Faulkes 2000). Nevertheless, continuing excavation of new burrows must occur in order to localize new food resources. The available data on *F. mechowii* suggest pronounced burrowing activity after the end of rains (Šumbera et al. 2012). Fractal dimensions of burrow systems are more complex in the rainy season compared to the dry season (Sichilima et al. 2008a).

Radiotracking of six individuals (trapped in the vicinity of Ndola, Zambia), revealed that wild *F. mechowii* constantly stay underground, spending most of their time in the nesting chambers (Lövy et al. 2013). Dependent on the individual, animals were moving within the burrow system outside of their nest for 3.2–8 h a day (Lövy et al. 2013). Little is known about surface activity in *F. mechowii*. Only very rarely are animals found wandering around outside their burrow systems. Respective individuals are often found to be large adult males (Kawalika and Burda 2007; Chimimba et al. 2010). How and to which degree dispersal and mate finding are accomplished above- or underground remains elusive.

Diet.—*Fukomys mechowii* primarily feeds on underground plant storage organs such as the rhizomes, tubers and bulbs of diverse weeds, and on roots of shrubs and trees. It does not drink free water. While large, bulky food items are consumed in situ when encountered, smaller ones are transported into food chambers (Šumbera et al. 2012). Typically, one to two primary food chambers, mostly close to nesting chambers, occur in burrow systems (Sichilima et al. 2008a). However, *F. mechowii* also frequently supplements its diet by feeding on animal prey which is cached separately from plant material in the burrows and which may be immobilized for storage (Kawalika and Burda 2007). We observed that small food items, such as small shoots or grains, are grasped with both hands simultaneously and were invariably consumed in a sitting position.

Food stores may or may not be present in burrow systems and vary substantially in size. The mass of hoarded plant material in 32 burrows was found to be significantly greater during the rainy season (349 ± 322 g; range: 45–1,310) compared to the dry season (161 ± 128 g; range: 45–420) at Chingola, Zambia (Sichilima et al. 2008a). A study on two neighboring burrow systems at Ndola Hills Forest Reserve in Zambia in the dry season recovered 1,635 g (177 items) of hoarded plant material in one, and no notable caches at all in the other system (Šumbera et al. 2012). No indication of cropping of shoots growing from the food stores has been found (Sichilima et al. 2008a). Even if different food plants are hoarded within one burrow system, food items are sometimes encountered sorted, with tubers of only one species present in a specific chamber (Scharff et al. 2001). Animal prey is typically encountered in small stores of around 20 items (Burda and Kawalika 1993).

A great variety of plants are consumed. Naturally occurring plant species recorded in the storage chambers of *F. mechowii* from different Zambian localities included: Anacardiaceae (*Lannea discolor*), Apiaceae (*Steganotaenia*), Asteraceae (e.g., *Senecio*), Colchicaceae (*Gloriosa superba*), Dioscoreaceae (*Dioscorea bulbifera*, *D. cochleari-apiculata*, *Tacca*), Fabaceae (*Albizia*, *Mucuna*, *Rhynchosia*, *Sphenostylis marginata*, *Tephrosia*), Hypoxidaceae (e.g., *Hypoxis*), Iridaceae (e.g., *Gladiolus*), undetermined Liliaceae, Myrtaceae (*Syzygium guineense*), Orchidaceae (e.g., *Eulophia*), Phyllanthaceae (*Pseudolachnostylis*), Rubiaceae (*Crossopteryx febrifuga*), Vitaceae (*Cissus*), Zingiberaceae (*Aframomum biauriculatum*), and rhizomes of grasses (Poaceae) such as *Tristachya* (Scharff et al. 2001; Sichilima et al. 2008a; Šumbera et al. 2012). Identified food plants obtained from storage chambers at the Bateké plateau close to Kinshasa in the Democratic Republic of Congo

included: Amaryllidaceae (*Crinum jagus*), Anisophylleaceae (*Anisophyllea*), Aparagaceae (*Asparagus africanus*), Orchidaceae (*Brachycorythis*, *Eulophia*), Zingiberaceae (*Aframomum*), and the toxic fern *Pteridium aquilinum* (Dennstaedtiaceae—Kisasa et al. 2004; Palata-Kabudi et al. 2005). Besides the diverse native plants consumed, *F. mechowii* also regularly relies on cultivated species, such as cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), potato (*Solanum tuberosum*), groundnut (*Arachis hypogaea*), ginger (*Zingiber officinale*), and corn (*Zea mays*—Scharff et al. 2001; Palata-Kabudi et al. 2005; Sichilima et al. 2008a). In forested areas, rhizomes are fed on more frequently than in open habitats (Scharff et al. 2001). Animal prey primarily consists of invertebrates, especially earthworms and scarabaeid beetle larvae (Kawalika and Burda 2007). However, *F. mechowii* is also reported to occasionally feed on toads and snakes (e.g., *Atractaspis*) and readily consumes meat in captivity (Burda and Kawalika 1993).

Diseases and parasites.—Despite their social lifestyle, transmission rates and general exposure to parasites and pathogens appear to be comparatively low in *Fukomys*, as their major histocompatability (MHC) genes do not show signatures of strong positive selection (Kundu and Faulkes 2004). Mites of the family Trombiculidae are the only known ectoparasites of *F. mechowii* (Scharff et al. 2001; Šklíba et al. 2016). Documented endoparasites are few and include cestodes (*Inermicapsifer madagascariensis*, *Raillietina*) and nematodes (*Protospirura muricola*, *Capillaria*) found in the small intestine and the abdominal cavity (Scharff et al. 1997, 2001). Such helminths occurred in 12 individuals out of a sample of 35 wild-caught specimens (34%—Scharff et al. 2001). There are no reports of zoonoses transferred from *F. mechowii* to humans.

Interspecific interactions.—Burrow systems of *Fukomys mechowii* provide shelter for diverse commensals, among them invertebrates, amphibians, and snakes (Šklíba et al. 2016). Apart from humans who hunt mole-rats for their highly valued meat (Burda and Kawalika 1993) or persecute them as serious agricultural and horticultural pests, there are no predators of *F. mechowii* documented. However, they probably fall prey to various opportunistic predators during surface activity.

HUSBANDRY

Fukomys mechowii is easily sustained in captivity (Kawalika 2004; Begall et al. 2018). It can be fed with carrots and potatoes as staple food, while other tubers and cucumber, lettuce, and parsley might be offered in addition to that. The diet might be supplemented by providing cereals, peanuts, and animal protein (Bennett et al. 1994; Kawalika and Burda 2007). Families of *F. mechowii* can be kept in simple glass terraria of various sizes that are filled with a layer of sawdust or horticultural peat (minimum 5 cm). Terraria measuring 2 m by 0.5 m provide adequate space for large families of up to 14 animals (Scharff et al. 1999). It is typically sufficient to clean the enclosures and to change the substrate every 2 or 3 months (Matschei and Bäthe 2012). A nesting chamber must be provided but might be as simple as a flowerpot or a wooden box. Tissue paper or wood wool should be regularly offered as nesting material (Bennett and Aguilar 1995). Temperature levels should be adjusted to 26 ± 2°C (*SD*), at least 45% air humidity is advisable (Bennett and Aguilar 1995). Family groups of *F. mechowii* are highly xenophobic toward unfamiliar conspecifics in captivity, requiring strict separation of individual families (Begall et al. 2018). This necessitates that pairings must be undertaken while both partners are separated from their natal groups (Knafla 2008). Animals are easily handled by grasping the skin at the back (with thumb and index finger) near the tail base.

Anesthesia of *F. mechowii* can be accomplished by administration of ketamine (6 mg/kg body mass) and xylazine (2.5 mg/kg body mass). At this dose, the loss of the pedal withdrawal reflex lasts on average for 29 ± 2 (*SD*) min and the loss of the righting reflex for 140 ± 41 (*SD*) min (Garcia Montero et al. 2015).

Despite its modest husbandry demands, *F. mechowii* is only rarely housed in captivity (Matschei and Bäthe 2012). Publicly displayed animals are kept in the zoos of Peoria (Illinois), Prague (Czech Republic), and Osnabrück (Germany) and family groups employed for research at the Universities of Duisburg-Essen (Germany) and České Budějovice (Czech Republic). Only a few private holders keep and breed the species. All captive individuals in Europe are descended from animals captured in Eastern Zambia (see Scharff et al. 1999).

BEHAVIOR

Grouping behavior.—*Fukomys mechowii* is a highly social species living in families grouped around a single reproductive pair (Burda and Kawalika 1993; Scharff et al. 2001; Kawalika and Burda 2007; Sichilima et al. 2008b; Šumbera et al. 2012). Dispersal of nonreproductive animals is delayed so that several adult individuals originating from different litters coexist in one family group and assist in raising their siblings (Kawalika and Burda 2007). Data on wild congeneric *F. damarensis* indicate that these helpers spend more time foraging than breeders (Francioli et al. 2020). Moreover, laboratory studies on the same species showed that the breeding female's time spent resting and feeding and its fecundity positively correlate with the number of helpers in a group (Houslay et al. 2020). Sizes of wild *F. mechowii* family groups are reported to range from 3 to approximately 20, on average 9–13 animals (Scharff et al. 2001; Sichilima et al. 2008b; Šumbera et al. 2012). These data, based on altogether 41 families, are comparable to the more thoroughly studied *F. damarensis* in which, based on 110 families, a mean family size of 11 was estimated (Bennett and Faulkes 2000). At one locality (Chichele, Zambia), the study area was difficult to survey so that the 40 animals trapped in this area could have originated from two neighboring families implying that at least one of these groups consisted of 20 or more animals (Scharff et al. 2001). Experienced local mole-rat

hunters have reported large family sizes of up to about 40–60 animals, but these claims remain unconfirmed (Burda and Kawalika 1993).

Morphological examination of animals captured in the field (Scharff et al. 2001) indicates that *F. mechowii* lives almost exclusively in monogamous families with only one breeding pair. Sichilima et al. (2008b) reported two simultaneously breeding females in 4 out of 32 families (16%) but did not further investigate the exact family group affiliation of the respective animals. Microsatellite analyses of two wild groups from Zambia also supported the assumption that a nonrelated founder pair and their offspring constitute a respective family group (Šumbera et al. 2012). The same study also demonstrated that following replacement of the reproductive male, the new breeder and its offspring may integrate into the existing group (Šumbera et al. 2012). A higher turnover rate of breeding males compared to females is typical for species of *Fukomys* (Caspar et al. 2021). This, in combination with the particularly extreme sexual dimorphism in *F. mechowii*, might point to males having to regularly fight off competitors that try to take over the reproductive female and burrow system they occupy (Caspar et al. 2021).

Reproductive behavior.—Mating in *Fukomys mechowii* is aseasonal and restricted to the breeding pair of a group (see "Ontogeny and Reproduction"). Currently, studies on reproductive behavior have only been conducted on captive animals. In the majority of cases when two unfamiliar mole-rats of opposite sex are placed together, courtship starts soon after the introduction (Scharff et al. 1999). Reproductive males stay faithful to their partner when presented with an unfamiliar female in the presence of the respective male's family, whereas nonbreeders eventually start copulating (Knafla 2008). The incest taboo of *F. mechowii* is probably based on individual recognition of family members mediated by body odors and not on reproductive suppression (Heth et al. 2002; see "Communication"). *Fukomys mechowii* is able to remember its siblings (and avoids mating with them) after a separation of more than 4 months (Bappert and Burda 2005).

Both sexes might initiate copulation (Burda and Kawalika 1993). In newly founded pairs, initiation by females prevails, whereas the opposite appears to be the case for established pairs (Scharff et al. 1999). When initially paired, partners engage in extensive cheek-rubbing and later anogenital sniffing (Scharff et al. 1999). Before copulation starts, the pair typically chases each other for a brief period of time while emitting cooing to squeaking vocalizations (Bennett and Aguilar 1995; Scharff et al. 1999; Bednářová et al. 2013). This behavior can be observed in both newly formed and established pairs. The female invites mating by presenting its rump to the male while raising the tail, often exhibiting a conspicuous twitching of the whole body. It eventually adopts lordosis, the male starts mounting, and intromission is achieved. The male executes one to two pelvic thrusts per second, the frequency of which increases toward ejaculation (Bennett and Aguilar 1995). The females terminate copulation by drawing away from the male. Before that, it sometimes emits a distinct squeal (Bennett and Aguilar 1995; Bednářová et al. 2013). Several mounting events (each typically between 5 and 15 s long) might closely follow each other (Scharff et al. 1999), intermitted by chasing bouts. In newly founded pairs, an initial phase of sustained sexual activity lasting up to 8 h has been observed (Scharff et al. 1999).

Communication.—The vocal repertoire of *Fukomys mechowii* consists of 14 true vocalizations and four sounds not produced by the larynx (Bednářová et al. 2013). Additionally, one seismic signal (of an unclear function) has been recorded which is produced when the animals beat their chests against the tunnel floor in situations of distress (Bednářová et al. 2013). Two of the mechanical sounds are produced when the animals chatter or grind their incisors, a behavior that probably occurs in all subterranean rodents. These sounds have often been observed in the context of aggressive encounters among bathyergids and were interpreted as an emphasis of the agonistic attitude of the emitter (Bednářová et al. 2013). However, *F. mechowii* chatters its teeth also under relaxed conditions, likely to sharpen the ever-growing incisors, and it may thus not always represent a communicative signal (Bednářová et al. 2013). Two further mechanically produced sounds (hiss and snort) are both related to exhalation of air and are produced during aggressive encounters (Bednářová et al. 2013). The 14 true vocalizations of *F. mechowii* are emitted in the contexts of contact, aggression, distress, mating, and alarm (Bednářová et al. 2013).

The loudest calls display frequencies below 5 kHz, and the fundamental frequency of most calls is below 1 kHz (Bednářová et al. 2013). Therefore, the vocalizations are in the range of best hearing for the species and are effectively propagated in the peculiar acoustic environment of the tunnel system (Lange et al. 2007; Gerhardt et al. 2017).

Little data exist on olfactory communication, and it is unclear to which extent social behavior is mediated by odors derived from such things as feces, urine, or male facial secretions. Urine of *F. mechowii* does not contain major urinary proteins (Hagemeyer et al. 2011). Family members of *F. mechowii* can recognize each other individually by means of anogenital odor. This has been demonstrated by habituation–discrimination tests during which the animals could discriminate smells from familiar siblings (Heth et al. 2002).

Miscellaneous behavior.—Under laboratory conditions in intact families, *Fukomys mechowii* rests on average 84% of the time (Dammann et al. 2011). Only nonreproductive females spend significantly less time resting (74%) and more time being active (locomotion: 20%; feeding: 6%) compared to males and reproductive females (Dammann et al. 2011). When resting, the mole-rats usually gather in groups to huddle, a behavioral strategy to conserve heat (Šumbera 2019). When within a huddling group, individual *F. mechowii* often stretch out their bodies. However, when sleeping alone, they curl up (Lövy et al. 2013).

Brain nuclei and related terminal networks supposedly controlling the sleep–wake cycles in *F. mechowii* are similar to those of other mole-rats and other rodent species in general (Bhagwandin et al. 2013). Two different chronotypes can be differentiated: some animals have rhythmic activity patterns with most activity occurring during the light phase while others are arrhythmic with no clear and predictable activity patterns (Bhagwandin et al. 2011). Rapid eye movement (REM) sleep and non-REM sleep as identified by electroencephalogram and electromyogram analyses of individually kept animals showed that the two chronotypes do not differ in their amount of REM sleep (Bhagwandin et al. 2011). However, the arrhythmic chronotype spent more time in a state of waking, whereas the rhythmic chronotype had longer non-REM sleep phases.

GENETICS

Diploid (2n) chromosome number in *Fukomys mechowii* is 40 (Macholán et al. 1993). This is the lowest chromosome number so far recorded among bathyergid taxa and diagnostic for the species (Ingram et al. 2004). All chromosomes are biarmed (NF = 80), with X chromosomes in females being heteromorphic at times (Macholán et al. 1993). Both the X and Y chromosomes are notably large due to translocation of several autosomal elements (Deuve et al. 2006).

The mitochondrial cytochrome-*b* and 12 S genes, and the nuclear intron 1 of the transthyretin gene (TTR) of *F. mechowii* have been used to infer its phylogenetic position among other *Fukomys* species (Faulkes et al. 1997; Ingram et al. 2004; Van Daele et al. 2007). These analyses indicate that *F. mechowii* belongs to the basal-most offshoot of the Southern Hemisphere *Fukomys* lineage, which also includes *F. bocagei* and *F. vandewoestijneae* (Faulkes et al. 1997; Ingram et al. 2004; Van Daele et al. 2007). This so-called *mechowii* clade presumably split from other *Fukomys*, in the late Pliocene (Van Daele et al. 2007; Bryja et al. 2018). However, molecular studies also demonstrated that giant mole-rats traditionally comprised under *F. mechowii* are paraphyletic and encompass an Eastern (Zambia, presumably eastern Democratic Republic of Congo) and a Western (Angola, western Democratic Republic of Congo) clade. The Eastern lineage is more closely related to the small-bodied *F. vandewoestijneae* than to the Western giant mole-rat clade (Visser et al. 2019; J. Krásová, in litt., University of South Bohemia, České Budějovice, Czech Republic, 24 May 2021). A proper taxonomic revision of the *mechowii* group acknowledging this issue is still pending and will have consequences for giant mole-rat nomenclature (see "Nomenclatural Notes"). Information on population genetics in *F. mechowii* is sparse. A considerable number of microsatellite loci in this species have been characterized (Burland et al. 2001; Ingram 2006; Bray et al. 2011).

CONSERVATION

The population status of *Fukomys mechowii* is considered "Least Concern" (LC) with a supposedly stable population trend by the International Union for Conservation of Nature Red List of Threatened Species (Maree and Faulkes 2016). This classification was justified by the wide distribution of the species, because it appears to be highly abundant in all regions from where it has been recorded, and the fact that it colonizes agricultural lands. However, there have been no systematic surveys to determine population densities of *F. mechowii*. It likely occurs in protected areas in Zambia, but no specific conservation measures have been taken so far. It is regularly hunted for its meat in rural areas (described in detail by Kawalika 2004), but probably without significant consequences for the integrity of local populations (Maree and Faulkes 2016).

REMARKS

Fukomys mechowii is an animal of great significance to the rural population of Zambia, for example, in the Kasama region. It is an important agricultural pest species, being responsible for local crop failure rates of up to 95% (Kawalika 2004). On the other hand, its meat is highly valued, being the only readily available source of animal protein in some areas. Furthermore, *F. mechowii* is relevant to local shamanist lore. Encountering a straying mole-rat is considered a bad omen (Kawalika 2004).

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LITERATURE CITED

Agrawal V.C. 1967. Skull adaptations in fossorial rodents. Mammalia 31:300–312.

Allen G.M. 1939. A checklist of African mammals. Bulletin of the Museum of Comparative Zoology at Harvard College 83:1–763.

American Society of Mammalogists. 2020. Mammal diversity database. http://www.mammaldiversity.org. Accessed 14 Apr 2020.

Ansell W.F.H. 1978. Mammals of Zambia. National Parks and Wildlife Service, Chilanga, Zambia.

Ansell W.F.H., Dowsett R.J. 1988. Mammals of Malawi – an annotated checklist and atlas. The Trendrine Press, St. Ives, United Kingdom.

Banke J., Mess A., Zeller U. 2001. Functional morphology of the rostral head region of *Cryptomys hottentotus* (Bathyergidae, Rodentia). In: Denys C., Granjon L., Poulet A., editors. African small mammals. IRD Editions. Colloques et Séminaires, Paris, France; p. 231–241.

Bappert M., Burda H. 2005. Forgotten siblings or how the long memory of the eusocial giant mole-rat *Cryptomys mechowi* (Rodentia: Bathyergidae) serves to maintain incest avoidance. Mammalian Biology 70(Suppl.):5.

Bednářová R., Hrouzková-Knotková E., Burda H., Sedláček F., Šumbera R. 2013. Vocalizations of the giant mole-rat (*Fukomys mechowii*), a subterranean rodent with the richest vocal repertoire. Bioacoustics 22:87–107.

Begall S., Berendes M., Schielke C.K.M., Henning Y., Laghanke M., Scharff A., van Daele P., Burda H. 2015. Temperature preferences of

African mole-rats (family Bathyergidae). Journal of Thermal Biology 53:15–22.
Begall S., Burda H., Šumbera R. 2018. Graumulle – *Cryptomys* und *Fukomys*. VerlagsKG Wolf, Magdeburg, Germany.
Begall S., Nappe R., Hohrenk L., Schmidt T.C., Burda H., Sahm A., Szafranski K., Dammann P. 2021. Life expectancy, family constellation and stress in giant mole-rats (*Fukomys mechowii*). Philosophical Transactions of the Royal Society of London, B: Biological Sciences 376:20200207.
Bennett N.C., Aguilar G.H. 1995. The reproductive biology of the Zambian mole-rat, *Cryptomys mechowi* (Rodentia: Bathyergidae). South African Journal of Zoology 30:1–4.
Bennett N.C., Aguilar G.H., Jarvis J.U.M., Faulkes C.G. 1994. Thermoregulation in three species of Afrotropical subterranean mole-rats (Rodentia: Bathyergidae) from Zambia and Angola and scaling within the genus *Cryptomys*. Oecologia 97:222–227.
Bennett N.C., Burda H. 2013. *Cryptomys mechowi*. In: Happold M., Happold D.C.D., editors. Mammals of Africa. 3. Rodents, hares and rabbits. Bloomsbury Publishing, London, United Kingdom; p. 659–660.
Bennett N.C., Faulkes C.G. 2000. African mole-rats: ecology and eusociality. Cambridge University Press, Cambridge, United Kingdom.
Bennett N.C., Molteno A.J., Spinks A.C. 2000. Pituitary sensitivity to exogenous GnRH in giant Zambian mole-rats, *Cryptomys mechowi* (Rodentia: Bathyergidae): support for the "socially-induced infertility continuum". Journal of Zoology 252:447–452.
Beolens B., Watkins M., Grayson M. 2009. The eponym dictionary of mammals. Johns Hopkins University Press, Baltimore, Maryland, USA.
Bhagwandin A., Gravett N., Bennett N.C., Manger P.R. 2013. Distribution of parvalbumin, calbindin and calretinin containing neurons and terminal networks in relation to sleep associated nuclei in the brain of the giant Zambian mole-rat (*Fukomys mechowii*). Journal of Chemical Neuroanatomy 52:69–79.
Bhagwandin A., Gravett N., Lyamin O.I., Oosthuizen M.K., Bennett N.C., Siegel J.M., Manger P.R. 2011. Sleep and wake in rhythmic versus arrhythmic chronotypes of a microphthalmic species of African mole rat (*Fukomys mechowii*). Brain Behavior and Evolution 78:162–183.
Bocage J.V.B. 1890. Les rats-taupes d'Angola. Jornal de Sciencias Mathematicas, Physicas e Naturaes, Serié 2, 1:269–277.
Brants A. 1827. Het geslacht der muizen door Linnaeus opgesteld: volgens de tegenswoordige toestand der wetenschap in familien, geslachten en soorten verdeeld. Akademische Buchdruckerei, Berlin, Prussia.
Bray T.C., Bennett N.C., Bloomer P. 2011. Low levels of polymorphism at novel microsatellite loci developed for bathyergid mole-rats from South Africa. Conservation Genetics Resources 3:221–224.
Bryja J., Konvičková H., Bryjová A., Mikula O., Makundi R., Chitaukali W.N., Šumbera R. 2018. Differentiation underground: range-wide multilocus genetic structure of the silvery mole-rat does not support current taxonomy based on mitochondrial sequences. Mammalian Biology 93:82–92.
Burda H., Honeycutt R.L., Begall S., Grütjen O., Scharff A. 2000. Are naked and common mole-rats eusocial and if so, why? Behavioral Ecology and Sociobiology 47:293–303.
Burda H., Kawalika M. 1993. Evolution of eusociality in the Bathyergidae. The case of the giant mole rats (*Cryptomys mechowi*). Naturwissenschaften 80:235–237.
Burgin C.J., Wilson D.E., Mittermeier R.A., Rylands A.B., Lacher T.E., Sechrest W. 2020. Illustrated mammals of the World. Volume 1: Monotremata to Rodentia. Lynx Edicions, Barcelona, Spain.
Burland T.M., Bishop J.M., O'Ryan C., Faulkes C.G. 2001. Microsatellite primers for the African mole-rat genus *Cryptomys* and cross-species amplification within the family Bathyergidae. Molecular Ecology Notes 1:311–314.
Caspar K.R., Müller J., Begall S. 2021. Effects of sex and breeding status on skull morphology in cooperatively breeding Ansell's mole-rats and an appraisal of sexual dimorphism in the Bathyergidae. Frontiers in Ecology and Evolution 9:638754.
Chimimba C.T., Sichilima A.M., Faulkes C.G., Bennett N.C. 2010. Ontogenetic variation and craniometric sexual dimorphism in the social giant mole-rat, *Fukomys mechowii* (Rodentia: Bathyergidae), from Zambia. African Zoology 45:160–176.
Cox P.G., Faulkes C.G., Bennett N.C. 2020. Masticatory musculature of the African mole-rats (Rodentia: Bathyergidae). PeerJ 8:e8847.
Dammann P., Šumbera R., Maßmann C., Scherag A., Burda H. 2011. Extended longevity of reproductives appears to be common in *Fukomys* mole-rats (Rodentia, Bathyergidae). PLoS One 6(4):e18757.
Deuve J.L., Bennett N.C., O'Brien P.C.M., Ferguson-Smith M.A., Faulkes C.G., Britton-Davidian, J., Robinson T.J. 2006. Complex evolution of X and Y autosomal translocations in the giant mole-rat, *Cryptomys mechowi* (Bathyergidae). Chromosome Research 14:681–691.
Ellerman J.R., Morrison-Scott T.C.S., Hayman, R.W. 1953. Southern African mammals 1758–1951: a reclassification. London (United Kingdom): Trustees of the British Museum, London, United Kingdom.
Faulkes C.G., Bennett N.C., Bruford M.W., O'Brien H.P., Aguilar G.H., Jarvis J.U.M. 1997. Ecological constraints drive social evolution in the African mole-rats. Proceedings of the Royal Society of London, B: Biological Sciences 264:1619–1627.
Faulkes C.G., Mgode G.F., Le Comber S.C., Bennett N.C. 2010a. Cladogenesis and endemism in Tanzanian mole-rats, genus *Fukomys*: (Rodentia Bathyergidae): a role for tectonics? Biological Journal of the Linnean Society 100:337–352.
Faulkes C.G., Sichilima A.M., Van Sandwyk J., Lutermann H., Bennett N.C. 2010b. Control of ovulation in female giant mole-rats, *Fukomys mechowii* (Rodentia: Bathyergidae). Journal of Zoology 282:64–74.
Faulkes C.G., Verheyen E., Verheyen W., Jarvis J.U.M., Bennett N.C. 2004. Phylogeographical patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae). Molecular Ecology 13:613–629.
Fitzinger L.J. 1867. Versuch einer natürlichen Anordnung der Nagethiere (Rodentia). Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche Classe. Abt. 1, Mineralogie, Botanik, Zoologie, Geologie und Paläontologie 55:453–515.
Francioli Y., Thorley J., Finn K., Clutton-Brock T.H., Zöttl M. 2020. Breeders are less active foragers than non-breeders in wild Damaraland mole-rats. Biology Letters 16:20200475.
Garcia Montero A., Burda H., Begall S. 2015. Chemical restraint of African mole-rats (*Fukomys* sp.) with a combination of ketamine and xylazine. Veterinary Anaesthesia and Analgesia 42:187–191.
Garcia Montero A., Vole C., Burda H., Malkemper E.P., Holtze S., Morhart M., Saragusty J., Hildebrandt T.B., Begall S. 2016. Non-breeding eusocial mole-rats produce viable sperm - spermiogram and functional testicular morphology of *Fukomys anselli*. PLoS One 11:e0150112.
Gerhardt P., Henning Y., Begall S., Malkemper E.P. 2017. Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency cut-offs. Journal of Experimental Biology 220:4377–4382.
Gippoliti S., Amori G. 2011. A new species of mole-rat (Rodentia, Bathyergidae) from the Horn of Africa. Zootaxa 2918:39–46.
Gomes Rodrigues H., Šumbera R., Hautier L. 2016. Life in burrows channeled the morphological evolution of the skull in rodents: the case of African mole-rats (Bathyergidae, Rodentia). Journal of Mammalian Evolution 23:175–189.
Gray J.E. 1843. List of the specimens of Mammalia in the collection of the British museum. George Woodfall and Son, London, United Kingdom.
Gray J.E. 1864. Notes on the species of sand-moles (*Georychus*). Proceedings of the Zoological Society of London 1864:123–125.
Hagemeyer P., Begall S., Janotova K., Todrank J., Heth G., Jedelsky P.L., Burda H., Stopka P. 2011. No major urinary proteins (MUPs) to help signal individual identity or biological state in some subterranean rodents. Journal of Chemical Ecology 37:687–694.

Henning Y., Mladěnková N., Burda H., Szafranski K., Begall S. 2018. Retinal S-opsin dominance in Ansell's mole-rats (*Fukomys anselli*) is a consequence of naturally low serum thyroxine. Scientific Reports 8:4337.

Heth G., Todrank J., Burda H. 2002. Individual odor similarities within colonies and across species of *Cryptomys* mole rats. Journal of Mammalogy 83:569–575.

Hinton M.A.C. 1921. Some new African mammals. Annals and Magazine of Natural History, Series 9, 40:368–373.

Hoesch W., von Lehmann E. 1956. Zur Säugetier-Fauna Südwestafrikas. Bonner Zoologische Beitraege 7:8–57.

Honeycutt R.L., Allard M.W., Edwards S.V., Schlitter D.A. 1991. Systematics and evolution of the family Bathyergidae. In: Sherman P.W., Jarvis J.U.M., Alexander R.D., editors. The biology of the naked mole-rat. Princeton University Press, Princeton, New Jersey, USA; p. 45–65.

Houslay T.M., Vullioud P., Zöttl M., Clutton-Brock T.H. 2020. Benefits of cooperation in captive Damaraland mole-rats. Behavioral Ecology 31:711–718.

Illiger J.K.W. 1811. Prodromus systematis mammalium et avium additis terminis zoographicis utriusque classis, eorumque versione germanica. C. Salfeld, Berlin, Germany.

Ingram C.M. 2006. The evolution of nuclear microsatellite DNA markers and their flanking regions using reciprocal comparisons within the African mole-rats (Rodentia: Bathyergidae). Ph.D. dissertation, Texas A&M University, College Station, Texas, USA.

Ingram C.M., Burda H., Honeycutt R.L. 2004. Molecular phylogenetics and taxonomy of the African mole-rats, genus *Cryptomys* and the new genus *Coetomys* Gray, 1864. Molecular Phylogenetics and Evolution 31:997–1014.

Kawalika M. 2004. Rodents of Ndola (Copperbelt Province, Zambia). Ph.D. dissertation, University of Duisburg-Essen, Essen, Germany.

Kawalika M., Burda H. 2007. Giant mole-rats, *Fukomys mechowii*, 13 years on the stage. In: Begall S., Burda H., Schleich C.E., editors. Subterranean rodents – news from underground. Springer-Verlag, Berlin, Germany; p. 205–219.

Kingdon J. 1974. East African mammals—an atlas of evolution in Africa, vol. II, part B (Hares and Rodents). University of Chicago Press, Chicago, Illinois, USA.

Kisasa R., Palata J.C., Pwema V. 2004. Premiers essais d'élevage du rat-taupe (*Cryptomys mechowi*, Rongeur: Bathyergidae) à Kinshasa, République démocratique du Congo. Tropicultura 22:180–184.

Knafla A. 2008. Sozialer stress und sexualverhalten bei eusozialen graumullen. State examination thesis, University of Duisburg-Essen, Essen, Germany.

Kock D., Ingram C.M., Frabotta L.J., Honeycutt R.L., Burda H. 2006. On the nomenclature of Bathyergidae and *Fukomys* n. gen. (Mammalia: Rodentia). Zootaxa 1142:51–55.

Kott O., Moritz R.E., Šumbera R., Burda H., Němec P. 2014. Light propagation in burrows of subterranean rodents: tunnel system architecture but not photoreceptor sensitivity limits light sensation range. Journal of Zoology 294:67–75.

Kott O., Šumbera R., Nemec P. 2010. Light perception in two strictly subterranean rodents: life in the dark or blue? PLoS One 5(7):e11810.

Kotzé S.H., Van Der Merwe E.L., Bennett N.C., O'Riain M.J. 2010. The comparative anatomy of the abdominal gastrointestinal tract of six species of African mole-rats (Rodentia, Bathyergidae). Journal of Morphology 271:50–60.

Krehbiehl M. 2010. Anatomie der rostralen und caudalen Tasthaare beim Sambischen Riesengraumull (*Fukomys mechowii*) – Sinushaare oder Leithaare? Ph.D. dissertation, Veterinary College Hanover, Hanover, Germany.

Kundu S., Faulkes C.G. 2004. Patterns of MHC selection in African mole-rats, family Bathyergidae: the effects of sociality and habitat. Proceedings of the Royal Society of London, B: Biological Sciences 271:273–228.

Kverková K., Bělíková T., Olkowicz S., Pavelková Z., O'Riain J., Šumbera R., Burda H., Bennett H.C., Němec R. 2018. Sociality does not drive the evolution of large brains in eusocial African mole-rats. Scientific Reports 8:1–14.

Landry S.O. 1957. Factors affecting the procumbency of rodent upper incisors. Journal of Mammalogy 38:223–234.

Lange S., Burda H., Wegner R.W., Dammann P., Begall S., Kawalika M. 2007. Living in a "stethoscope": burrow-acoustics promote auditory specializations in subterranean rodents. Naturwissenschaften 94:134–138.

Lesson R.P. 1826. Voyage autour du monde: exécuté par ordre du roi, sur la corvette de Sa Majesté, la Coquille, pendant les années 1822, 1823, 1824, et 1825. Tome I, part I. Imprimerie de Firmin Didot, Paris, France.

Lövy M., Šklíba J., Šumbera R. 2013. Spatial and temporal activity patterns of the free-living giant mole-rat (*Fukomys mechowii*), the largest social bathyergid. PLoS One 8(1):e55357.

Macholán M., Burda H., Zima J., Misek I., Kawalika M. 1993. Karyotype of the giant mole-rat, *Cryptomys mechowi* (Rodentia, Bathyergidae). Cytogenetic and Genome Research 64:261–263.

Maree S., Faulkes C.G. 2016. *Fukomys mechowi*. The IUCN Red List of Threatened Species 2016: e.T5756A22185092. https://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T5756A22185092.en. Accessed 1 Aug 2021.

Matschei C., Bäthe R. 2012. Riesengraumull und Mashona-Graumull – zwei Sandgräberarten. Mitteilungen der Bundesarbeitsgruppe (BAG) Kleinsäuger e.V 2:10–14.

McIntosh A.F., Cox P.G. 2016. Functional implications of craniomandibular morphology in African mole-rats (Rodentia: Bathyergidae). Biological Journal of the Linnean Society 117:447–462.

Monadjem A., Taylor P.J., Denys C., Cotterill F.P. 2015. Rodents of sub-Saharan Africa: a biogeographic and taxonomic synthesis. de Gruyter, Berlin, Germany.

Morlok W.F. 1983. Vergleichend- und funktionell-anatomische Untersuchungen an Kopf, Hals und Vorderextremität subterraner Nagetiere (Mammalia, Rodentia). Courier Forschungsinstitut Senckenberg 64:1–237.

Ogilby W. 1838. On a collection of Mammalia procured by Captain Alexander during his journey into the country of the Damaras. Proceedings of the Zoological Society of London 1838:5–15.

Okrouhlík J., Burda H., Kunc P., Knížková I., Šumbera R. 2015. Surprisingly low risk of overheating during digging in two subterranean rodents. Physiology & Behavior 138:236–241.

Oliveriusová L., Němec P., Králová Z., Sedláček F. 2012. Magnetic compass orientation in two strictly subterranean rodents: learned or species-specific innate directional preference? Journal of Experimental Biology 215:649–654.

Palata-Kabudi J.C., Kisasa R., Poso O., Pwema V. 2005. Regime alimentaire du rat-taupe geant, *Cryptomys mechowi*, sur le plateau de Bateke, Republique Democratique du Congo. Mammalia 69:445–450.

Peichl L., Němec P., Burda H. 2004. Unusual cone and rod properties in subterranean African mole-rats (Rodentia, Bathyergidae). European Journal of Neuroscience 19:545–558.

Peters W. 1881. Über die von Herrn Major v. Mechow von seiner letzten Expedition nach Westafrika mitgebrachten Säugetiere. Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin 1881:131–134.

Prochel J., Begall S., Burda H. 2013. Morphology of the carpal region in some rodents with special emphasis on hystricognaths. Acta Zoologica 95:220–238.

Sahm A., Bens M., Henning Y., Vole C., Groth M., Schwab M., Hoffmann S., Platzer M., Szafranski K., Dammann P. 2018. Higher gene expression stability during aging in long-lived giant mole-rats than in short-lived rats. Aging (Albany NY). 10:3938–3956.

Sahm A., Platzer M., Koch P., Henning Y., Bens M., Groth M., Burda H., Begall S., Ting S., Goetz M., et al. 2021. Increased longevity due to sexual activity in mole-rats is associated with transcritpional changes in the HPA stress axis. eLife, 10:e57843. doi: 10.7554/eLife.57843.

Scharff A., Begall S., Grütjen O., Burda H. 1999. Reproductive characteristics and growth of Zambian giant mole-rats, *Cryptomys mechow* (Rodentia: Bathyergidae). Mammalia 63:217–230.

Scharff A., Burda H., Tenora F., Kawalika M., Barus V. 1997. Parasites in social subterranean Zambian mole-rats (*Cryptomys* spp., Bathyergidae, Rodentia). Journal of Zoology 241:571–577.

Scharff A., Locker-Grütjen O., Kawalika M., Burda H. 2001. Natural history of the giant mole-rat, *Cryptomys mechowi* (Rodentia: Bathyergidae), from Zambia. Journal of Mammalogy 82:1003–1015.

Schmitt M., Bappert M.T., Kruger C., Burda H. 2009. The mole-rat's pseudo thumb. Mammalian Biology 74(Suppl.):23.

Sichilima A.M., Bennett N.C., Faulkes C.G., Le Comber S.C. 2008a. Evolution of African mole-rat sociality: burrow architecture, rainfall and foraging in colonies of the cooperatively breeding *Fukomys mechowii*. Journal of Zoology 275:276–282.

Sichilima A.M., Faulkes C.G., Bennett N.C. 2008b. Field evidence for aseasonality reproduction and colony size in the Afrotropical giant mole-rat, *Fukomys mechowii* (Rodentia: Bathyergidae). African Zoology 43:144–149.

Šklíba J., Jirků M., Šumbera R. 2016. Burrow systems of mole-rats as refuges for frogs in the Miombo woodlands of south-east Africa. Journal of Tropical Ecology 32:158–161.

Šumbera R. 2019. Thermal biology of a strictly subterranean mammalian family, the African mole-rats (Bathyergidae, Rodentia) - a review. Journal of Thermal Biology 79:166–189.

Šumbera R., Mazoch V., Patzenhauerova H., Lövy M., Skliba J., Bryja J., Burda H. 2012. Burrow architecture, family composition and habitat characteristics of the largest social African mole-rat: the giant mole-rat constructs really giant burrow systems. Acta Theriologica 57:121–130.

Thomas O. 1906. New African mammals of the genera *Cercopithecus*, *Scotophilus*, *Miniopterus*, *Crocidura*, *Georychus*, and *Heliophobius*. Annals and Magazine of Natural History, Series 7, 17:173–179.

Thomas O. 1917. Notes on *Georychus* and its allies. Annals and Magazine of Natural History, Series 8, 20:441–444.

Thomas O., Wroughton R.C. 1905. On a second collection of mammals obtained by Dr. W. J. Ansorge in Angola. Annals and Magazine of Natural History, Series 7, 16:169–178.

Trouessart E.L. 1881. Catalogue des mammifères vivants et fossiles: Ordre des Rongeurs. Bulletin de la Société d'Études Scientifiques d'Angers 10:58–212.

Van Daele P.A.A.G., Blonde P., Stjernstedt R., Adriaens D. 2013. A new species of African mole-rat (*Fukomys*, Bathyergidae, Rodentia) from the Zaire-Zambezi watershed. Zootaxa 3636:171–189.

Van Daele P.A.A.G., Dammann P., Meier J.L., Kawalika M., Van De Woestijne C., Burda H. 2004. Chromosomal diversity in mole-rats of the genus *Cryptomys* (Rodentia: Bathyergidae) from the Zambezian region: with descriptions of new karyotypes. Journal of Zoology 264:317–326.

Van Daele P.A.A.G., Herrel A., Adriaens D. 2008. Biting performance in teeth-digging African mole-rats (*Fukomys*, Bathyergidae, Rodentia). Physiological and Biochemical Zoology 82:40–50.

Van Daele P.A.A.G., Verheyen E., Brunain M., Adriaens D. 2007. Cytochrome b sequence analysis reveals differential molecular evolution in African mole-rats of the chromosomally hyperdiverse genus *Fukomys* (Bathyergidae, Rodentia) from the Zambezian region. Molecular Phylogenetics and Evolution 45:142–157.

Van Wassenbergh S., Heindryckx S., Adriaens D. 2017. Kinematics of chisel-tooth digging by African mole-rats. Journal of Experimental Biology 220:4479–4485.

Visser J.H., Bennett N.C., Jansen van Vuuren B. 2019. Phylogeny and biogeography of the African Bathyergidae: a review of patterns and processes. PeerJ 7:7730.

Wagner J.A. 1843. Die Säugthiere in Abbildungen nach der Natur, mit Beschreibungen. Supplementband. Dritte Abtheilung: Die Beutelthiere und Nager (erster Abschnitt). Voß'sche Buchhandlung, Erlangen, Bavaria.

Wallace E.D., Bennett N.C. 1998. The colony structure and social organization of the giant Zambian mole-rat, *Cryptomys mechowi*. Journal of Zoology 244:51–61.

Willingstorfer W.J., Burda H., Winckler J. 1998. Ovarian growth and folliculogenesis in breeding and nonbreeding females of a social rodent, the Zambian common mole-rat, *Cryptomys* sp. Journal of Morphology 237:33–41.

Winkler A.J., Denys C., Avery D.M. 2010. Rodentia. In: Werdelin L., Sanders W.J., editors. Cenozoic mammals of Africa. University of California Press, Berkeley, California, USA; p. 263–304.

Zelová J., Šumbera R., Okrouhlík J., Burda H. 2010. Cost of digging is determined by intrinsic factors rather than by substrate quality in two subterranean rodent species. Physiology & Behavior 99:54–58.

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Fukomys anselli (Rodentia: Bathyergidae)

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Abstract: *Fukomys anselli* (Burda, Zima, Scharff, Macholán, and Kawalika 1999) is a bathyergid commonly known as Ansell's mole-rat. This tooth-digging subterranean rodent lives in cooperatively breeding family groups. It is a small-bodied representative of the genus *Fukomys*, whose members are distributed in sub-Saharan Africa. *Fukomys anselli* is endemic to central Zambia and occurs in mesic woodland and agricultural areas. In rural settings, *F. anselli* is frequently hunted for consumption, but it is unclear whether this significantly impacts its population integrity. *Fukomys anselli* is listed as "Least Concern" (LC) with a declining population trend by the International Union for Conservation of Nature Red List of Threatened Species.

Key words: eusociality, mole-rat, sub-Saharan Africa, subterranean rodent, tooth-digger

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Fukomys anselli Burda, Zima, Scharff, Macholán, and Kawalika 1999

Ansell's Mole-rat

Cryptomys anselli Burda, Zima, Scharff, Macholan, and Kawalika 1999:36. Type locality "Court of the Chainama Hills Golf Club in the north-eastern part of Lusaka, Zambia."
Coetomys anselli: Ingram, Burda, and Honeycutt 2004:1008. Name combination.
Fukomys anselli: Kock, Ingram, Frabotta, Honeycutt, and Burda 2006:54. First use of current name combination.

Fig. 1.—Adult female *Fukomys anselli* photographed in captivity at the Department of General Zoology, University of Duisburg-Essen, Essen, Germany. Note the cylindrical body shape, microphthalmy, and procumbent incisors. The characteristic white dorsal head patch is not visible. Photograph by Sarah M. Wilms used with permission.

CONTEXT AND CONTENT. Order Rodentia, suborder Hystricomorpha, infraorder Hystricognathi, family Bathyergidae, subfamily Bathyerginae, genus *Fukomys*. No subspecies of *F. anselli* are recognized.

NOMENCLATURAL NOTES. The genus name comprises the term "fuko" which derives from mfuko, a vernacular name for mole-rat in certain Zambian Bantu languages such as Bemba and Kaonde, and *mys* which translates to mouse from ancient Greek (Kock et al. 2006). The latter is a common suffix of scientific names given to rodents. The epitheton *anselli* honors the British zoologist William Frank Harding Ansell (1923–1996) who was a specialist in mammals of the Zambezian region (Burda et al. 1999). Trivial names of the species are Ansell's mole-rat and Zambian mole-rat (Dando and Van Daele 2020). However, as numerous mole-rat species are found in the Republic of Zambia, including other endemics, the latter name is misleading. Furthermore, Zambian mole-rat is also in use as a trivial name for the congeneric species *F. amatus* (Wroughton, 1907).

Van Daele et al. (2007) remarked that *Cryptomys molyneuxi* Chubb, 1908, might be a senior synonym of *F. anselli*. However, Burda et al. (1999) rejected this notion based on biogeographical considerations and instead suggested that *C. molyneuxi* is a junior synonym of *F. amatus*. Before *F. anselli* was formally described, mole-rats of respective populations from the Lusaka region were at times also erroneously reported as *Cryptomys* (*hottentotus*) *amatus* (= *Fukomys amatus*—e.g., Bennett et al. 1994; Faulkes et al. 1997; see Burda et al. 1999).

DIAGNOSIS

Robust species assignment of most small-bodied (< 200 g) Zambian mole-rats, including *Fukomys anselli*, can only be achieved based on karyotyping (*F. anselli*: 2n = 68) or DNA sequence markers. *Fukomys anselli* is essentially indistinguishable from the parapatric Kafue mole-rat (*F. kafuensis*) based on gross morphological grounds. However, the former appears to consistently exhibit a thicker walled infraorbital foramen than the latter (Burda et al. 1999), and there are subtle species-specific morphological characters of the middle ear ossicles distinguishing them as well (Lange and Burda 2005). Nevertheless, *F. anselli* can be readily identified on the basis of its unique karyotype (compare *F. kafuensis*: 2n = 58).

GENERAL CHARACTERS

Fukomys anselli is a small African mole-rat, with adults displaying uniformly short, primarily ochre-brown pelage (Fig. 1). However, most, though not all, individuals display a conspicuous white marking on the forehead (Bennett and Burda 2013). This head spot is highly variable in both size and shape (Burda et al. 1999). It rarely extends to the rhinarium, eyes, or cranial portions of the neck and almost never spreads caudally along the spine as is often found in the Micklem's mole-rat (*F. micklemi*) and the Damaraland mole-rat (*F. damarensis*). Head patches are present at birth and retain their shape throughout life. However, there is a pronounced change of general fur coloration during ontogeny. The pups' pelage is typically dark gray to black at birth, slowly takes on a brown hue after weaning, and eventually turns ochre when attaining sexual maturity (Burda et al. 1999; see "Ontogeny and Reproduction"). The corners of the mouth in some adult males are framed in a dark rusty-brown color. These markings result from waxy glandular secretions which agglutinate the pelage in the perioral region and have been linked to reproductive status in some studies (Sichilima et al. 2011; Šklíba et al. 2012), an association we have not observed in captive animals. Apart from that, no sexual dichromatism is observable. Hands and feet lack fur and are instead dorsally covered and fringed by sparse pale hairs. Similarly, the short tail is naked except for white caudal bristles (guard hairs). The eyelids, rhinarium, and the immediate area surrounding the external ear opening are naked. As with all bathyergids, *F. anselli* lacks auricles. Two pairs of pectoral and one pair of inguinal nipples are present which are visible through the pelage in reproductive females (Burda 1989; Bennett and Burda 2013).

The general habitus of *F. anselli* is typical for the genus *Fukomys*. Body size, as approximated by body mass, is sexually dimorphic and may vary with reproductive status in the wild (Šklíba et al. 2012). Generally, higher mean body weights (± *SD*) for males (96.1 g ± 14.7, range: 80–145, *n* = 40) than for females (79.1 g ± 12.7, range: 65–122, *n* = 100) were reported for another sample of wild-caught *F. anselli*, from unspecified localities and without differentiation into reproductive status groups (Bennett and Burda 2013). In a sample of individuals captured 15 km west of Lusaka, the mean mass (± *SE*; range; *n*) of adult males was found to be 63.0 ± 18.3 g (36.7–110.3; 87), whereas that of adult females was 52.9 ± 11.8 g (35.1–77.8; 86—Sichilima et al. 2011). Additionally, nonpregnant reproductive females were found to show a higher body mass (mean: 62.3 g; range: 42.3–83.3; *n* = 17) than nonreproductive females (mean: 45.0 g; range: 35.1–59.5; *n* = 50) in the same study. A similar pattern was reported for a smaller sample of males captured at unspecified localities (De Bruin et al. 2012). Mean body mass (± *SD*) of reproductive males (81.4 ± 13.7 g, *n* = 18) was significantly higher than that of nonreproductive males (39.8 ± 19.0 g, *n* = 49). Reproductive females of the same sample also displayed exceedingly greater mean body masses (63.9 ± 11.4 g, *n* = 19) than nonreproductive females (33.5 ± 11. 8 g, *n* = 64—De Bruin et al. 2012). However, reproductive status assignment in these two studies is questionable. The authors regarded individuals ≥ 35 g as adults, whereas data from captive animals indicate that adulthood is reached at roughly two times this body mass (Begall and Burda 1998; see "Ontogeny and Reproduction"). This practice skews the results toward lower weights in nonreproductive animals, as some immature individuals will invariably be counted as adult nonreproductive individuals because diagnostic traits of breeders are missing. Furthermore, available data from captive *F. anselli* do not suggest that breeders grow significantly larger than fully adult nonreproductive animals of the same sex (Caspar et al. 2021).

Additional standard body measurements (mm; mean with *SD* and range presented in parentheses) for 20 male and 30 female specimens of *F. anselli*, respectively, derived from unspecified native localities (Bennett and Burda 2013) were: head–body length, 121.5 (10.9, 109–135), 119.3 (8.0, 108–132); tail length, 17.9 (1.9, 15.6–21.7), 18.3 (2.3, 13.9–22.9); length of hind foot, 22.6 (3.1, 21.8–25.8), 23.3 (0.9, 21.8–25.2).

The skull of *F. anselli* (Fig. 2) exhibits the morphology typical of the genus and resembles that of other tooth-digging bathyergids (see "Form and Function"). It displays hystricognathy (Gomes Rodrigues et al. 2016) and hystricomorphy, but the small infraorbital foramen, as characteristic for mole-rats of the *Cryptomys* and *Fukomys* genera, is only penetrated by a rudimentary extension of the zygomaticomandibularis muscle (Van Daele et al. 2008). The infraorbital foramen is thick-walled and its form ranges from elliptical to drop-shaped (Burda et al. 1999). In line

Fig. 2.—Dorsal, ventral, and lateral views of the skull of an adult male *Fukomys anselli* from the research collection of the Department of General Zoology, University of Duisburg-Essen, Essen, Germany. Greatest length of skull is 36.42 mm. Photographs by KRC.

with the mole-rats' microphthalmy, the bony orbit is reduced. Sexual dimorphism in the skull is pronounced. Males display notably larger skulls (10% wider and 4.5% longer) than females relative to their body size (Caspar et al. 2021). The facial portion of the skull is hypertrophied, and exhibits widened zygomatic arches in males compared to females. These traits among others have been interpreted as adaptions for intrasexual combat in male *F. anselli* (Caspar et al. 2021). The crania of reproductive individuals do not differ from those of same-sex nonreproductive individuals (Caspar et al. 2021).

Basic cranial measurements (mm, mean with *SD*, and range presented in parentheses) of 10 males and 10 females, respectively, from unspecified areas in Zambia (Bennett and Burda 2013) were: greatest skull length, 33.9 (1.9, 29.0–38.8) and 31.8 (1.7, 29.8–34.7); greatest skull width, 26.5 (2.3, 22.6–30.0) and 23.9 (1.8, 22.1–26.1); length of toothrow (P4–M3), 6.1 (0.3, 5.6–6.9) and 6.0 (0.4, 5.4–6.8). Additionally, linear measurements from the crania and mandibles of 40 captive *F. anselli* are available (Caspar et al. 2021).

Dentition is typical of the genus *Fukomys*. Each jaw quadrant carries one incisor, one molariform premolar, and three molars. Incisors do not have grooves and the roots extend caudally beyond the molar alveoli (Honeycutt et al. 1991). The single premolar is larger than the molars, which decrease in size from anterior to posterior (Honeycutt et al. 1991). Molars are highly simplified and appear as enamel cylinders enclosing a dentine core. Traces of reentrant folds may be observable on the labial molar surface (Honeycutt et al. 1991). Although the incisors are hypertrophied in males compared to females, there is no sexual dimorphism apparent in the cheek dentition (Caspar et al. 2021).

DISTRIBUTION

Fukomys anselli is endemic to central Zambia, its range encompassing the nation's capital Lusaka, its type locality (Fig. 3). It is recorded in the Lusaka, Central, and Southern provinces (Bennett and Burda 2013). In the south part of the range, its distribution is commonly assumed to be restricted by the Kafue River and most likely also by the Zambezi River (Bennett and Burda 2013). However, according to Dando and Van Daele (2020), *F. anselli* also occurs south of the Kafue River in the northern part of the Southern Province of Zambia. Respective animals were diagnosed as *F. anselli* based on cytochrome-*b* sequence data (P. Van Daele, in litt., University of Ghent, Ghent, Belgium, 6 July 2020; but see "Molecular genetics" on the utility of this method to diagnose the species). In general, the range boundaries of *F. anselli* are not well defined. It is primarily found within a radius of about 100 km around Lusaka (Bennett and Burda 2013) but has also been recorded from Kaindu, and several other sites in the western Central Province (Van Daele et al. 2004). Whether its range is continuous remains to be determined. North of Lusaka, small species of *Fukomys* (probably *F. anselli*) extend into the Kabwe area, where they live sympatrically with the giant mole-rat (*F. mechowii*—Kawalika 2004). The entire range exhibits temperate subtropical climate and is situated in the Southern African high plateau region (ca. 1,300 m elevation—Šklíba et al. 2012). Mean annual precipitation in the area is about 820 mm (Dando and Van Daele 2020).

Principally, *F. anselli* inhabits the seasonal Miombo woodlands abundant in the Zambezi area, but it is also found in more open habitats and wetlands (dambo—Dando and Van Daele 2020). In addition, it is frequently encountered on agricultural lands (Scharff 1998; Sichilima et al. 2011) and ventures well into the suburbs of Lusaka (Burda et al. 1999).

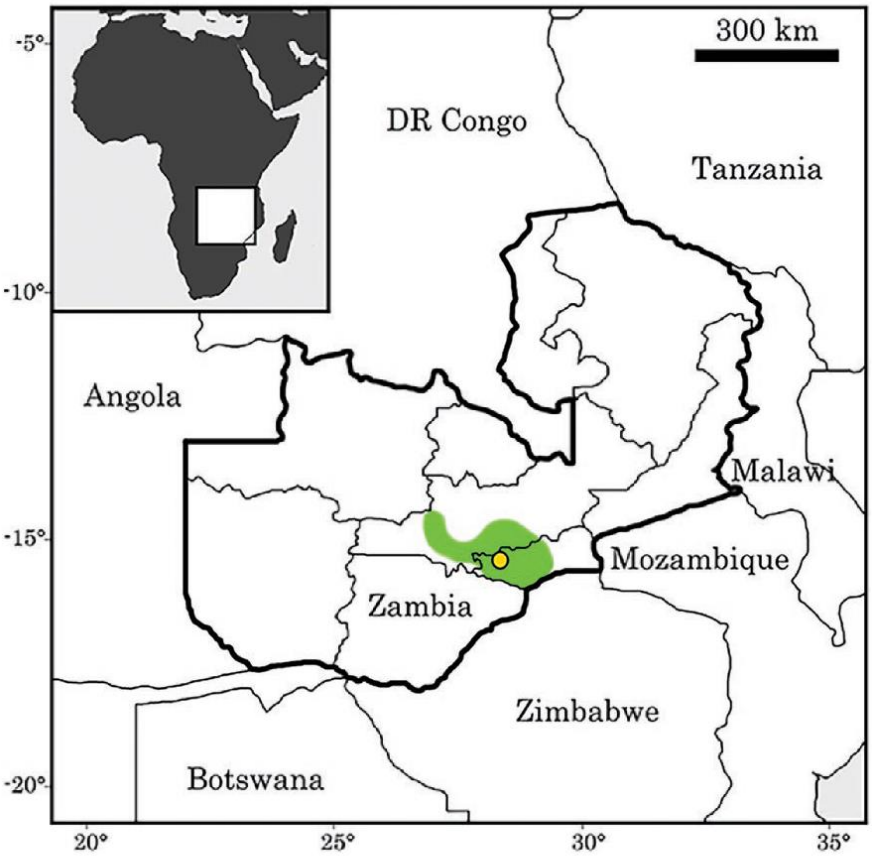

Fig. 3.—Geographic distribution of *Fukomys anselli*. The yellow dot highlights the location of Zambia's capital Lusaka, the species' type locality. Range boundaries are largely conjectural and based on Dando and Van Daele (2020).

FOSSIL RECORD

African mole-rats (Bathyergidae) have long been considered to be an ancient group of hystricomorph rodents. Early molecular studies supported this view by placing their emergence into the early Eocene, about 45 Ma (Faulkes et al. 2004; Ingram et al. 2004). However, a more recent analyses dated the origin of the clade to the early Miocene, about 21 Ma (Bryja et al. 2018). The oldest fossil remains of bathyergids also date to the early Miocene and are assigned to genera which cannot be securely placed in respect to crown-group taxa (Winkler et al. 2010). Current fossil evidence indicates that the family was restricted to sub-Saharan Africa since its emergence. The sister genera *Cryptomys* and *Fukomys* represent the most recent and most successful radiation of crown-group bathyergids. Given the topology of the bathyergid family tree and distribution patterns of extant genera, the *Cryptomys–Fukomys* clade was suggested to have originated in Africa south to the Kalahari Desert (Faulkes et al. 2004). The Palaeo-Zambezi river could have acted as a barrier separating ancestral populations of these genera (Ingram et al. 2004). Conflicting molecular data suggest the clade originated either in the middle Miocene, 11–17 Ma (Faulkes et al. 2004; Ingram et al. 2004), or in the latest Miocene, 6 Ma (Bryja et al. 2018). The oldest known fossils of small bathyergids resembling modern *Cryptomys* and *Fukomys* date to the early Pliocene of South Africa (Winkler et al. 2010). Problematically, an assignment of fossils to either genus is not possible, because both are currently only distinguished based on ethology, karyology, and molecular data (Kock et al. 2006; Monadjem et al. 2015). Although extant species of *Cryptomys* are restricted to South Africa and southern Zimbabwe, species of the genus *Fukomys* colonized diverse habitats of varying humidity, primarily west to the African Rift Valley, as far north as into the northern tropical savannahs (Faulkes et al. 2010). However, fossil material that could potentially be assigned to *Fukomys* (but was originally classified as *Cryptomys*) is sparse (Monadjem et al. 2015), not referable to extant species, and does not elucidate phylogeographic patterns within the genus.

FORM AND FUNCTION

As with all African mole-rats, *Fukomys anselli* exhibits a strictly subterranean lifestyle and displays striking anatomical specializations to life underground (Honeycutt 2017) which poses specific demands on respiration, thermoregulation, and sensory systems (Begall et al. 2018). Its body is cylindrical, its extremities and tail are short, the testes are abdominal, eyes are small, pinnae are missing, and the ear is exclusively sensitive to comparatively low frequencies. All these characters together represent an adaptive syndrome to life underground (Burda 2006), though they also can occur separately in epigeic mammals. The skin of all African mole-rats is only loosely attached to the underlying musculature by connective tissue (Begall et al. 2018). Thus, it can be widely displaced from the rump, which reduces friction on the body when moving in narrow underground tunnels. The reticular dermis of *F. anselli* contains a remarkably dense network of elastic fibers that are missing, for instance, in the guinea-pig (*Cavia porcellus*—Hesselmann 2010). The velvety, evenly short fur brushes easily in both directions and thus facilitates locomotion in the burrow system (Kingdon 1974). Well-innervated guard hairs, probably enhancing tactile sensing, cover the entire body (Krehbiehl 2010). The density of guard hairs is especially high at the tail, allowing the animal to move backwards with the same speed and ease as it moves forward (Krehbiehl 2010).

When burrowing, soil is initially loosened by the incisors. Accordingly, *F. anselli*, as most bathyergids, employs a chisel-tooth mediated mode of digging (McIntosh and Cox 2016a). As typical for subterranean rodents, hairy lip-folds close medially behind the procumbent incisors, even at full gape, thereby preventing ingestion of dirt during the digging process (Banke et al. 2001). To avoid obstruction of the airways, the nostrils can be sealed as well (Banke et al. 2001). Loosened soil is pushed below the rump by the forelimbs and is dispensed posteriorly by the hindlimbs (Kingdon 1974). To allow for efficient shoveling, both forefeet and hind feet are laterally widened by a sesamoid bone (prepollex and prehallux, respectively—Schmitt et al. 2009). Furthermore, the os lunatum and os scaphoideum of the carpus are fused, which might increase the stability of the palm when digging (Prochel et al. 2013). While the forefeet display pointed claws, the ones of the hind feet are dorsoventrally flattened and appear nail-like, assisting in effectively dispensing loosened soil (Kingdon 1974). The shoulder girdle is translocated cranially, compared to epigeic rodents, and exhibits ossified mesoscapular segments, situated between the shoulder blades and clavicles. Both characters are associated with a burrowing lifestyle and are frequently observed in different subterranean mammal clades (Morlok 1983). The angulus caudalis of the scapula is elongated to enlarge the attachment site for the teres major muscle, a forelimb retractor that facilitates digging (Morlok 1983). However, most important for burrowing are the adaptations found in the animals' skull and dentition.

Compared to its body size, the skull of *F. anselli* is notably large and its jaws are elongated, permitting a wide gape (Morlok 1983; McIntosh and Cox 2016b). The mandible is highly mobile due to an unfused mandibular symphysis and enlarged, flattened glenoid fossae (Gomes Rodrigues et al. 2016). Similar to other chisel-tooth digging rodents, the skull is deep and broad with widened zygomatic arches for supporting the substantially hypertrophied jaw musculature (Gomes Rodrigues et al. 2016; McIntosh and Cox 2016a). The relative size of the temporalis muscle is increased, allowing sustained high bite forces at the wide gapes (approaching 70°) that typically occur during tooth-digging (McIntosh and Cox 2016b; Van Wassenbergh et al. 2017). Relative to body mass, *Fukomys* mole-rats display the second most massive jaw musculature of all subterranean rodents, being surpassed only by the naked mole-rat (*Heterocephalus glaber*—Morlok 1983).

As typical for tooth-digging rodents, *F. anselli* shows pronounced upper incisor procumbency that facilitates digging while reducing friction on the nasal region (Agrawal 1967). However, contrary to some claims (e.g., Landry 1957), procumbency in the genus *Fukomys* is not notably expressed in comparison to most other bathyergids (McIntosh and Cox 2016a). The roots of both the upper and lower incisors in *F. anselli* are remarkably deep and extend caudally beyond the respective molar rows, a character uniquely shared by tooth-digging bathyergids among rodents (Ellerman 1940). The extreme depth of the tooth alveoli could serve to increase mechanical resilience and facilitate force dissipation in the skull (Landry 1957). Other closely related *Fukomys* species are among the mammal species with the highest relative bite force (Van Daele et al. 2008).

Thermoneutrality of *F. anselli* has been determined to be dependent on reproductive status, with the thermoneutral zone of breeders being elevated (28–33°C) compared to nonreproductive animals (26–30°C—Schielke et al. 2017). Mean body temperature (± *SD*) at thermoneutrality is on average 35 ± 1.1°C and 36.1 ± 0.9°C at ambient temperatures (T_a) of 30°C and 32.5°C, respectively. At lower ambient temperatures, body temperature decreases and can be as low as 33.2 ± 0.9°C at T_a = 10°C, probably indicating heterothermy. At such low T_as in combination with starvation, *F. anselli* shows signs of torpor. At T_a = 35°C, body temperature increases to 37 ± 0.6°C (Marhold and Nagel 1995). Basal metabolic rate (0.76 ± 0.08 ml O_2 g^{-1} h^{-1}) is lowest at T_a = 32.5°C, and only slightly higher (0.86 ± 0.08 ml O_2 g^{-1} h^{-1}) at T_a = 30°C. It is significantly lower than expected according to allometric equations for mammals or rodents in particular. The mean (± *SD*) mass-specific resting metabolic rate is significantly higher in reproductive *F. anselli* (1.17 ± 0.17 ml O_2 × g^{-1} × h^{-1}) compared to nonreproductive animals (0.89 ± 0.19 ml O_2 × g^{-1} × h^{-1}—Schielke et al. 2017). Resting metabolic rate is downregulated by low levels of thyroxine, a thyroid hormone (Henning et al. 2014, 2018). Thermal conductance (0.144 ml O_2 g^{-1} × h^{-1} × C^{-1}) is significantly higher than predicted based on body mass compared to other mammals.

At thermoneutrality (T_a = 32.5°C), heart rate (200 ± 23 *SD*) and respiratory rate (74 ± 26 *SD*) are significantly lower than expected for similar-sized mammals (Marhold and Nagel 1995). Even lower respiratory rates (36 ± 5 *SD*) were measured in sleeping *F. anselli* (Garcia Montero et al. 2015). Physiological measurements have been discussed in relation to the hypercapnic and hypoxic conditions and high humidity in sealed tunnel systems (Šumbera 2019). Under laboratory testing conditions (temperature gradient: 16–37°C), *F. anselli* prefers ambient temperatures of about 29°C, thus near its thermoneutral zone (Begall et al. 2015). In the field, *F. anselli* is most active when soil temperatures 10 cm below the surface (i.e., at depths of the foraging tunnels) reach maximum values (ca. 25°C in the study of Šklíba et al. 2014). The animals start shivering at around T_a = 20°C (Marhold and Nagel 1995).

The eye of *F. anselli* is tiny (equatorial diameter: ca. 2 mm); however, it possesses all structures that are typical for a mammalian eye (e.g., stratified retina, iris, lens, eye bulb—Cernuda-Cernuda et al. 2003, Peichl et al. 2004). Previous reports about *F. anselli* assumed that the animals were blind (e.g., Eloff 1951), but behavioral studies showed that they can at least distinguish between light and darkness (Wegner et al. 2006a). The photoreceptor layer is dominated by rods that are fewer in number than in nocturnal, epigeic rodents, but individual rods are larger (Peichl et al. 2004). The proportion of cones (ca. 10%) among the total amount of photoreceptors is higher than in diurnal mammals. Most cones (about 90%) express S-opsins and the adaptive value has puzzled researchers (Peichl et al. 2004) because blue light propagates much less efficiently in underground tunnels than light of longer wavelengths (Kott et al. 2014). More recently, the high proportion of S-opsins has been attributed to unusually low levels of thyroxine (T4) in *F. anselli* (Henning et al. 2014, 2018). T4-treatment increased S-opsin expression significantly in adult *F. anselli* (but not resting metabolic rate) indicating that the unique mammalian opsin expression pattern of *F. anselli* is a side effect of keeping the basal metabolic rate low (Henning et al. 2018). In the subcortical visual system of *F. anselli*, structures needed for coordinating visuomotor reflexes are reduced, while those involved in photoperiod perception and discrimination of form and brightness, and detection of movement are normally developed (although, due to the small size of the eye, the resolution is low—Němec et al. 2004). In accordance, light exposure for 1 h after 3 days in constant darkness led to cell activity (indicated by c-Fos labeling) in the visual system (compared to control animals that were not stimulated), especially of neurons involved in light–dark discrimination and image forming (Oelschläger et al. 2000). The suprachiasmatic nucleus did not react consistently in controls and stimulated animals, which might indicate that entrainment to the photoperiod is difficult in some individuals (Oelschläger et al. 2000).

Hearing and acoustic properties of underground tunnels have been extensively studied in *F. anselli*. The frequency range of best hearing identified by means of different methods (evoked-potential recordings of the auditory brainstem, cochlear distortion products, and behavioral audiogram) is approximately between 0.6 and 1 kHz (Müller and Burda 1989; Kössl et al. 1996; Brückmann and Burda 1997). Cochlear place-frequency mapping revealed an overrepresentation of this frequency range in the cochlea, leading some authors to claim that *F. anselli* possesses an acoustic fovea (Müller et al. 1992, but see Kössl et al. 1996). Despite that, the hearing threshold at these frequencies is still located at about 30 dB sound pressure level (Gerhardt et al. 2017). The high-frequency cutoff is located at about 4 kHz, leading to an extremely narrow hearing range (Müller and Burda 1989; Gerhardt et al. 2017). The restricted hearing range of *F. anselli* corresponds well with tunnel acoustics (Burda 2006; Lange et al. 2007) and is also reflected by its ear morphology. Ear pinnae are lacking, and sound location is, thus, supposedly severely restricted (Lange 2006). The outer ear canal is rather small in diameter and filled with cerumen which hinders transmission of air-borne sound to the tympanic membrane. The tympanic membrane is relatively large and almost perfectly round with no pars flaccida (Burda et al. 1992; Lange 2006). The middle ear of *F. anselli* is of the freely mobile type, that is, the malleus and incus are fused, the middle ear muscles are reduced, and the manubrium runs parallel to the crus longum of the incus (Burda et al. 1992). The stapes footplate is relatively large (Burda et al. 1992; Lange and Burda 2005). The cochlea comprises 3.5 turns and has the shape of a cone (Lange 2006). The highest density of inner and outer hair cells is located near the apex where low-frequency hearing is transduced (Lange 2006). Many measurements of the membranous labyrinth (including the sensory epithelia) of the vestibular organ are expanded in *F. anselli* compared to the laboratory Norway rat (*Rattus norvegicus*) indicating a higher mechanical sensitivity (Lindenlaub et al. 1995).

Fukomys anselli was the first mammal for which a magnetic sense has been demonstrated under controlled laboratory conditions (Burda 1987; Burda et al. 1990; Marhold et al. 1997a). It shows a significant preference to build its nest in southeastern direction when placed in a circular arena. Under artificially produced magnetic conditions (shift of the horizontal component of the magnetic field) the animals change its nest-building preference, accordingly, thus demonstrating a polarity compass (Burda et al. 1990). Reversal of the inclination does not lead to a change in their nest-building preference (Marhold et al. 1997a). Because the experiments were conducted in complete darkness, it can be assumed that the magnetic compass of *F. anselli* is light-independent (Marhold et al. 1997a). Further research focused on the mechanisms of magnetoreception in *F. anselli* (Marhold et al. 1997b; Němec et al. 2001; Wegner et al. 2006b) and provided evidence for the animals' magnetoreceptors being located in the eyes, probably the cornea (Caspar et al. 2020).

As alluded to already, sensory adaptations to the subterranean lifestyle are also reflected in the brain of *F. anselli*. A complete brain atlas of the species highlighting some of these peculiarities is available (Dollas et al. 2019). The size of the brain (ca. 1.26 g)

and the number of neurons in specific brain regions conform to rodent scaling rules (Kverková et al. 2018).

ONTOGENY AND REPRODUCTION

Ontogeny.—Ontogenetic data on *Fukomys anselli* exclusively derive from captive animals. The body mass of newborns is on average 7.9 g (range 5.7–10.7) and is negatively correlated with the number of young per litter (Begall and Burda 1998). The mean length of neonates (from nose to tip of the tail) is 59.9 mm including a head length of 15.4 mm and a tail length of 5.9 mm (Burda 1989). Neonates are hairless and their eyes are closed, with incisors already piercing their lips (Burda 1989). Postpartum growth is slow (mean Gompertz growth constant K = 0.006—Begall and Burda 1998), and development is correlated with body mass rather than with age (Burda 1989). The young tend to spontaneously leave the nest at 5 days of age and are able to return on their own (Burda 1989). However, they may be retrieved by family members when detected (Begall and Burda 2011). They start to self-scratch and clean themselves beginning at 2 days of age (Burda 1989). Eyes are opened when young attain a body mass of about 13 g (median 23 days after birth; range 13–50 days). Pelage starts to develop at 3–5 days of age, and the hair coat is fully developed at 8–10 days of age. Initially, the fur is dark gray (at times almost black, except for the white head spot, if present) and later the coat color changes to grayish brown, brown, and finally ochre (Burda 1989; Burda et al. 1990). The young are nursed until reaching a body mass of 34.1 ± 0.3 g corresponding to 72–105 days of age (Burda 1989). They start to eat lettuce and oat flakes (while still being suckled) at 19 days of age and carrots at 25–30 days of age. Autocoprophagy and begging for feces starts at an age of around 1 month (Burda 1989).

Captive *F. anselli* can be mated at an age of 18 months (Bappert et al. 2012). The maximum recorded life span of *F. anselli* in captivity is 19.9 years in females and 22.2 years in males (observed by SB). Notably, the life span of reproductive animals is on average two times that of nonbreeding *F. anselli* (Dammann and Burda 2006). Certain markers of glycation and advanced glycation end-products increase significantly with advancing age and unexpectedly reach higher levels in breeders compared to nonbreeders (Dammann et al. 2011).

Reproduction.—*Fukomys anselli* is monogamous and displays reproductive division of labor with only one male (i.e., "king") and one female (i.e., "queen") reproducing in each family group (Patzenhauerová et al. 2013). Offspring may remain lifelong with the parents and support them raising their siblings. Thus, *F. anselli*, as all *Fukomys* mole-rats, lives in communities that some authors have characterized as eusocial (Burda 1999; for discussions on the definition and applicability of the term "eusociality," see Burda et al. 2000; see also "Behavior"). This social system is mediated by individual recognition combined with incest avoidance (see "Behavior"). The physiological and neurobiological mechanisms of eusociality in *Fukomys* are not well understood yet. The distribution of oxytocin and vasopressin immunoreactive neurons in the hypothalamus does not differ between reproductive and nonreproductive animals (Valesky et al. 2012).

Fukomys anselli was at times classified as a spontaneous ovulator because mating has no significant effect on urine progesterone levels of previously nonreproductive females. Additionally, males lack ornamentation of the penis and baculum, characteristics of many mammals exhibiting induced ovulation (Bennett et al. 2010). However, it also has been noted that copulation led to a marked increase in urinary progesterone and estradiol of sexually experienced females (Hagemeyer et al. 2009). Furthermore, sexually active females had significantly higher urinary estradiol and progesterone levels than adult but sexually quiescent females. The ovaries of nonreproductive females contain all stages of follicular development (up to tertiary follicles, and even luteinized unruptured follicles) but no true corpora lutea as can be found in reproductive females (Willingstorfer et al. 1998). Therefore, it has been concluded that ovulation of *F. anselli* (indicated by true corpora lutea) might be induced by repeated copulations (Hagemeyer et al. 2009). The luteinized unruptured follicles probably produce progesterone, which in turn could prevent ovulation (pregnancy block). Because luteinized unruptured follicles are also found in other hystricognath rodents (Weir and Rowlands 1974), they might represent a plesiomorphic trait in bathyergids. Burda (1999) suggested that this block of ovulation is overcome in reproductive females by the luteolytic effect of oxytocin which is usually released during copulation. Testes are significantly larger in reproductive males compared to nonreproductive males; however, nonreproductive male *F. anselli* also produce viable sperm (Garcia Montero et al. 2016). Females possess a uterus duplex (Willingstorfer et al. 1998) and a baubellum (Thomas 1917). The following values are given as mean ± *SD* with ranges presented in parentheses. Gestation lasts on average 98 ± 9 days (84–112 days—Burda 1989; Begall and Burda 1998). Females may give birth in a sitting position (Begall et al. 2018). The shortest interbirth interval is 3 months (Begall et al. 2018) indicating that the respective females conceived in postpartum estrus. Litter size is on average 2.4 ± 0.9 young (1–5—Begall and Burda 1998). The average number of young per litter increases with the number of parturitions for a particular female (Begall and Burda 1998). Ovaries begin to atrophy at the age of 3–6 years (Willingstorfer et al. 1998); however, we (HB and SB) observed that females can reproduce up to an age of at least 15 years in captivity.

ECOLOGY

Population characteristics.—As other subterranean mammals, *Fukomys anselli* is a typical K-strategist, and its populations seem to be locally stable. The most comprehensive insights into the species' population structure were provided by a 12-month field study conducted in the suburbs of Lusaka

that involved the capture of 33 families of *F. anselli* (Sichilima et al. 2011). The average family size was 8.7 ± 2.2 individuals (mean ± *SE*, range 6–16, *n* = 33). The sex ratio of the population was skewed toward females at 0.83:1. Pregnant and lactating females were found throughout the study period from February 2009 to February 2010, indicating an aseasonal pattern of breeding. Autopsy of individuals (*n* = 288) from the 33 colonies revealed a total of 19 pregnant females. Nine of these were in the latter stages of pregnancy and mean litter size was determined as 2.7 young (range 1–4). Maximum colony size for *F. anselli* is expected to be about 25 animals, based on reports from local mole-rat hunters and on fecundity and longevity in captive *F. anselli* (Burda et al. 2000). Unfortunately, there is no reliable marker to estimate the age structure of families, and it is not yet known how new families are founded in the field.

The sex ratio of neonate *F. anselli* in captivity is 0.85 (98 males:115 females—Begall and Burda 1998). The total mortality of young is 34%, and because the mortality of males is higher than that of females, the sex ratio shifts to 0.7 at the time of weaning (8 weeks—Begall and Burda 1998). This corresponds to the sex ratio (0.77) of subadult and adult *F. anselli* trapped in the field (Burda 1989). A similar sex ratio (0.83) among adult *F. anselli* was also reported by the most comprehensive field study so far (Sichilima et al. 2011).

Space use.—Each *Fukomys anselli* family group occupies and maintains an extensive burrow system. Based on data from nine families it was found that these burrows cover an area of 6,920 ± 4,480 *SD* (range 1,930–19,100) m^2 and consist of 0.5–2.8 km of tunnels (mean 1.2 ± 0.6 *SD* km, *n* = 15), which are densely branched and reticulated especially around nest chambers (Šklíba et al. 2012). The floor of most tunnels is located at depths of 10–12 cm. The average tunnel diameter is 4.6 (range 3.8–6.8) cm. Deeper tunnels are located mostly in the vicinity of nests. Typically, each burrow system has one currently used nest (a chamber with fresh bedding) 47 ± 17 *SD* (range 25–90) cm underground. The nest chamber is usually surrounded by an elaborate three-dimensional network of tunnels on several levels. Food stores and toilets (defecation chambers) are typically found within a distance of 2 m from the nest that is currently in use (Šklíba et al. 2012). Burrow systems of neighboring family groups are often interconnected by a freely passable tunnel (Šklíba et al. 2012). Stealing of food from storage chambers through interconnecting tunnels by mole-rats from neighboring families has been anecdotally reported (Šklíba et al. 2016b).

Data on the stability of burrow systems of *F. anselli* are missing yet judging from family sizes and the extent of burrow systems, they appear to be maintained for several years. This would be in line with observations on the more extensively studied *F. damarensis* (Bennett and Faulkes 2000). Plastic materials found in nests of *F. anselli* have been interpreted as an indication of surface activity, which likely has an essential role in pair formation and dispersal (Scharff and Grütjen 1997).

Diet.—*Fukomys anselli* is herbivorous and primarily feeds on underground plant storage organs such as the rhizomes, tubers and bulbs of diverse weeds, and roots of shrubs. It was found that occasionally seeds of the Mobola plum (*Parinari curatellifolia*) were gathered (apparently aboveground) and consumed (Scharff and Grütjen 1997; Scharff 1998). When bulky food items with a mass > 30 g are encountered underground, they are typically consumed on site. Correspondingly, tunnels are constructed in complicated branching patterns in soil patches rich in tubers (Šklíba et al. 2012). Smaller food items, however, are often transported into food chambers. These are located in close proximity, typically within a radius of 2 m, to the nesting chamber of the burrow system (Šklíba et al. 2012). A study conducted at Lusaka East Forest Reserve sampled burrows of 16 *F. anselli* family groups and recovered 84 ± 73 *SD* (8–216) food items per chamber, with a total weight of 169 ± 177 *SD* (3–618) g in each (Šklíba et al. 2012). The average weight of food items (excluding grass corms) was found to be 3.0 ± 3.5 *SD* (0.1–33) g. Extensive analyses of food composition and foraging preferences in wild *F. anselli* have not been reported in the literature at this time. It is known that tubers of the genera *Dolichos* (Fabaceae) and *Hypoxis* (Hypoxidaceae) next to sedge rhizomes (Cyperaceae) constitute an important food source for the species (Scharff 1998; Šklíba et al. 2012). Agricultural crops, such as sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), potatoes (*Solanum tuberosum*), and carrots (*Daucus carota*), are also readily consumed (Scharff 1998). As with other bathyergids, *F. anselli* does not drink free water. In our studies, we observed small food items, such as grains, are grasped with both hands simultaneously and are invariably consumed in a sitting position.

Diseases and parasites.—Despite their social habits, general exposure to and transmission rates of parasites and pathogens are assumed to be low in *Fukomys* (specifically in *F. damarensis*), as their major histocompatability (MHC) alleles do not show patterns indicative of strong positive selection (Kundu and Faulkes 2004). Although known from other species of the genus (e.g., Lutermann et al. 2015), no ectoparasites have been found in association with *F. anselli* (Scharff et al. 1997; Šklíba et al. 2016b). The few documented gastrointestinal endoparasites in *F. anselli* and the closely allied species *F. kafuensis* include cestodes (*Inermicapsifer*, *Rodentolepis*) and nematodes (*Hexametra*, *Protospirura numidica*, *Protospirura muricola*, *Protospirura*, *Mammalakis zambiensis*—Scharff et al. 1997; Lutermann et al. 2018). The abundance and prevalence of helminth parasites in *F. anselli* is low, as typical for subterranean rodents, but tends to increase during the dry season (Lutermann et al. 2018). Lung infections with adiaspores of the saprophytic soil fungus *Emmonsia parva* were found in all individuals within a sample of 20 wild-caught *F. anselli* (Hubálek et al. 2005). However, granulomatous alteration of the lung tissue in these animals was minimal. There are no reports of zoonoses transferred from *F. anselli* to humans (Scharff 1998).

Interspecific interactions.—Burrow systems of *Fukomys* mole-rats provide shelter for diverse commensals, among them invertebrates, amphibians, and snakes. Most abundant among frog species found in burrow systems of *Fukomys anselli* is the Senegal running frog (*Kassina senegalensis*—Šklíba et al. 2016a). Apart from humans who hunt mole-rats for their valued meat (Burda and Kawalika 1993 with reference to *F. mechowii*), *F. anselli* has no known predators. However, data on other small-bodied bathyergids suggest that canids, owls, and snakes may prey on it (de Graaff 1964).

HUSBANDRY

Fukomys anselli is easily sustained in captivity at an ambient temperature of 25 ± 2 (*SD*) °C and (at least) 45% relative humidity. Animals can be fed with carrots and potatoes as staple food, supplemented with sweet potatoes and other tubers. At least once per week they should receive cereals and vegetables (cucumber, lettuce, parsley, radish, etc.), occasionally complemented with apple slices. It is not necessary to provide free water. *Fukomys anselli* families may be kept in simple glass terraria of various sizes adapted to the dimensions of the group (e.g., a family with 12 family members can be kept in a terrarium of the size 120 cm by 50 cm). A nesting chamber (e.g., flowerpot or wooden box) needs to be provided. Saw dust or horticultural peat can be used as substrate (minimum 5 cm high). Tissue papers or wood wool should be regularly offered as nesting material. Similar to cleaning schedules recommended for other bathyergids, it is sufficient to clean the terraria and to change the substrate every 1–3 months (Matschei and Bäthe 2012). Under laboratory conditions, *F. anselli* may very rarely develop infections with *Candida* and *Yersinia enterocolitica* (Scharff 1998). The animals can be handled by grasping the skin at the back (with thumb and index finger) near the tail. Groups of *F. anselli* are highly xenophobic toward unfamiliar conspecifics in captivity, requiring strict separation of individual families (Burda 1989, 1995; Begall et al. 2018). This necessitates that pairings must be undertaken while both partners are separated from their natal groups. This provided, opposite-sex animals from different families can be easily paired (Burda 1989, 1995).

Chemical restraint of *F. anselli* can be accomplished by administration of ketamine (6 mg/kg body mass) and xylazine (2.5 mg/kg body mass). At this dose the pedal withdrawal reflex lasts on average for 20 ± 15 (*SD*) min (Garcia Montero et al. 2015).

Fukomys anselli is publicly displayed at the zoos of Basel (Switzerland), Berlin (Germany), Leipzig (Germany), and Wuppertal (Germany), with all animals originally deriving from the same breeding stock currently housed at the University of Duisburg-Essen. Colonies employed for research are kept at the Universities of Duisburg-Essen (Germany) and České Budějovice (Czech Republic), and at the Center of Advanced European Study and Research in Bonn (Germany). A few individuals are also privately housed.

BEHAVIOR

Grouping behavior.—*Fukomys anselli* families consist of the breeding pair and their nonreproductive offspring (Burda 1989; Patzenhauerová et al. 2013). The breeding pair is monogamous, and dispersal of nonreproductive animals is delayed so that several generations of adult *F. anselli* overlap (Burda et al. 2000; Patzenhauerová et al. 2013). Due to the reproductive division of labor and strong philopatry of offspring, *F. anselli* is considered a eusocial mammal by some authors (Burda et al. 2000). Data on wild congeneric *F. damarensis* indicate that nonbreeders spend more time foraging than breeders (Francioli et al. 2020). Furthermore, laboratory studies on *F. damarensis* showed that the amount of time that breeding females spent resting and feeding and their fecundity positively correlate with the number of nonreproductive animals in a group (Houslay et al. 2020). Mean family size in the field (Lusaka Province, Zambia) is 15 animals (n = 10 families—Scharff 1998), 8.5 animals (n = 13 functional families—Patzenhauerová et al. 2013), and 8.7 animals (n = 33 families—Sichilima et al. 2011). The maximum number of animals in a family captured in the field was 47 (Scharff 1998); however, animals from different families might have been mixed up resulting in an exceptionally large number. In the vast majority of cases only one female per family is reproductively active (Scharff 1998; Šklíba et al. 2012; Patzenhauerová et al. 2013), with only one study reporting the occurrence of multiple breeding females (Sichilima et al. 2011). Therein, three of the 33 sampled families (9%) were reported to encompass two reproductive females, but the exact family group affiliation of the respective animals was not further investigated and might have been erroneous. With one reproductive female and an assumed interlitter interval of 3 months (i.e., 2–3 litters per year), it is expected that an average *F. anselli* family of 13 animals covers about 6.5 generations of pups (Burda et al. 2000). Radiotracking of breeders and nonbreeders from two families in the field showed that nonbreeding females were tracked at distances of more than 90 m from the nest, whereas all other family members were tracked at shorter distances (Šklíba et al. 2016b). The authors interpret this behavior as females nonbreeders actively searching for underground dispersal opportunities. In other *Fukomys* species, there are anecdotal reports of females that dispersed and monopolized abandoned parts of the burrow system, probably waiting for dispersing males that may prefer to disperse aboveground (*Fukomys damarensis*—Hazell et al. 2000; *Fukomys mechowii*—Šumbera et al. 2012; Lövy et al. 2013). Neighboring burrow systems of *F. anselli* were frequently found to be interconnected (Škliba et al. 2012), which might also facilitate dispersal but may render families vulnerable to invasion by foreign animals. As with other *Fukomys* species, molecular analyses of parentage revealed higher turnover rates of breeding males compared to females in *F. anselli* (Patzenhauerová et al. 2013). This, in combination with pronounced sexual dimorphism, points to males having to regularly

fight off competitors that try to take over the reproductive female and burrow system they occupy (Caspar et al. 2021).

However, successful immigrations into wild groups appear to be rare. Only three individuals from three different groups could be identified as immigrants in a large sample of wild *F. anselli* (total sample size: 119 animals from 16 groups—Patzenhauerová et al. 2013).

Reproductive behavior.—In the laboratory, within complete families (i.e., families including the reproductive pair) of *Fukomys anselli*, mating occurs only between the reproductive pair and never between parents and offspring or between siblings (Burda 1995; Bappert et al. 2012). However, when two unfamiliar individuals of opposite sex are placed together, in the majority of cases courtship starts soon and the female eventually conceives (Burda 1989, 1990, 1995). Reproductive males, in contrast to nonbreeders, stay "faithful," when presented with mating opportunities (Bappert et al. 2012). Nonreproductive brothers and a sister may be separated for up to 18 days before the siblings start copulating and eventually produce offspring upon reintroduction (Burda 1995). However, it is unlikely that such a long separation (and subsequent reintroduction) occurs under natural conditions. Anogenital smear from an opposite-sex conspecific of a different family elicits longer sniffing time compared to that from an opposite-sex sibling (Heth et al. 2002). If siblings are separated for more than 18 days and then the anogenital smear is presented, the siblings sniff longer at the odor from the separated sibling compared to that of a sibling that had remained in contact (Heth et al. 2004). Thus, the incest taboo of *F. anselli* is based on individual recognition of family members mediated by body odors, not on reproductive suppression (Burda 1995; Heth et al. 2004).

In newly established families, it is usually the female that solicits mating. The animals show a more or less extended courtship phase where they extensively nuzzle and sniff at each other, especially the anogenital areas and the partner's cheeks (Burda 1989). If the female becomes sexually aroused, her entire body starts to twitch conspicuously; at one point she adopts lordosis and the male starts mounting. As soon as intromission takes place, the male engages in pelvic thrusting. Mounting lasts 5–15 s (Burda 1989; Begall et al. 2018). Males may mount females also during late stages of pregnancy, and even during parturition.

Both parents and also elder siblings from previous litters are involved in taking care of the offspring, for example, by retrieving young that are found outside of the communal nest (Burda 1989). The young are cleaned primarily by the mother (Burda 1989). Infanticide and cannibalism have been occasionally observed if the neonates were born with a body mass of less than 7 g (Burda 1989).

Communication.—A study of 12 captive animals (six adults, six juveniles) belonging to three families revealed that *Fukomys anselli* emits 13 true vocalizations, produced in the larynx, and one mechanical sound (Credner et al. 1997). A more recent study, which included 41 adults and 10 juveniles from 15 families in different (and also artificially induced) contexts, revealed 16 calls and four mechanically produced air-borne sounds (Schmidt 2014). Probably all subterranean rodents produce sounds by grinding or chattering their incisors. These sounds have often been observed in the context of aggressive encounters among bathyergids and were interpreted as emphasizing the agonistic attitude of the emitter. However, because *F. anselli* grinds its teeth mostly when resting, perhaps to sharpen the ever-growing incisors, it may not always represent a communication signal (Credner et al. 1997). Two further mechanically produced sounds (hiss and snort) are both related to exhalation of air (Schmidt 2014). The 16 vocalizations are emitted in the contexts of physical contact, mating, aggression, conflict or distress, and alarm (Schmidt 2014). The peak frequencies of the vocalizations often range below 5 kHz (fundamental frequencies: < 1 kHz) and are accordingly located in the species' auditory range, simultaneously conforming well to tunnel acoustics (Credner et al. 1997; Schmidt 2014). Young of *F. anselli* emit five structurally different true vocalizations and two mechanically produced sounds (Schmidt 2014). Seismic signals are likely produced when animals are thumping the tunnel floor with the chest (also termed "pumping"), a behavior related to distress which is performed by several bathyergid species (Poduschka 1978; Schmidt 2014).

Behavioral tests using urine, anogenital smear, and feces showed that all three substances deliver certain information about the sender, but anogenital smear yields the best results for individual recognition via odors (Hagemeyer et al. 2004; Hagemeyer 2010). Urine of *F. anselli* does not contain major urinary proteins (Hagemeyer et al. 2011).

Miscellaneous behavior.—Studies of circadian rhythms in captive *Fukomys anselli* (and other bathyergids) yielded equivocal results: some animals were reported to be arrhythmic and incapable of light entrainment (Gattermann and Burda 1994), others are able to entrain to the photoperiod under 12L:12D, but they displayed very weak free-running circadian rhythms in constant darkness (Fritzsche et al. 1997; de Vries et al. 2008). Furthermore, *F. anselli* kept in Germany were more active during the day (Fritzsche et al. 1997), whereas the animals studied in the laboratory in South Africa were rather nocturnal (de Vries et al. 2008). The only field study on chronobiology indicates that *F. anselli* has rather strong circadian rhythms with activity peaking around 1400 h (Šklíba et al. 2014). Under laboratory conditions, there is no significant difference between activity budgets of reproductive and nonreproductive animals (Dammann and Burda 2006; Schielke et al. 2012). When resting, individuals usually gather in groups to huddle, a behavioral strategy to conserve heat (Šumbera 2019). Under laboratory conditions, *F. anselli* spends on average 90% of the time huddling in a communal nest (Dammann and Burda 2006; Begall et al. 2018).

GENETICS

Cytogenetics.—Diploid (2n) chromosome number in *Fukomys anselli* is 68 (Burda et al. 1999). Within the karyotypically diverse *Fukomys* genus this number of chromosomes is unique and diagnostic for the species (Ingram et al. 2004). Most chromosomes exhibit an acrocentric

organization. However, chromosome structure appears to be variable, with other chromosome types occurring in mixed proportions (FN = 79–82—Burda et al. 1999). Correspondingly, X chromosomes are structurally diverse as well, at times resulting in heteromorphic gonosomal pairs in females (Burda et al. 1999).

Molecular genetics.—The mitochondrial cytochrome-*b* and 12 S genes, and the nuclear intron 1 of the transthyretin gene (TTR) of *Fukomys anselli* have been sequenced and used to infer its phylogenetic position among mole-rats of the *Fukomys* genus (Faulkes et al. 1997 [included as *Cryptomys amatus*]; Ingram et al. 2004; Van Daele et al. 2007). *Fukomys anselli* is part of a species group formed by at least three parapatric, morphologically uniform populations of controversial taxonomic status that are exclusively distributed in Zambia ("*F. micklemi* clade"—Van Daele et al. 2007). Besides *F. anselli*, this group most prominently comprises *F. kafuensis* (2n = 58) and *F. micklemi* (2n = 56/60—Ingram et al. 2004; Van Daele et al. 2004). There is genetic evidence in support of grouping *F. kafuensis* and *F. micklemi* as sister species and for *F. anselli* branching off more basally (Ingram et al. 2004), but the phylogenetic relationships within this clade require further investigation (Van Daele et al. 2007). Indeed, mole-rats that are referred to as *F. anselli* and that share the diagnostic 2n = 68 karyotype do not form a well-supported clade in molecular analyses (Ingram et al. 2004; Van Daele et al. 2007) and might be paraphyletic in regards to other members of the *F. micklemi* group (Van Daele et al. 2007). Since sequence divergence is very low within the latter species group, the available genetic data might be too limited to robustly delineate species boundaries. In any case, a taxonomic revision of Zambezian *Fukomys* integrating molecular, morphological, and karyological data is direly needed (Van Daele et al. 2004) and might lead to a partial reclassification of 2n = 68 cytotype populations currently assigned to *F. anselli*. The *F. micklemi* clade encompassing *F. anselli* is of recent origin and presumably diverged from its sister taxon, *F. damarensis* of the Kalahari Desert, in the middle of the Pleistocene (Van Daele et al. 2007).

Population genetics.—Microsatellite patterns in wild *Fukomys anselli* indicate monopolization of reproduction by a single pair at a time, limited dispersal, and high levels of genetic relatedness between members of studied colonies (n = 16—Patzenhauerová et al. 2013). This stands in support of a high prevalence of extreme reproductive skew and monogamy in *F. anselli*. Several nuclear microsatellites of *F. anselli* have been characterized (Ingram 2006) but different microsatellite loci identified in other bathyergids can also be utilized to study this species (Patzenhauerová et al. 2013).

CONSERVATION

The population status of *Fukomys anselli* is considered "Least Concern" (LC) with a decreasing population trend by the International Union for Conservation of Nature Red List of Threatened Species (Dando and Van Daele 2020). However, estimates of population size and density remain tentative as there have been no systematic surveys. *Fukomys anselli* is regularly trapped and consumed by locals but to which degree this affects the integrity of the population remains unknown (Dando and Van Daele 2020). No specific measures are in effect to conserve the species, but it occurs in at least one protected area, the Lusaka East Forest Reserve (Škliba et al. 2012; Dando and Van Daele 2020).

REMARKS

Fukomys anselli is routinely hunted by locals for consumption and sometimes offered by street and market vendors. Because the animals also occur in cultivated fields, gardens, parks, and golf courses, they are regarded as a pest species (Scharff 1998; Begall et al. 2018). Capturing wild *F. anselli* is complicated because the animals are cautious, quickly perceive danger, and plug the entrances of their tunnels (Begall et al. 2018). Single individuals of a burrow system can be captured by means of Hickman traps (Scharff 1998). The traditional method often used by locals (especially, to catch all animals of a tunnel system) requires a lot of patience: After removal of the upper layers of soil one puts three or four sticks into the earth directly above a straight running tunnel. If an animal passes the tunnel, the sticks will move, and the hunter will cut the retreat by quickly striking a hoe behind the animal (Scharff 1998). Then, the mole-rat can be dug out and the procedure can start again to catch another family member.

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LITERATURE CITED

Agrawal V.C. 1967. Skull adaptations in fossorial rodents. Mammalia 31:300–312.

Banke J., Mess A., Zeller, U. 2001. Functional morphology of the rostral head region of *Cryptomys hottentotus* (Bathyergidae, Rodentia). In: Denys C., Granjon L., Poulet A., editors. African small mammals. IRD ed. Colloques et Séminaires, Paris, France; p. 231–241.

Bappert M.-T., Burda H., Begall S. 2012. To mate or not to mate? Mate preference and fidelity in monogamous Ansell's mole-rats, *Fukomys anselli*, Bathyergidae. Folia Zoologica 61:71–83.

Begall S., Berendes M., Schielke C.K., Henning Y., Laghanke M., Scharff A., van Daele P., Burda H. 2015. Temperature preferences of African mole-rats (family Bathyergidae). Journal of Thermal Biology 53:15–22.

Begall S., Burda H. 1998. Reproductive characteristics and growth rate in the eusocial Zambian common mole-rat (*Cryptomys* sp., Bathyergidae). Zeitschrift für Säugetierkunde 63:297–306.

Begall S., Burda H. 2011. Der Ansell-Graumull – ein Überlebenskünstler unter Tage. Zeitschrift des Kölner Zoos 54:89–103.

Begall S., Burda H., Šumbera R. 2018. Graumulle – *Cryptomys* und *Fukomys*. VerlagsKG Wolf, Magdeburg, Germany.
Bennett N.C., Aguilar G.H., Jarvis J.U., Faulkes C.G. 1994. Thermoregulation in three species of Afrotropical subterranean mole-rats (Rodentia: Bathyergidae) from Zambia and Angola and scaling within the genus *Cryptomys*. Oecologia 97:222–227.
Bennett N.C., Burda H. 2013. *Cryptomys anselli*. In: Happold M., Happold D.C.D., editors. Mammals of Africa. Vol. 3. Rodents, hares and rabbits. Bloomsbury Publishing, London, United Kingdom; p. 649–650.
Bennett N.C., Faulkes C.G. 2000. African mole-rats: ecology and eusociality. Cambridge University Press, Cambridge, United Kingdom.
Bennett N.C., van Sandwyk J., Lutermann H. 2010. The pattern of ovulation in Ansell's mole-rats: phylogenetic or ecological constraints? Journal of Zoology (London) 281:66–73.
Brückmann G., Burda H. 1997. Hearing in blind subterranean Zambian mole-rats (*Cryptomys* sp.): collective behavioural audiogram in a highly social rodent. Journal of Comparative Physiology, A. Sensory, Neural, and Behavioral Physiology 181:83–88.
Bryja J., Konvičková H., Bryjová A., Mikul O., Makundi R., Chitaukali N., Šumbera R. 2018. Differentiation underground: range-wide multilocus genetic structure of the silvery mole-rat does not support current taxonomy based on mitochondrial sequences. Mammalian Biology 93:82–92.
Burda H. 1987. Magnetische Navigation bei den Graumullen, *Cryptomys hottentotus* (Bathyergidae)? Zeitschrift für Säugetierkunde 61:12.
Burda H. 1989. Reproductive biology (behaviour, breeding, and postnatal development) in subterranean mole-rats, *Cryptomys hottentotus* (Bathyergidae). Zeitschrift für Säugetierkunde 54:360–376.
Burda H. 1990. Constraints of pregnancy and evolution of sociality in mole-rats. With special reference to reproductive and social patterns in *Cryptomys hottentotus* (Bathyergidae, Rodentia). Zeitschrift für zoologische Systematik und Evololutionsforschung 28:26–39.
Burda H. 1995. Individual recognition and incest avoidance in eusocial common mole-rats rather than reproductive suppression by parents. Experientia 51:411–413.
Burda H. 1999. Syndrome of eusociality in African subterranean mole-rats (Bathyergidae, Rodentia), its diagnosis and aetiology. In: Wasser S.P., editor. Evolutionary theory and processes: modern perspectives. Kluwer Academic, Dordrecht, The Netherlands; p. 385–418.
Burda H. 2006. Ear and eye in subterranean mole-rats, *Fukomys anselli* (Bathyergidae) and *Spalax ehrenbergi* (Spalacidae): progressive specialization or regressive degeneration? Animal Biology 56:475–486.
Burda H., Bruns V., Hickman G.C. 1992. The ear in subterranean Insectivora and Rodentia in comparison with ground-dwelling representatives. I. Sound conducting system of the middle ear. Journal of Morphology 214:49–61.
Burda H., Honeycutt R.L., Begall S., Grütjen O., Scharff A. 2000. Are naked and common mole-rats eusocial and if so, why? Behavioral Ecology and Sociobiology 47:293–303.
Burda H., Kawalika M. 1993. Evolution of eusociality in the Bathyergidae. The case of the giant mole rats (*Cryptomys mechowi*). Die Naturwissenschaften 80:235–237.
Burda H., Marhold S., Westenberger T., Wiltschko R., Wiltschko W. 1990. Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). Experientia 46:528–530.
Burda H., Zima J., Scharff A., Macholán M., Kawalika M. 1999. The karyotypes of *Cryptomys anselli* sp. nova and *Cryptomys kafuensis* sp. nova: new species of the common mole-rat from Zambia (Rodentia, Bathyergidae). Zeitschrift für Säugetierkunde 64:36–50.
Caspar K.R., Moldenhauer K., Moritz R.E., Němec P., Malkemper E.P., Begall S. 2020. Eyes are essential for magnetoreception in a mammal. Journal of the Royal Society, Interface 17:20200513.
Caspar K.R., Müller J., Begall S. 2021. Effects of sex and breeding status on skull morphology in eusocial Ansell's mole-rats and an appraisal of sexual dimorphism in the Bathyergidae. Frontiers in Ecology and Evolution 9:638754.
Cernuda-Cernuda R., García-Fernández J.M., Gordijn M.C., Bovee-Geurts P.H., DeGrip W.J. 2003. The eye of the African mole-rat *Cryptomys anselli*: to see or not to see? The European Journal of Neuroscience 17:709–720.
Credner S., Burda H., Ludescher F. 1997. Acoustic communication underground: vocalization characteristics in subterranean social mole-rats (*Cryptomys* sp., Bathyergidae). Journal of Comparative Physiology, A. Sensory, Neural, and Behavioral Physiology 180:245–255.
Dammann P., Burda H. 2006. Sexual activity and reproduction delays aging in a mammal. Current Biology 16:R117–R118.
Dammann P., Sell D.R., Begall S., Strauch C., Monnier V.M. 2011. Advanced glycation end-products as markers of aging and longevity in the long-lived Ansell's mole-rat (*Fukomys anselli*). The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 67:573–583.
Dando T., Van Daele P.A.A.G. 2020. *Fukomys anselli*. The IUCN Red List of Threatened Species 2020:e.T44858A22184572 https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T44858A22184572.en. Accessed 1 Oct 2020.
De Bruin P.R., Viljoen H., Sichilima A.M., Bennett N.C. 2012. Socially induced infertility in Ansell's mole-rat: are there depressed hormone levels in non-reproductive males and females? Journal of Zoology (London) 286:15–21.
de Graaff G. 1964. A systematic revision of the Bathyergidae (Rodentia) of Southern Africa Ph.D. dissertation, University of Pretoria, Pretoria, South Africa.
de Vries J.L., Oosthuizen M.K., Sichilima A.M., Bennett N.C. 2008. Circadian rhythms of locomotor activity in Ansell's mole-rat: are mole-rat's clocks ticking? Journal of Zoology (London) 276:343–349.
Dollas A., Oelschläger H.H.A., Begall S., Burda H., Malkemper E.P. 2019. Brain atlas of the African mole-rat *Fukomys anselli*. The Journal of Comparative Neurology 527:1885–1900.
Ellerman J.R. 1940. The families and genera of living rodents, with a list of named forms (1758–1936). Vol. 1. Rodents other than Muridae. Trustees of the British Museum (Natural History), London, United Kingdom.
Eloff G. 1951. Orientation in the mole-rat *Cryptomys*. British Journal of Psychology 42:134–145.
Faulkes C.G., Bennett N.C., Bruford M.W., O'Brien H.P., Aguilar G.H., Jarvis J.U.M. 1997. Ecological constraints drive social evolution in the African mole-rats. Proceedings of the Royal Society of London, B: Biological Sciences 264:1619–1627.
Faulkes C.G., Mgode G.F., Le Comber S.C., Bennett N.C. 2010. Cladogenesis and endemism in Tanzanian mole-rats, genus *Fukomys*: (Rodentia Bathyergidae): a role for tectonics? Biological Journal of the Linnean Society 100:337–352.
Faulkes C.G., Verheyen E., Verheyen W., Jarvis J.U., Bennett N.C. 2004. Phylogeographical patterns of genetic divergence and speciation in African mole-rats (family: Bathyergidae). Molecular Ecology 13:613–629.
Francioli Y., Thorley J., Finn K., Clutton-Brock T., Zöttl M. 2020. Breeders are less active foragers than non-breeders in wild Damaraland mole-rats. Biology Letters 16:20200475.
Fritzsche P., Miethe G., Gattermann R. 1997. Circadian activity rhythm and entrainment in the subterranean common mole-rat (*Cryptomys hottentotus*). Biological Rhythm Research 6:116.
Garcia Montero A., Burda H., Begall S. 2015. Chemical restraint of African mole-rats (*Fukomys* sp.) with a combination of ketamine and xylazine. Veterinary Anaesthesia and Analgesia 42:187–191.
Garcia Montero A., Vole C., Burda H., Malkemper E.P., Holtze S., Morhart M., Saragusty J., Hildebrandt T.B., Begall S. 2016. Non-breeding eusocial mole-rats produce viable sperm–spermiogram and functional testicular morphology of *Fukomys anselli*. PLoS One 11:e0150112.
Gattermann R., Burda H. 1994. Soziale isolation und Circadianrhythmik beim eusozialen Graumull. Verhandlungen der Deutschen Zoologischen Gesellschaft 87:39.
Gerhardt P., Henning Y., Begall S., Malkemper E.P. 2017. Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem potentials reveal extremely poor high-frequency cut-offs. Journal of Experimental Biology 220:4377–4382.

Gomes Rodrigues H., Šumbera R., Hautier L. 2016. Life in burrows channelled the morphological evolution of the skull in rodents: the case of African mole-rats (Bathyergidae, Rodentia). Journal of Mammalian Evolution 23:175–189.

Hagemeyer P. 2010. Pheromones in social mole-rats and implications for the study of mammalian chemical communication Ph.D. dissertation, University of Duisburg-Essen, Essen, Germany.

Hagemeyer P., Begall S., Janotova K., Todrank J., Heth G., Jedelsky P.L., Burda H., Stopka P. 2011. Searching for major urinary proteins (MUPs) as chemosignals in urine of subterranean rodents. Journal of Chemical Ecology 37:687–694.

Hagemeyer P., Lange S., Broecker-Preuss M., Burda H. 2009. The influence of olfactory stimulus and sexual activity on gonadal steroids in eusocial mole-rats. Folia Zoologica 58:65–74.

Hagemeyer P., Peuckmann S., Papenfuhs N., Burda H. 2004. Chemical communication in blind, subterranean eusocial mole-rats (genus *Coetomys*): which odour source codes information for individual recognition? Advances in Ethology 38:56.

Hazell R.W.A., Bennett N.C., Jarvis J.U.M., Griffin M.. 2000. Adult dispersal in the co-operatively breeding Damaraland mole-rat (*Cryptomys damarensis*): a case study from the Waterberg region of Namibia. Journal of Zoology (London) 252:19–25.

Henning Y., Mladěnková N., Burda H., Szafranski K., Begall S. 2018. Retinal S-opsin dominance in Ansell's mole-rats (*Fukomys anselli*) is a consequence of naturally low serum thyroxine. Scientific Reports 8:4337.

Henning Y., Vole C., Begall S., Bens M., Broecker-Preuss M., Sahm A., Szafranski K., Burda H., Dammann P. 2014. Unusual ratio between free thyroxine and free triiodothyronine in a long-lived mole-rat species with bimodal ageing. PLoS One 9:e113698.

Hesselmann F. 2010. Vergleich der mikroskopischen Anatomie der Haut und Hautanhangsorgane des Hausmeerschweinchens (*Cavia aperea* f. *porcellus*) und des Ansells Kleingraumulls (*Fukomys anselli*) unter Berücksichtigung ihrer Anpassung an unterschiedliche Lebensräume Ph.D. dissertation, Tierärztliche Hochschule Hannover, Hannover, Germany.

Heth G., Todrank J., Begall S., Wegner R.E., Burda H. 2004. Genetic relatedness discrimination in a eusocial rodent, *Cryptomys anselli* mole-rats. Folia Zoologica 53:269–278.

Heth G., Todrank J., Burda H. 2002. Individual odor similarities within colonies and across species of *Cryptomys* mole rats. Journal of Mammalogy 83:569–575.

Honeycutt R.L. 2017. Bathyergidae. In: Wilson D.E., Lacher T.E., Jr., Mittermeier R.A., editors. Handbook of mammals of the world. Vol. 6. Lagomorphs and rodents. Lynx Edicions, Barcelona, Spain; p. 352–370.

Honeycutt R.L., Allard M.W., Schlitter D.A., Edwards S.V. 1991. Systematics and evolution of the family Bathyergidae. In: Sherman P.W., Jarvis J.U.M., Alexander, R.D., editors. The biology of the naked mole-rat. Princeton University Press, Princeton, New Jersey, USA; p. 45–65.

Houslay T.M., Vullioud P., Zöttl M., Clutton-Brock T.H. 2020. Benefits of cooperation in captive Damaraland mole-rats. Behavioral Ecology 31:711–718.

Hubálek Z., Burda H., Scharff A., Heth G., Nevo E., Sumbera R., Pesko J., Zima J. 2005. Emmonsiosis of subterranean rodents (Bathyergidae, Spalacidae) in Africa and Israel. Medical Mycology 43:691–697.

Ingram C.M. 2006. The evolution of nuclear microsatellite DNA markers and their flanking regions using reciprocal comparisons within the African mole-rats (Rodentia: Bathyergidae) Ph.D. dissertation, Texas A&M University, College Station, Texas, USA.

Ingram C.M., Burda H., Honeycutt R.L. 2004. Molecular phylogenetics and taxonomy of the African mole-rats, genus *Cryptomys* and the new genus *Coetomys* Gray, 1864. Molecular Phylogenetics and Evolution 31:997–1014.

Kawalika M. 2004. Rodents of Ndola (Copperbelt Province, Zambia) Ph.D. dissertation, University of Duisburg-Essen, Essen, Germany.

Kingdon J. 1974. East African mammals - an atlas of evolution in Africa. Vol. II. Part B (hares and rodents). University of Chicago Press, Chicago, Illinois, USA.

Kock D., Ingram C.M., Frabotta L.J., Honeycutt R.L., Burda H. 2006. On the nomenclature of Bathyergidae and *Fukomys* n. gen. (Mammalia: Rodentia). Zootaxa 1142:51–55.

Kössl M., Frank G., Burda H., Müller M. 1996. Acoustic distortion products from the cochlea of the blind African mole rat, *Cryptomys* spec. Journal of Comparative Physiology, A. Sensory, Neural, and Behavioral Physiology 178:427–434.

Kott O., Moritz R.E., Šumbera R., Burda H., Němec P. 2014. Light propagation in burrows of subterranean rodents: tunnel system architecture but not photoreceptor sensitivity limits light sensation range. Journal of Zoology (London) 294:67–75.

Krehbiehl M. 2010. Anatomie der rostralen und caudalen Tasthaare beim Sambischen Riesengraumull (*Fukomys mechowii*) – Sinushaare oder Leithaare? Ph.D. dissertation, Hochschule Hannover, Hannover, Germany.

Kundu S., Faulkes C.G. 2004. Patterns of MHC selection in African mole-rats, family Bathyergidae: the effects of sociality and habitat. Proceedings of the Royal Society of London, B: Biological Sciences 271:273–278.

Kverková K., Bělíková T., Olkowicz S., Pavelková Z., O'Riain M.J., Šumbera R., Burda H., Bennett N.C., Němec P. 2018. Sociality does not drive the evolution of large brains in eusocial African mole-rats. Scientific Reports 8:9203.

Landry S.O. 1957. Factors affecting the procumbency of rodent upper incisors. Journal of Mammalogy 38:223–234.

Lange S. 2006. Sinnesökologie afrikanischer Sandgräber (Bathyergidae) am Beispiel von Hör- und Geruchssinn Ph.D. dissertation, University of Duisburg-Essen, Essen, Germany.

Lange S., Burda H. 2005. Comparative and functional morphology of the middle ear in Zambian mole-rats (*Coetomys* – *Cryptomys*, Bathyergidae). Belgian Journal of Zoology 135:5–10.

Lange S., Burda H., Wegner R.E., Dammann P., Begall S., Kawalika M. 2007. Living in a "stethoscope": burrow-acoustics promote auditory specializations in subterranean rodents. Die Naturwissenschaften 94:134–138.

Lindenlaub T., Burda H., Nevo E. 1995. Convergent evolution of the vestibular organ in the subterranean mole-rats, *Cryptomys* and *Spalax*, as compared with the aboveground rat, *Rattus*. Journal of Morphology 224:303–311.

Lövy M., Sklíba J., Sumbera R. 2013. Spatial and temporal activity patterns of the free-living giant mole-rat (*Fukomys mechowii*), the largest social bathyergid. PLoS One 8:e55357.

Lutermann H., Carpenter-Kling T., Ueckermann E.A., Gutjahr G., Bennett N.C. 2015. Ectoparasite burdens of the Damaraland mole-rat (*Fukomys damarensis*) from Southern Africa. The Journal of Parasitology 101:666–670.

Lutermann, H., Haukisalmi V., Junker K. 2018. First report of gastrointestinal parasites from Ansell's mole-rat (*Fukomys anselli*) in Zambia. Journal of Parasitology 104:566–570.

Marhold S., Burda H., Kreilos I., Wiltschko W. 1997b. Magnetic orientation in common mole-rats from Zambia. In: Orientation and navigation: birds, humans and other animals. Paper No. 5. Royal Institute of Navigation, Oxford, United Kingdom.

Marhold S., Nagel A. 1995. The energetics of the common mole rat *Cryptomys*, a subterranean eusocial rodent from Zambia. Journal of Comparative Physiology, B. Biochemical, Systemic, and Environmental Physiology 164:636–645.

Marhold S., Wiltschko W., Burda H. 1997a. A magnetic polarity compass for direction finding in a subterranean mammal. Naturwissenschaften 84:421–423.

Matschei C., Bäthe R. 2012. Riesengraumull und Mashona-Graumull – zwei Sandgräberarten. Mitteilungen der Bundesarbeitsgruppe (BAG) Kleinsäuger e. V 2:10–14.

McIntosh A.F., Cox P.G. 2016a. The impact of gape on the performance of the skull in chisel-tooth digging and scratch digging mole-rats (Rodentia: Bathyergidae). Royal Society Open Science 3:160568.

McIntosh A.F., Cox P.G. 2016b. Functional implications of craniomandibular morphology in African mole-rats (Rodentia: Bathyergidae). Biological Journal of the Linnean Society 117:447–462.

Monadjem A., Taylor P.J., Denys C., Cotterill F.P. 2015. Rodents of Sub-Saharan Africa: a biogeographic and taxonomic synthesis. Walter de Gruyter, Berlin, Germany.

Morlok W.F. 1983. Vergleichend- und funktionell-anatomische Untersuchungen an Kopf, Hals und Vorderextremität subterraner Nagetiere (Mammalia, Rodentia). Courier Forschungsinstitut Senckenberg 64:1–237.

Müller M., Burda H. 1989. Restricted hearing range in a subterranean rodent, *Cryptomys hottentotus*. Die Naturwissenschaften 76:134–135.

Müller M., Laube B., Burda H., Bruns V. 1992. Structure and function of the peripheral auditory system in the African mole rat (*Cryptomys hottentotus*): evidence for a low frequency acoustic fovea. Journal of Comparative Physiology, A. Sensory, Neural, and Behavioral Physiology 171:469–476.

Němec P., Altmann J., Marhold S., Burda H., Oelschlager H.H. 2001. Neuroanatomy of magnetoreception: the superior colliculus involved in magnetic orientation in a mammal. Science 294:366–368.

Němec P., Burda H., Peichl L. 2004. Subcortical visual system of the African mole-rat *Cryptomys anselli*: to see or not to see? The European Journal of Neuroscience 20:757–768.

Oelschläger H.H., Nakamura M., Herzog M., Burda H. 2000. Visual system labeled by c-Fos immunohistochemistry after light exposure in the 'blind' subterranean Zambian mole-rat (*Cryptomys anselli*). Brain, Behavior and Evolution 55:209–220.

Patzenhauerová H., Šklíba J., Bryja J., Šumbera R. 2013. Parentage analysis of Ansell's mole-rat family groups indicates a high reproductive skew despite relatively relaxed ecological constraints on dispersal. Molecular Ecology 22:4988–5000.

Peichl L., Nemec P., Burda H. 2004. Unusual cone and rod properties in subterranean African mole-rats (Rodentia, Bathyergidae). The European Journal of Neuroscience 19:1545–1558.

Poduschka W. 1978. Abwehrreaktion der Mullratte, *Cryptomys hottentotus* (Lesson, 1826). Säugetierkundliche Mitteilungen 26:260–268.

Prochel J., Begall S., Burda H. 2013. Morphology of the carpal region in some rodents with special emphasis on hystricognaths. Acta Zoologica 95:220–238.

Scharff A. 1998. Systematik und Verhaltensökologie sambischer Sandgräber (Bathyergidae, Rodentia) Ph.D. dissertation, University of Essen, Essen, Germany.

Scharff A., Burda H., Tenora F., Kawalika M., Barus V. 1997. Parasites in social subterranean Zambian mole-rats (*Cryptomys* spp., Bathyergidae, Rodentia). Journal of Zoology (London) 241:571–577.

Scharff A., Grütjen O. 1997. Evidence for aboveground activity of Zambian mole rats (*Cryptomys*, Bathyergidae, Rodentia). Zeitschrift für Säugetierkunde 62:253–254.

Schielke C.K.M., Begall S., Burda H. 2012. Reproductive state does not influence activity budgets of eusocial Ansell's mole-rats, *Fukomys anselli* (Rodentia, Bathyergidae): a study of locomotor activity by means of RFID. Mammalian Biology 77:1–5.

Schielke C.K.M., Burda H., Henning Y., Okrouhlík J., Begall S. 2017. Higher resting metabolic rate in long-lived breeding Ansell's mole-rats (*Fukomys anselli*). Frontiers in Zoology 14:45.

Schmidt J. 2014. Das Vokalisationsrepertoire von Ansell-Graumullen (*Fukomys anselli*) State examination thesis, Universität Duisburg-Essen, Essen, Germany.

Schmitt M., Bappert M.-T., Krüger C., Burda H. 2009. The mole-rat's pseudo thumb. Mammalian Biology-Zeitschrift für Säugetierkunde 74:23.

Sichilima A.M., Bennett N.C., Faulkes C.G. 2011. Field evidence for colony size and aseasonality of breeding and in Ansell's mole-rat, *Fukomys anselli* (Rodentia: Bathyergidae). African Zoology 46:334–339.

Šklíba J., Jirků M., Šumbera R. 2016a. Burrow systems of mole-rats as refuges for frogs in the Miombo woodlands of south-east Africa. Journal of Tropical Ecology 32:158–161.

Šklíba J., Lövy M., Burda H., Šumbera R. 2016b. Variability of space-use patterns in a free living eusocial rodent, Ansell's mole-rat indicates age-based rather than caste polyethism. Scientific Reports 6:37497.

Šklíba J., Lövy M., Hrouzková E., Kott O., Okrouhlík J., Šumbera R. 2014. Social and environmental influences on daily activity pattern in free-living subterranean rodents: the case of a eusocial bathyergid. Journal of Biological Rhythms 29:203–214.

Šklíba J., Mazoch V., Patzenhauerová H., Hrouzková E., Lövy M., Kott O., Šumbera R. 2012. A maze-lover's dream: burrow architecture, natural history and habitat characteristics of Ansell's mole-rat (*Fukomys anselli*). Mammalian Biology 77:420–427.

Šumbera R. 2019. Thermal biology of a strictly subterranean mammalian family, the African mole-rats (Bathyergidae, Rodentia) - a review. Journal of Thermal Biology 79:166–189.

Šumbera R., Mazoch V., Patzenhauerová H., Lövy M., Šklíba J., Bryja J., Burda H. 2012. Burrow architecture, family composition and habitat characteristics of the largest social African mole-rat: the giant mole-rat constructs really giant burrow systems. Acta Theriologica 57:121–130.

Thomas O. 1917. Notes on *Georychus* and its allies. The Annals and Magazine of Natural History; Zoology, Botany, and Geology 20:441–444.

Valesky E.M., Burda H., Kaufmann R., Oelschläger H.H. 2012. Distribution of oxytocin- and vasopressin-immunoreactive neurons in the brain of the eusocial mole rat (*Fukomys anselli*). Anatomical Record 295:474–480.

Van Daele P.A.A.G., Dammann P., Meier J.L., Kawalika M., Van De Woestijne C., Burda H. 2004. Chromosomal diversity in mole-rats of the genus *Cryptomys* (Rodentia: Bathyergidae) from the Zambezian region: with descriptions of new karyotypes. Journal of Zoology (London) 264:317–326.

Van Daele P.A., Herrel A., Adriaens D. 2008. Biting performance in teeth-digging African mole-rats (*Fukomys*, Bathyergidae, Rodentia). Physiological and Biochemical Zoology 82:40–50.

Van Daele P.A., Verheyen E., Brunain M., Adriaens D. 2007. Cytochrome b sequence analysis reveals differential molecular evolution in African mole-rats of the chromosomally hyperdiverse genus *Fukomys* (Bathyergidae, Rodentia) from the Zambezian region. Molecular Phylogenetics and Evolution 45:142–157.

Van Wassenbergh S., Heindryckx S., Adriaens D. 2017. Kinematics of chisel-tooth digging by African mole-rats. The Journal of Experimental Biology 220(Pt 23):4479–4485.

Wegner R.E., Begall S., Burda H. 2006a. Light perception in 'blind' subterranean Zambian mole-rats. Animal Behaviour 72:1021–1024.

Wegner R.E., Begall S., Burda H. 2006b. Magnetic compass in the cornea: local anaesthesia impairs orientation in a mammal. The Journal of Experimental Biology 209(Pt 23):4747–4750.

Weir B.J., Rowlands J.W. 1974. Functional anatomy of the hystricomorph ovary. Symposia of the Zoological Society of London 34:303–332.

Willingstorfer W.J., Burda H., Winckler J. 1998. Ovarian growth and folliculogenesis in breeding and nonbreeding females of a social rodent, the Zambian common mole-rat, *Cryptomys* sp. Journal of Morphology 237:33–41.

Winkler A.J., Denys C., Avery D.M. 2010. Rodentia. In: Werdelin L., Sanders W.J., editors. Cenozoic mammals of Africa. University of California Press, Berkeley, California, USA; p. 263–304.

Wroughton R.C. 1907. On a collection of mammals made by S.A. Neave in Rhodesia, north of the Zambesi, with field notes by the collector. Manchester Memoirs 51:1–39.

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Discussion

It is not without irony that both of the two taxa discussed in the Mammalian Species Accounts may both not represent valid species. As detailed in the article, the giant mole-rat will need to be split into two species, *F. mechowii* (Peters, 1881) in the West (Angola, Western Democratic Republic of Congo; type locality: Malange, Angola) and *F. mellandi* (Thomas, 1906) in the East (Zambia; type locality: Mpika, Zambia). *F. mellandi* is more closely related to the small-bodied *F. vandewoestijneae* Van Daele, 2013 than it is to *F. mechowii*. This implies that *F. vandewoestijneae* is either a recently evolved dwarf among his larger relatives or that giant size (> 500 g) evolved twice in *Fukomys*. Given that all giant mole-rats in captivity, including zoo-housed groups, are of Zambian descent, the current denomi-nation of these animals as *F. mechowii* (incl. Chapter 2.1) will soon be outdated and needs to be changed to *F. mellandi*. The now imminent splitting of giant mole-rats has already been anticipated by Van Daele et al. (2007) and Kawalika and Burda (2007) but has not been appropriately discussed by various later papers on the phylogeny and taxonomy of *Fukomys* mole-rats (e.g., Visser et al., 2019). The immediate goal of future taxonomic work on giant mole-rats will be to delineate the ranges and diagnostic characters of the two species. Anecdotal evidence suggests that Eastern and Western giant mole-rats are hardly distinguishable based on external morphology (Van Daele et al., 2007) and a study presenting data on skull measurements from species of the *Mechowii* clade did not recover Eastern and Western giant mole-rats clustering separately (Van Daele et al., 2013). It should be noted, however, that this study did not aim to differentiate between giant mole-rat populations and pooled both sexes for the morphometric analyses, complicating the identification of potential species-specific patterns.

Just like *F. mechowii*, *F. anselli* needs to undergo taxonomic revision in the near future. However, its case appears to be more complicated. As detailed in Chapter 2.1, *F. anselli* (or Central Zambian mole-rats with the diagnostic karyotype of 2n = 68, to be precise) is not consistently recovered as monophyletic. If larger molecular datasets continue to be indicative of that, two options present themselves for the taxonomy of this species and its relatives: The name *F. anselli* is retained for the most inclusive taxon encompassing animals from the type locality (Lusaka, Zambia) but not other named lineages. That, however, would likely necessitate the designation of various other Central Zambian *Fukomys* species (or subspecies, dependent on the rank one might give *F. anselli*) with increasingly small ranges, given the convoluted branching patterns within the *Micklemi* species group, to which *F. anselli* belongs (Van Daele et al., 2007). Second, the complete *Micklemi* group will be lumped into a single species, which, considering the principle of priority, should carry the name *Fukomys micklemi* (Chubb, 1909). The latter option was already cautiously advocated by Van Daele et al. (2007). For the *F. anselli* research community, which has produced a substantial bibliography on animals deriving from the type locality, such a nomenclatural adjustment could proof to be a great inconvenience, but it indeed appears to be a sensible step.

The description of *F. anselli* dates back to a time in which mole-rat species were designated solely based on their karyotype (other examples include *F. darlingi* – Aguilar, 1993; *F. kafuensis* – Burda et al., 1999; *F. micklemi* - Van Daele et al., 2004; *F. whytei* – Burda et al., 2005). Molecular datasets were not yet widely available and morphological evidence was not deemed relevant due to the close resemblance of various small-bodied *Fukomys* populations (Burda et al., 1999). At the same time, it was often not discussed why a species designation based on karyotype is justified. Karyotypic integrity in *Fukomys* is extremely fragile (Van Daele et al., 2004) and it remains to be clarified how stable specific karyotypes in specific lineages are. In fact, when convenient, populations exhibiting different chromosomal numbers have been grouped within one species, as has been the case in *F. damarensis* (2n = 74/78 – Nevo et al., 1986), *F. foxi* (2n = 66/70 – Williams et al., 1983), and also in populations assigned to *F. micklemi* (2n = 56/58/60 – compare Van Daele et al., 2004, 2019), that are closely affiliated with *F. anselli*. Remarkably, different karyotypes for *F. micklemi* have even been reported from the species' type locality: While Meier (2002) determined a karyotype of 2n = 58 for animals from Kataba, Van Daele et al. provide 2n = 60. In line with that, karyotypic instability within *Fukomys* has been described to peak among the *Micklemi* species group (Van Daele et al., 2007). Variation in karyotypes should therefore not be seen as a crucial obstacle to integrate *F. anselli* into *F. micklemi*. Interestingly, many other subterranean rodents are also renowned for their karyotypic diversity, particularly the blind mole-rats of the genera *Nannospalax* and *Spalax* (Nevo et al., 1986; Arslan et al., 2016). During the 1990s, it was advocated to designate each known cytotype in blind mole-rats as a species, which would have resulted in well over 50 species (Nevo et al., 1994). Nowadays it is acknowledged, that the 73 chromosomal races (including 12 different diploid chromosomal numbers) described in blind mole-rats are only of limited taxonomic value and lose out against molecular data when it comes to species designation (Arslan et al., 2016). I am in favor of adopting these principles in *Fukomys* taxonomy as well.

When it comes to morphological differences, very little is known about the variation in all alleged species of the *Micklemi* group. Laboratory lineages of *F. micklemi* (2n = 60) and *F. anselli* can be quickly differentiated based on pelage coloration but not necessarily through other traits (pers. obs.). Whether fur color can also inform robust differentiation between these species in the wild is not known but appears doubtful, due to the great intraspecific phenotypic variability of *Fukomys* populations. For instance, animals captured close to the type locality of *F. micklemi* (Western Province of Zambia, left bank of the Zambezi River close to the Kataba River) have been reported to be either blueish black (Chubb, 1909) or dark grey (Meier, 2002), with the lab lineage deriving from the same locality showing two pelage color morphs, one light ochre brown, one dark brown (pers. obs., compare Chapter 2.4). Furthermore, preliminary observations on skull morphology suggest no differentiation between the two lineages in that respect (Müller, 2021). A

thorough morphological study of animals deriving from the different lineages assigned to the *Micklemi* clade is necessary to identify how much these mole-rats vary in traits that might inform a species diagnosis. Such work needs to either include data collection in the wild or in museum collections as the few lineages held in captivity only represent fractions of the total diversity that is to be expected in the *Micklemi* clade. Tragically, the opportunity to collect these data together with karyological studies in diverse lineages of the *Micklemi* clade has not been seized in the past (Van Daele et al., 2004).

It still is a long way to have established a robust taxonomy of the genus *Fukomys*. Apart from the aforementioned issues of species designations based on karyology alone, there have been recent attempts to introduce *Fukomys* species solely on morphological grounds ("*Fukomys ilariae*", a notorious taxon described by Gippoliti & Amori, 2011 from one damaged and idiosyncratic museum voucher) or markedly limited molecular data ("*Fukomys choma*", a nomen nudum introduced by Visser et al., 2019 based on cytochrome b sequences). This has to be avoided in the future. A revision of the currently accepted species or efforts to describe new ones should be integrative and rely on several lines of evidence instead of just one to avoid nomenclatural chaos in potential future revisions. Recently, Kitchener et al. (2017, 2022) presented a revised taxonomy of the family Felidae including a traffic light system for taxonomic certainty. It increases transparency by recognizing knowledge gaps and suggests to group (sub)species into three grades of taxonomic robustness, based on the availability of data on morphology, genetics, and biogeography for the respective populations. Such an approach can be particularly valuable for morphologically variable taxa with a long taxonomic and nomenclatural history, as is the case with common mole-rats (Ellerman et al., 1953). This example might act as an instructive guide for a thorough future revision of the remarkably diverse genus *Fukomys*, including *F. anselli* and *F. mechowii*.

2.2 – Magnetoreception

Eyes are essential for magnetoreception in a mammal

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- **Conception** – 0 %, the study was conceived by REM, PN, and SB.
- **Data collection** – 50%: I collected the majority of data for experimental series 2.
- **Data analyses** – 25 %: Data analysis was undertaken by SB, REM, EPM, KM, and me.
- **Writing the manuscript** – 85 %: I wrote the initial draft of the manuscript with input from all coauthors, particularly SB and EPM.
- **Revising the manuscript** – 65 %: I revised the manuscripts following the reviewer's comments together with input from all coauthors.

_____ _____

Signature of Ph.D. student Signature of supervisor

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THE ROYAL SOCIETY PUBLISHING

Eyes are essential for magnetoreception in a mammal

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Several groups of mammals use the Earth's magnetic field for orientation, but their magnetosensory organ remains unknown. The Ansell's mole-rat (*Fukomys anselli*, Bathyergidae, Rodentia) is a microphthalmic subterranean rodent with innate magnetic orientation behaviour. Previous studies on this species proposed that its magnetoreceptors are located in the eye. To test this hypothesis, we assessed magnetic orientation in mole-rats after the surgical removal of their eyes compared to untreated controls. Initially, we demonstrate that this enucleation does not lead to changes in routine behaviours, including locomotion, feeding and socializing. We then studied magnetic compass orientation by employing a well-established nest-building assay under four magnetic field alignments. In line with previous studies, control animals exhibited a significant preference to build nests in magnetic southeast. By contrast, enucleated mole-rats built nests in random magnetic orientations, suggesting an impairment of their magnetic sense. The results provide robust support for the hypothesis that mole-rats perceive magnetic fields with their minute eyes, probably relying on magnetite-based receptors in the cornea.

1. Introduction

Magnetoreception, the ability to perceive magnetic fields, is a sensory modality that occurs in all major vertebrate groups as well as in a range of invertebrate taxa [1–3]. The hunt for a magnetic sense in mammals has gained pace since the late 1980s. By now magnetoreception has been shown in numerous mammal groups (reviewed in [4]). Still, even though rodents are readily available for experiments under laboratory conditions, only a few attempts have been made to explore the underlying physiology of this sensory modality in mammals. Past studies employed methods spanning electrophysiology [5], pharmacological inhibition [6] and neural activity mapping [7,8], but their paucity contrasts with the great number of methodologically diverse experiments performed with birds [9–11]. Therefore, the location, structure and functional properties of mammalian magnetoreceptors are still elusive [4].

A popular hypothesis proposes that magnetite crystals (Fe_3O_4) linked to mechanosensitive ion channels could enable magnetically modulated neuronal excitation, rendering magnetoreception possible [3,12]. Such magnetite receptors would function light independently and could be located anywhere in the body. In mammals, there is evidence consistent with magnetite-mediated magnetoreception in bats [13,14] and in Ansell's mole-rats (*Fukomys anselli*), a subterranean rodent from the woodlands of Central Zambia [15].

Ansell's mole-rat is a model species in the study of magnetoreception because of its strong innate preference to build nests in the south-eastern

sector of a circular arena [15]. This orientation bias is species-specific, with other African mole-rats displaying deviating magnetic preferences in analogous experimental settings [16]. Nest-building assays have revealed several properties of the Ansell's mole-rat's magnetic compass: it works in total darkness and responds to changes in field polarity but not in inclination [17]; it is affected by strong magnetic pulses [18] but is insensitive to radiofrequency magnetic fields [19]. Responsiveness to radiofrequencies was tested by applying a broadband field of 0.1–10 MHz with an intensity of 85 nT or 1.315 MHz fields of either 480 nT or 4800 nT intensity, but none affected the magnetic orientation of the mole-rats [19]. The mentioned characteristics of the mole-rat's magnetic sense fit magnetite-based receptors but seem incompatible with the radical pair mechanism of magnetoreception [19], which is prominently hypothesized to be present in birds [10,11] and also in murid rodents [20]. However, the anatomical locus of the corresponding receptor cells remains unknown.

The eye has a crucial role in the avian magnetic compass response ([10,11] but see [21]) and has therefore also attracted attention as a potential location of magnetoreceptors in mammals (e.g. [6]). Although Ansell's mole-rat spends most of its life in darkness and visual input is of marginal behavioural relevance for the species (cf. [22]), its minute eyes show no sign of qualitative regression. Instead, they conform to the mammalian ground pattern in displaying all typical anatomical features [23]. Wegner *et al.* [6] reported that local lidocaine anaesthesia of the cornea led to a loss of directional nest building in Ansell's mole-rats. The treatment did not impair light–dark discrimination, suggesting that at least some functions of the retina were unaffected. The authors concluded that the mole-rat magnetoreceptors are located in the cornea, but the utility of lidocaine for behavioural testing has significant drawbacks. First, induced anaesthesia, even after repeated applications, only lasts up to 15 min [24]. Second, lidocaine is lipophilic and can diffuse widely, potentially affecting untargeted tissues. Lidocaine further binds to serum proteins in the blood and rapidly crosses the blood–brain barrier, where it may induce non-specific effects on the central nervous system [25]. Therefore, ablation experiments should be preferred over local anaesthesia in experiments aimed to narrow down the site of magnetoreceptors [26]. To test the hypothesis that the magnetoreceptors of Ansell's mole-rats are indeed located in the eye, we assessed magnetic orientation during nest building in subjects with surgically removed eyes.

2. Materials and methods

2.1. Subjects

We studied 40 (series 1 : 6 males, 6 females; series 2 : 16 males, 12 females) adult Ansell's mole-rats (*Fukomys anselli*, Bathyergidae, Rodentia) from the breeding stock of the Department of General Zoology at the University of Duisburg-Essen. All subjects were born in captivity and socially housed as either pairs or family groups of variable size. In enucleated mole-rats (series 1 : 12 subjects; series 2 : 10 subjects), the eyes were removed entirely. Surgical removal of the miniscule eyes is uncomplicated, as no bony orbit, is present and the ocular musculature, apart from the musculus retractor bulbi, is severely reduced [23]. Excision of the eye was accomplished under general anaesthesia and after application of a local muscle relaxant by a single cut with curved microsurgical scissors. This procedure sectioned the optic nerve and the three oculomotor cranial nerves as well as the part of the ophthalmic branch of the trigeminal nerve that innervates the eye, including the cornea. Other portions of the ophthalmic branch of the trigeminal nerve, which innervate the snout region, and other cranial nerves remained intact so that the neurological effects of the surgery remained precisely localized and impairment of animal welfare minimal. The enucleation procedure is further described in [27]. The recovery period between surgery and testing was a minimum of three weeks and 18 months in the first and second series of experiments, respectively. During recovery and following the experiments, the animals were socially housed in their home terraria, analogous to control group subjects. All surgeries and experiments conformed to the relevant ethical standards and were approved by the animal welfare officer of the University of Duisburg-Essen and the LANUV NRW, Germany (series 1: 50.05-230-37/06; series 2: 84-02.04.2015.A387).

2.2. Ethograms

Using video recordings, we compared the behaviour of enucleated ($n = 6$) and control ($n = 8$) adult mole-rats of four families from experimental series 2 in their home terraria for a minimum of 435 min (maximum 1338 min). The analysis of the videos was performed manually and blindly with respect to the experimental group (the resolution of the videos was not sufficient to resolve the mole-rats' minute eyes) by one observer (Katharina Schröer) who was not further involved in the project. Behaviours were evaluated separately for each individual on a minute by minute basis. The behaviour that lasted longest during the respective minute was noted; comparatively rare events (grooming, sniffing, and social play) were noted even if they occurred only shortly during the respective minute interval. The time budgets of the following routine behaviours were determined for each animal: resting, feeding (including food transport), locomotion (including digging), sniffing, grooming (auto- and allogrooming) and social play (e.g. sparring with teeth, play fighting). The fraction of each behavioural category per total amount of recording time was calculated. Statistical differences between the experimental groups were tested in *R* version 3.6.0 [28] with a binomial general linear model with experimental group and type of behaviour as factors.

2.3. Nest-building assay

We used the established nest-building assay to study magnetic compass orientation in Ansell's mole-rat (e.g. [6,15,17,19]). When being offered nesting material, mole-rats will typically start constructing nests within a short period of time, irrespective of their sex and reproductive status. The disposition to build nests also does not differ between animals tested in pairs, in groups or alone. Each pair was treated as a statistical unit. Animals were placed singly or in pairs (table 1) inside an opaque circular plastic arena (80 cm diameter, 30 cm height) and were given approximately 60 min to build a nest from paper scraps, which were evenly distributed across the arena. A white PVC cylinder (diameter 15 cm; height 20 cm) was placed in the middle of the arena to prevent nest construction in the centre. When paper scraps were used to construct a clearly demarcated nest mound (rather than a diffuse, potentially random aggregation of material), its position was documented photographically. In case no unequivocal nest was constructed ($n = 10$ for controls, $n = 8$ for enucleated subjects in series 2), animals repeated trials under the respective magnetic condition until a valid nest was built.

In experimental series 1, mole-rat pairs were tested under ambient local geomagnetic field conditions (51°27′50.3″ N 7°00′18.9″ E, 48.6 µT, 66° inclination). Tests were performed in 2006 and took place in a non-magnetic unshielded greenhouse

Table 1. Basic information on study animals used in series 1 (2006) and series 2 (2018/2019). Data on age refer to the time of testing. μ and r represent the direction and lengths of the angular mean vector regarding magnetic north, respectively.

Series 1 (2006)						
ID	sex	age (months)	μ (control)	r (control)	μ (enucl.)	r (enucl.)
0287	F	30	119°	0.99	285°	0.32
3854	M	30				
7556	F	82	89°	0.68	250°	0.11
0919	M	60				
2668	F	47	182°	0.59	41°	0.38
3900	M	60				
2848	F	72	127°	0.46	150°	0.31
3369	M	32				
9546	F	56	134°	0.58	37°	0.32
3919	M	59				
8118	F	91	105°	0.43	106°	0.67
0466	M	106				

Series 2 (2018/2019)					
ID	sex	age (months)	enucleated/control	μ	r
2654	F	55	both enucleated	47°	0.06
0328	M	55			
6125	F	66	both enucleated	125°	0.21
6752	M	39			
5496	F	69	both enucleated	71°	0.60
0861	M	54			
1334	F	75	both enucleated	250°	0.27
0772	M	78			
0772	F	31	both control	111°	0.98
5813	M	43			
6767	F	26	both control	142°	0.80
0816	M	51			
4780	M	69	both control	196°	0.58
0726	M	21			
0724	F	102	both control	150°	0.51
0717	M	23			
4295	F	57	enucleated	241°	0.60
2175	M	65	enucleated	30°	0.21
9074	F	90	control	245°	0.34
1641	M	58	enucleated	118°	0.37
5683	F	44	enucleated	218°	0.68
0528	F	151	control	216°	0.87
3360	M	184	enucleated	270°	0.08
6126	M	42	enucleated	214°	0.09
2731	M	27	control	18°	0.06
0737	F	25	control	185°	0.74
5222	M	26	control	121°	0.19
3493	M	24	control	195°	0.69

(made of plastic and aluminium) at Essen University campus, Germany. Animals were manually introduced into the arena from random directions. During testing, the arena was covered with a light-impervious lid to exclude possible visual orientation. A potential influence of ambient auditory stimuli from the campus environment could not be fully mitigated, but we made no observations potentially reflective of acoustically induced biases. Furthermore, Ansell's mole-rats have limited hearing capabilities especially at frequencies above 1 kHz [29]. Each mole-rat pair underwent four tests under control conditions and six tests after enucleation.

In experimental series 2, individual animals or pairs were tested in Essen-Haarzopf, a rural area under ambient geomagnetic field conditions (49 µT, 66° inclination) in the periphery of the city of Essen, Germany (51°25′09.6″ N 6°56′50.9″ E). Tests were performed in 2018–2019 and took place in a windowless wooden hut constructed completely of non-magnetic materials. The interior of the hut was shielded from radiofrequencies by a grounded Faraday cage made of aluminium mesh. Radiofrequency noise within the hut was low, peaking at 0.4 MHz with intensities of approximately 10 nT, but well below 1 nT for frequencies up to 100 MHz (discussed and visualized in [30]). To be able to distinguish topographic biases from magnetic orientation, each individual/pair was tested in the ambient magnetic field (mN = 360°) and three shifted fields with magnetic north at geographic east (mN = 90°), south (mN = 180°) and west (mN = 270°), respectively; the inclination and the intensity of the shifted magnetic fields remained unchanged (intensity: 48.5 ± 0.18 µT; inclination: 65.5 ± 0.05°). The intensity has been measured with a three-axial magnetic field sensor (FGM3D, Sensys, Bad Saarow, Germany). The sequence of magnetic conditions was randomized. The local magnetic field was manipulated by a double-wrapped three-axial four coil Merritt system (edge length: 3 m × 3 m × 3 m) powered by a programmable multichannel power supply (HMP4040, Rohde & Schwarz, Munich, Germany). Importantly, during the ambient magnetic field tests, the identical current was run antiparallelly through the coil system, so that possible side effects of the operational coils (noise, vibrations, heat, electric fields) were identical for all sessions. The experimental arena was placed within a square wooden box (edge length: 120 cm) equipped with four windows (100 cm length × 80 cm height), one on each cardinal direction, which were used to place animals into the arena from different topographical positions in a randomized sequence. These windows were closed during testing. The arena was fixed on a pedestal seated in a sandbox to minimize substrate vibrations.

During testing, an LED table lamp emitting monochromatic red light (Parathom R50 80.337 E14 Red 617 nm, 6 W, Osram, Munich, Germany) illuminated the room outside of the wooden box to allow the experimenters to operate. African mole-rats are not capable of perceiving red light [22,31]. Photon density (light level) within the wooden box under testing conditions was less than 0.001 µmol s^{-1} m^{-2} (measured by LI-COR photometer, model LI-250). The temperature of the hut was controlled by an air conditioning system (Model FTXS50, Daikin) and was measured before (mean: 26.37°C; s.d.: ± 1.74) and after (mean: 23.53°C; s.d.: ± 2.06) each experimental run. The air conditioner was deactivated during experiments to minimize directional acoustic cues and prevent disruption of the magnetic environment within the hut.

2.4. Data analysis

Nest orientations were scored (to the closest 5°) with reference to topographic north from photos by an experimenter (S.B.) blind to the magnetic condition and experimental group. We refer to topographic north as the direction of magnetic north before the field had been artificially rotated. After scoring, data from the four magnetic conditions were back-transferred by subtracting 90° (mN = east), 180° (mN = south) or 270° (mN = west) to obtain values with reference to magnetic north (for details confer [32]). Combining the data collected under different magnetic conditions but with reference to topographic north will be non-random, if the animals use non-magnetic cues for orientation. On the other hand, if the pooled data with reference to magnetic north will be non-random, the animal's orient by using magnetic cues. Mean vectors of the four trials of each individual/pair were calculated with respect to magnetic or topographic north via vector addition. Second-order statistics (Moore's modified Rayleigh test) using mean vectors and lengths of the respective mean vectors were used to detect significant deviations from a random distribution, with $\alpha = 0.05$. To test for a symmetric bimodal distribution, we employed Moore's modified Rayleigh test on doubled angles. All calculations were performed with Oriana 4.02 (Kovach Computing, United Kingdom). We performed model-fitting statistics in *R* using the package CircMLE, including all ten models available in the package [33]. Mann–Whitney U tests were used to test for differences in the length of the mean vectors of both experimental groups (GraphPad Prism V. 8.4.3).

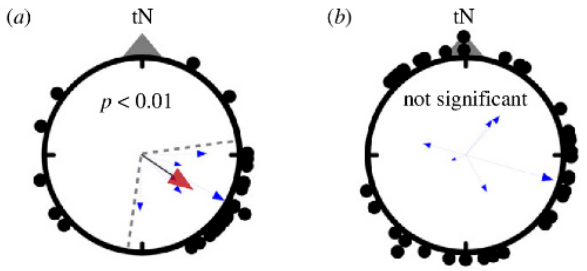

Figure 1. Pilot experiments showing a loss of directional preferences in the Ansell's mole-rat after enucleation. Nest distribution of six pairs of Ansell's mole-rats (*a*) before (control) and (*b*) after surgery (enucleation). Control trials ($n = 4$ per pair) exhibited a significant preference to build nests in the magnetic southeast, while the nests built by the same pairs after surgery ($n = 6$ per pair) were distributed randomly. The small blue arrows represent the mean vectors of each pair of Ansell's mole-rats and the arrow lengths reflect the r-values, a measure of the concentration of the nests. The red arrows are the weighted mean vector calculated over the mean vectors of tested pairs when significant; 95% confidence intervals of the weighted grand mean are indicated by dashed lines. The dots outside of the circles indicate the positions of all nests of each experimental group (controls: 24, enucleated: 36). The *p*-values indicate the results from Moore's modified Rayleigh tests performed on the mean vectors.

3. Results and discussion

In the first series of experiments performed in 2006, we tested six mole-rat pairs before and after enucleation using the nest-building assay in the ambient magnetic field (table 1). Before enucleation, the animals displayed a strong preference for the south-eastern sector of the arena (figure 1*a*; mean direction: 124°, 95% confidence interval (CI): 82°–188°, mean vector length $r = 0.549$, $n = 6$, Moore's modified Rayleigh test: $R^* = 1.231$, $p < 0.01$), in line with published magnetic preferences in Ansell's mole-rats [6,15,17,19]. By contrast, the distribution of nests built after enucleation was random (figure 1*b*, $r = 0.140$, $n = 6$, Moore's modified Rayleigh test: $R^* = 0.647$, $p > 0.1$). These results suggested an impairment of the magnetic sense but did not allow us to exclude topographic factors or series effects.

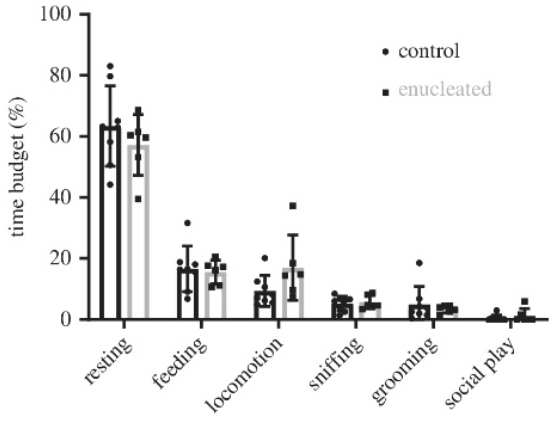

Figure 2. Enucleation does not affect the general behaviour of Ansell's mole-rats. Mean time budgets of six behaviours of enucleated and control Ansell's mole-rats observed in their home enclosures. All behaviours are expressed as the percentage of time spent per observation period. There were no significant differences between enucleated subjects and controls (binomial GLM for proportional data (behaviour × treatment): $t = -0.536$, $p = 0.594$). $n = 6$ (enucleated) and $n = 8$ (controls). Error bars represent the standard deviation.

To address these questions, we conducted a second series of experiments in 2018–2019, in which we tested a new cohort of enucleated ($n = 10$) and control ($n = 10$) animals with a coil setup that precisely controlled the magnetic field (table 1). Furthermore, to minimize unspecific effects of the surgery, the behavioural experiments were performed more than 1.5 years after enucleation. We ascertained whether the enucleated animals behaved normally by recording ethograms of the experimental and control subjects in their home enclosures. The measurements of the six quantified behaviours revealed no significant differences between enucleated subjects and controls (figure 2; binomial GLM: $t = -0.536$, $p = 0.594$), indicating that enucleation does not impact routine behaviour in captive settings.

In the nest-building assay, all animals were tested in four different magnetic field alignments to distinguish magnetic from topographic orientation responses. As in the first experimental series, control animals displayed a significant preference for the magnetic south-eastern sector of the arena (figure 3*a*; mean direction: 172°, CI: 119°–222°, $r = 0.441$, $n = 10$, Moore's modified Rayleigh test: $R^* = 1.268$, $p < 0.01$). By contrast, the magnetic distribution of nests built by enucleated mole-rats was indistinguishable from random (figure 3*b*; $r = 0.129$, $n = 10$, Moore's modified Rayleigh test: $R^* = 0.519$, $p > 0.5$). This difference was also expressed by the significantly shorter mean vectors of enucleated compared to control animals (figure 3*c*; median $r_{enucleated}$: 0.243, median $r_{control}$: 0.637, $n = 10$, one-tailed Mann–Whitney test: $U = 27.5$, $p = 0.045$). With respect to topographic north, the nest directions of the controls did not deviate from a random distribution (figure 3*d*; $r = 0.062$, $n = 10$, $R^* = 0.396$, $p > 0.5$). The nest directions of enucleated animals with respect to topographic north, however, did not appear random, so we used a model-fitting approach to identify the most likely underlying distribution. Three distributions were almost equally likely, with two of them being axial distributions (electronic supplementary material, table S1). Indeed, testing for an axial distribution using Moore's modified Rayleigh test on doubled angles revealed a preference to build nests along the topographic north-south axis in the enucleated group (figure 3*e*; mean direction = 176°/356°, CI: 272°–58°, $r = 0.429$, $n = 10$, $R^* = 1.302$, $p < 0.01$). Further, the mean vectors with respect to topographic north were significantly longer in the enucleated animals (figure 3*f*; median $r_{enucleated}$: 0.569, median $r_{control}$: 0.287, one-tailed Mann–Whitney test: $U = 18$, $p = 0.007$). These findings demonstrate that magnetic cues guided nest building in control animals, whereas enucleated animals appeared unable to perceive the magnetic field, leading them to fall back on individual topographic preferences. How such a topographic bias could emerge in the stimulus-deprived environment of the testing arena remains puzzling. Still, since there was no sign of a similar topographic preference in the controls, magnetic stimuli evidently represent more salient cues for animals with intact eyes.

In summary, we found a loss of magnetic directional preferences in two independent series of nest-building experiments, yet we did not detect other behavioural consequences of enucleation in mole-rats. All enucleated animals were fully immersed members of their respective family groups and many successfully bred and raised offspring. Enucleated subjects were equally motivated to build nests, arguing against stress or pain-related side effects of surgery, since these reduce nest-building behaviour in rodents [34]. As we carefully controlled for the influence of other sensory stimuli, we conclude that the removal of the eyes led to a permanent impairment of the magnetic sense. The effect of enucleation on magnetic orientation is comparable to lidocaine application onto the cornea, corroborating the conclusion by Wegner *et al.* [6] that this type of anaesthesia affected the eyes rather than adjacent tissues or the central nervous system. Thus, future screens for light independent, probably magnetite-based magnetoreceptors in this species should focus on the minute eyes of Ansell's mole-rat which are small enough (approx. 2 mm in diameter) to be investigated entirely by techniques such as high-throughput electron microscopy [35,36].

Due to the fact that enucleation has no perceivable influence on mole-rats' behaviours, apart from affecting magnetoreception, one might be tempted to speculate that the small but structurally complex eyes of these animals are still retained because of their involvement in sensing magnetic fields. It is unlikely, however, that ocular magnetoreception necessitates the eyes to display such a high degree of structural complexity. First, in contrast with light-dependent radical pair-based receptors [11], light-independent magnetite-based receptors do not require specific spatial receptor arrangements to function [12,37]. Second, the Middle East blind mole-rat (*Spalax ehrenbergi*), a distantly related underground-dwelling rodent of the muroid superfamily, has already been shown to respond to magnetic stimuli in total darkness, despite its highly regressed, subcutaneous eyes [38]. Whether impairments due to eye loss in free-living Ansell's mole-rats would be more severe, for example during surface activity, remains speculative since little is known about the behavioural ecology of this species in the wild. Why African mole-rats retain a structurally complex eye, while many other subterranean mammals do not, continues to be elusive [23].

There have been past attempts to detect magnetite in various rodent tissues [39] but, to our knowledge, no detailed surveys for ocular magnetite have been conducted in any

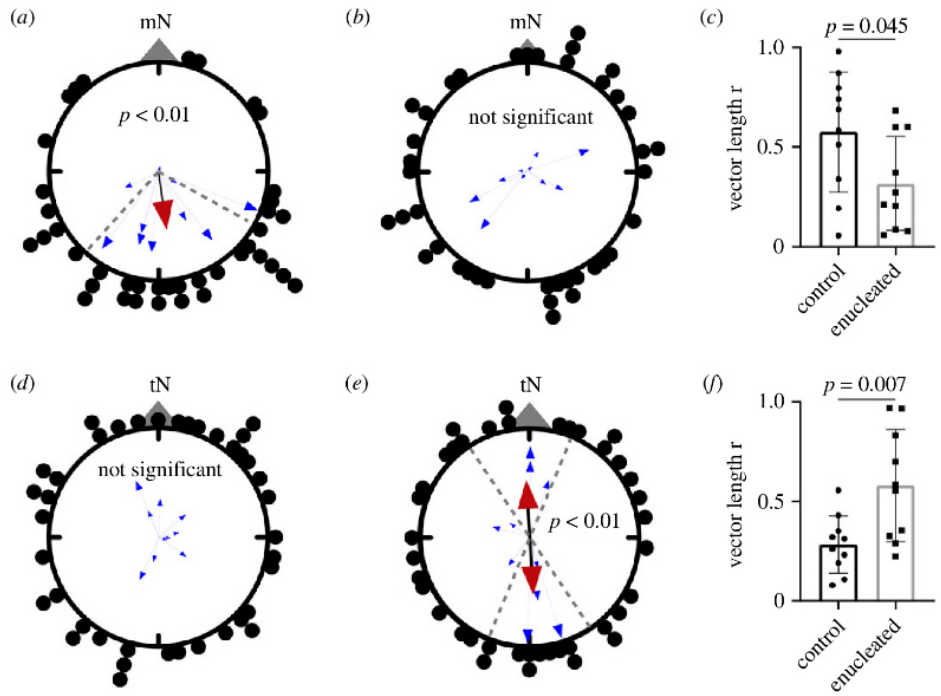

Figure 3. Enucleation results in a loss of magnetic directional preferences in the Ansell's mole-rat. (*a*,*b*) Nest distribution with respect to magnetic North (mN) of controls (*a*) and enucleated Ansell's mole-rats (*b*). (*c*) Mean angular vectors (calculated for each animal separately with respect to magnetic North) for controls and enucleated mole-rats. (*d*,*e*) Nest distribution with respect to topographic north (tN) in control (*d*) and enucleated mole-rats (*e*). (*f*) Mean angular vectors (with respect to topographic North) for controls and enucleated mole-rats. Small blue arrows represent the mean vectors of four nests built by individuals or pairs of Ansell's mole-rats and the arrow lengths reflect the *r*-values, a measure of the concentration of the nests. Red arrows represent weighted mean vector calculated over the mean vectors of tested individuals/pairs when significant. Dashed lines indicate 95% confidence intervals of the weighted grand mean. Dots outside the circles: positions of all 40 nests of each experimental group. The p-values indicate the results from Moore's modified Rayleigh tests (*a*,*e*) or one-tailed Mann–Whitney tests (*c*,*f*) performed on the mean vectors.

mammal species so far, including African mole-rats. Electron-dense crystalloid bodies were coincidentally noticed in retinal photoreceptors of Ansell's mole-rats, assumedly consisting of magnetite [40]. However, neither were the size and distribution of these particles quantified, nor has it been investigated whether they indeed contain iron. Later, ferric aggregates were identified by Prussian blue staining in the cornea of a single mole-rat eye, which have also been interpreted as magnetite crystals [6]. This intriguing, but non-replicated finding should be interpreted with caution because Prussian blue staining does not specifically detect magnetite. For example, iron-rich cells in the avian upper beak, once hypothesized to be magnetoreceptors and discovered via this method, were subsequently identified as macrophages, immune cells that accumulate ferric iron [41]. Although macrophages are typically absent from the rodent cornea, they invade corneal tissue during inflammation [42]. Besides that, the finding could be attributed to iron contamination from the laboratory environment, a familiar confounder in histological screens for magnetoreceptors [43].

If magnetite receptors are located in the cornea, they would most likely be innervated by the ophthalmic branch of the trigeminal nerve [44]. This would be consistent with the finding of magnetically induced neural activity in a part of the Ansell's mole-rat's superior colliculus that predominantly receives trigeminal input [7]. The trigeminal nerve has further been demonstrated to be involved in the magnetic sense in birds [9,45–48], but the exact location and structure of avian trigeminal magnetoreceptors is equally unknown [26]. The presence of similar trigeminal magnetoreception systems in birds and mammals appears conceivable, but ophthalmic nerve ablation experiments coupled with behavioural assays are needed to establish the role of trigeminal input for magnetoreception in mole-rats. Our study highlights the significance of the eye for mammalian magnetoreception, which could facilitate future research on its cellular basis. A thorough screen for magnetite in the mole-rat eye using electron-microscopic and spectroscopic methods is a warranted future experiment. A further candidate group to search for ocular magnetoreceptors besides mole-rats would be insectivorous bats, which are also microphthalmic and rely on a similar magnetic polarity compass system, probably based on magnetite [13]. By contrast to mole-rats, several bat species are widespread laboratory models in sensory biology and neurophysiology (e.g. *Carollia perspicillata*), their nervous systems are well characterized and they are readily available for a wide range of physiological methodologies. Ultimately, such experiments will contribute to characterize the function and significance of this enigmatic sensory channel within the mammalian radiation.

Ethics. All surgeries and experiments conformed to the relevant ethical standards and were approved by the animal welfare officer of the University of Duisburg-Essen and the LANUV NRW, Germany (series 1: 50.05-230-37/06; series 2: 84-02.04.2015.A387).

Data accessibility. The data are included in table 1.

Authors' contributions. R.E.M. and P.N. designed the experiments of series 1; S.B. designed the experiments of series 2; P.N. conducted the surgeries (series 1); R.E.M., K.M., K.R.C. and S.B. conducted the experiments; K.R.C., P.E.M., R.E.M., K.M. and S.B. analysed the data; P.E.M. created the figures; K.R.C., E.P.M. and S.B. drafted the manuscript with input from all others authors.

Competing interests. The authors declare no competing interests.

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References

1. Wiltschko R, Wiltschko W. 1995 *magnetic orientation in animals*, 297 p. Berlin, Germany: Springer.
2. Clites BL, Pierce JT. 2017 Identifying cellular and molecular mechanisms for magnetosensation. *Annu. Rev. Neurosci.* **40**, 231–250. (doi:10.1146/annurev-neuro-072116-031312)
3. Nordmann GC, Hochstoeger T, Keays DA. 2017 Magnetoreception—a sense without a receptor. *PLoS Biol.* **15**, e2003234. (doi:10.1371/journal.pbio.2003234)
4. Begall S, Burda H, Malkemper EP. 2014 Magnetoreception in mammals. In *Advances in the study of behavior*, vol. 46 (eds M Naguib, L Barrett, HJ Brockmann, S Healy, JC Mitani, TJ Roper), pp. 45–88. New York, NY: Academic Press.
5. Semm P, Schneider T, Vollrath L. 1980 Effects of an earth-strength magnetic field on electrical activity of pineal cells. *Nature* **288**, 607–608. (doi:10.1038/288607a0)
6. Wegner RE, Begall S, Burda H. 2006 Magnetic compass in the cornea: local anaesthesia impairs orientation in a mammal. *J. Exp. Biol.* **209**, 4747–4750. (doi:10.1242/jeb.02573)
7. Němec P, Altmann J, Marhold S, Burda H, Oelschläger HHA. 2001 Neuroanatomy of magnetoreception: the superior colliculus involved in magnetic orientation in a mammal. *Science* **294**, 366–368. (doi:10.1126/science.1063351)
8. Burger T, Lucová M, Moritz RE, Oelschläger HHA, Druga R, Burda H, Wiltschko W, Wiltschko R, Němec P. 2010 Changing and shielded magnetic fields suppress c-Fos expression in the navigation circuit: input from the magnetosensory system contributes to the internal representation of space in a subterranean rodent. *J. R Soc. Interface* **7**, 1275–1292. (doi:10.1098/rsif.2009.0551)
9. Wiltschko R, Wiltschko W. 2013 The magnetite-based receptors in the beak of birds and their role in avian navigation. *J. Comp. Physiol. A* **199**, 89–98. (doi:10.1007/s00359-012-0769-3)
10. Kishkinev DA, Chernetsov NS. 2015 Magnetoreception systems in birds: A review of current research. *Biol. Bullet. Rev.* **5**, 46–62. (doi:10.1134/S2079086415010041)
11. Hore PJ, Mouritsen H. 2016 The radical-pair mechanism of magnetoreception. *Ann. Rev. Biophys.* **45**, 299–344. (doi:10.1146/annurev-biophys-032116-094545)
12. Kirschvink JL, Gould JL. 1981 Biogenic magnetite as a basis for magnetic field detection in animals. *Biosystems* **13**, 181–201. (doi:10.1016/0303-2647(81)90060-5)
13. Holland RA, Kirschvink JL, Doak TG, Wikelski M. 2008 Bats use magnetite to detect the Earth's magnetic field. *PLoS ONE* **3**, e1676. (doi:10.1371/journal.pone.0001676)
14. Wang Y, Pan Y, Parsons S, Walker M, Zhang S. 2007 Bats respond to polarity of a magnetic field. *Proc. Biol. Sci.* **274**, 2901–2905. (doi:10.1098/rspb.2007.0904)
15. Burda H, Marhold S, Westenberger T, Wiltschko R, Wiltschko W. 1990 Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). *Experientia* **46**, 528–530. (doi:10.1007/BF01954256)
16. Oliveriusová L, Němec P, Králová Z, Sedláček F. 2012 Magnetic compass orientation in two strictly subterranean rodents: learned or species-specific innate directional preference? *J. Exp. Biol.* **215**, 3649–3654. (doi:10.1242/jeb.069625)
17. Marhold S, Wiltschko W, Burda H. 1997 A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften* **84**, 421–423. (doi:10.1007/s001140050422)
18. Marhold S, Burda H, Kreilos I, Wiltschko W. 1997 Magnetic orientation in common mole-rats from Zambia. In *Orientation and navigation: birds, humans and other animals*, pp. 1–9. Royal Institute of Navigation.
19. Thalau P, Ritz T, Burda H, Wegner RE, Wiltschko R. 2006 The magnetic compass mechanisms of birds and rodents are based on different physical principles. *J. R Soc. Interface* **3**, 583–587. (doi:10.1098/rsif.2006.0130)
20. Malkemper EP, Eder SH, Begall S, Phillips JB, Winklhofer M, Hart V, Burda H. 2015 Magnetoreception in the wood mouse (*Apodemus sylvaticus*): influence of weak frequency-modulated radio frequency fields. *Sci. Rep.* **4**, 9917. (doi:10.1038/srep09917)
21. Bojarinova J, Kavokin K, Pakhomov A, Cherbunin R, Anashina A, Erokhina M, Ershova M, Chernetsov N. 2020 Magnetic compass of garden warblers is not affected by oscillating magnetic fields applied to their eyes. *Sci. Rep.* **10**, 3473. (doi:10.1038/s41598-020-60383-x)
22. Kott O, Šumbera R, Němec P. 2010 Light perception in two strictly subterranean rodents: Life in the dark or blue? *PLoS ONE* **5**, e11810. (doi:10.1371/journal.pone.0011810)
23. Němec P, Cveková P, Benada O, Wielkopolska E, Olkowicz S, Turlejski K, Burda H, Bennett NC, Peichl L. 2008 The visual system in subterranean African mole-rats (Rodentia, Bathyergidae): retina, subcortical visual nuclei and primary visual cortex. *Brain Res. Bull.* **75**, 356–364. (doi:10.1016/j.brainresbull.2007.10.055)
24. Schønemann NK, van der Burght M, Arendt-Nielsen L, Bjerring P. 1992 Onset and duration of hypoalgesia of lidocaine spray applied to oral mucosa–a dose response study. *Acta Anaesthesiol. Scand.* **36**, 733–735. (doi:10.1111/j.1399-6576.1992.tb03554.x)
25. Pardridge WM, Sakiyama R, Fierer G. 1983 Transport of propranolol and lidocaine through the rat blood-brain barrier. Primary role of globulin-bound drug. *J. Clin. Invest.* **71**, 900–908. (doi:10.1172/JCI110844)
26. Engels S, Treiber CD, Salzer MC, Michalik A, Ushakova L, Keays DA, Mouritsen H, Heyers D. 2018 Lidocaine is a nocebo treatment for trigeminally mediated magnetic orientation in birds. *J. R. Soc. Interface* **15**, 20180124. (doi:10.1098/rsif.2018.0124)
27. Henning Y, Mladenkova N, Burda H, Szafranski K, Begall S. 2018 Retinal S-opsin dominance in Ansell's mole-rats (*Fukomys anselli*) is a consequence of naturally low serum thyroxine. *Sci. Rep.* **8**, 4337. (doi:10.1038/s41598-018-22705-y)
28. R Core Team. 2019 *R: A language and environment for statistical computing*. R version 3.6.0 ed2019.
29. Gerhardt P, Henning Y, Begall S, Malkemper EP. 2017 Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency hearing. *J. Exp. Biol.* **220**, 4377–4382. (doi:10.1242/jeb.164426)
30. Malewski S, Begall S, Burda H. 2018 Learned and spontaneous magnetosensitive behaviour in the Roborovski hamster (*Phodopus roborovskii*). *Ethology* **124**, 423–431. (doi:10.1111/eth.12744)
31. Peichl L, Němec P, Burda H. 2004 Unusual cone and rod properties in subterranean African mole-rats (Rodentia, Bathyergidae). *Eur. J. Neurosci.* **19**, 1545–1558. (doi:10.1111/j.1460-9568.2004.03263.x)
32. Muheim R, Edgar NM, Sloan KA, Phillips JB. 2006 Magnetic compass orientation in C57BL/6 J mice.

Learn. Behav. **34**, 366–373. (doi:10.3758/BF03193201)

33. Fitak RR, Johnsen S. 2017 Bringing the analysis of animal orientation data full circle: model-based approaches with maximum likelihood. *J. Exp. Biol.* **220**, 3878. (doi:10.1242/jeb.167056)
34. Jirkof P. 2014 Burrowing and nest building behavior as indicators of well-being in mice. *J. Neurosci. Methods* **234**, 139–146. (doi:10.1016/j.jneumeth.2014.02.001)
35. Graham BJ, Hildebrand DGC, Kuan AT, Maniates-Selvin JT, Thomas LA, Shanny BL, Lee WC. 2019 High-throughput transmission electron microscopy with automated serial sectioning. *bioRxiv*. 657346.
36. Titze B, Genoud C. 2016 Volume scanning electron microscopy for imaging biological ultrastructure. *Biol. Cell* **108**, 307–323. (doi:10.1111/boc.201600024)
37. Shaw J, Boyd A, House M, Woodward R, Mathes F, Cowin G, Saunders M, Baer B. 2015 Magnetic particle-mediated magnetoreception. *J. R Soc. Interface* **12**, 0499. (doi:10.1098/rsif.2015.0499)
38. Kimchi T, Terkel J. 2001 Magnetic compass orientation in the blind mole rat *Spalax ehrenbergi*. *J. Exp. Biol.* **204**, 751–758.
39. Mather JG. 1985 Magnetoreception and the search for magnetic material in rodents. In *Magnetite biomineralization and magnetoreception in organisms: A New biomagnetism* (eds JL Jones, DS Kirschvink, BJ MacFadden), pp. 509–536. New York, NY: Plenum Press.
40. Cernuda-Cernuda R, García-Fernández JM, Gordijn MCM, Bovee-Geurts PHM, DeGrip WJ. 2003 The eye of the african mole-rat *Cryptomys anselli*: to see or not to see? *Eur. J. Neurosci.* **17**, 709–720. (doi:10.1046/j.1460-9568.2003.02485.x)
41. Treiber CD *et al.* 2012 Clusters of iron-rich cells in the upper beak of pigeons are macrophages not magnetosensitive neurons. *Nature* **484**, 367–370. (doi:10.1038/nature11046)
42. Maruyama K *et al.* 2005 Inflammation-induced lymphangiogenesis in the cornea arises from CD11b-positive macrophages. *J. Clin. Invest.* **115**, 2363–2372. (doi:10.1172/JCI23874)
43. Edelman NB *et al.* 2015 No evidence for intracellular magnetite in putative vertebrate magnetoreceptors identified by magnetic screening. *Proc. Natl Acad. Sci. USA* **112**, 262–267. (doi:10.1073/pnas.1407915112)
44. Müller LJ, Marfurt CF, Kruse F, Tervo TM. 2003 Corneal nerves: structure, contents and function. *Exp. Eye Res.* **76**, 521–542. (doi:10.1016/S0014-4835(03)00050-2)
45. Lefeldt N, Heyers D, Schneider NL, Engels S, Elbers D, Mouritsen H. 2014 Magnetic field-driven induction of ZENK in the trigeminal system of pigeons (*Columba livia*). *J. R Soc. Interface* **11**, 20140777. (doi:10.1098/rsif.2014.0777)
46. Mouritsen H, Hore PJ. 2012 The magnetic retina: light-dependent and trigeminal magnetoreception in migratory birds. *Curr. Opin Neurobiol.* **22**, 343–352. (doi:10.1016/j.conb.2012.01.005)
47. Kishkinev D, Chernetsov N, Heyers D, Mouritsen H. 2013 Migratory reed warblers need intact trigeminal nerves to correct for a 1,000 km eastward displacement. *PLoS ONE* **8**, e65847. (doi:10.1371/journal.pone.0065847)
48. Heyers D, Zapka M, Hoffmeister M, Wild JM, Mouritsen H. 2010 Magnetic field changes activate the trigeminal brainstem complex in a migratory bird. *Proc. Natl Acad. Sci. USA* **107**, 9394. (doi:10.1073/pnas.0907068107)

Supplementary Material

Supplementary Table 1: Results of model-fitting using the CircMLE package (1) in *R*. The difference in the Akaike Information Criterion (delta AIC) is given with respect to a uniform (random) distribution. mN = magnetic north, tN = topographic north

Experimental series	Group	Reference direction	Best model	Delta AIC
1	Controls	tN = mN	M2A (unimodal)	9.141
	Enucleated	tN = mN	M1 (uniform)	0
2	Controls	mN	M2A (unimodal)	3.538
	Controls	tN	M1 (uniform)	0
	Enucleated	mN	M3A (homogenous symmetric bimodal)	1.928
	Enucleated	tN	M2B (symmetric modified unimodal)	
			M3A (homogenous symmetric bimodal)	3.03
				2.747
			M3B (symmetric bimodal)	2.526

1. Fitak RR, Johnsen S. Bringing the analysis of animal orientation data full circle: model-based approaches with maximum likelihood. *Journal of Experimental Biology*. 2017;220(21):3878.

Discussion

Although the results presented in Chapter 2.2 are straight forward, I want to address two methodological caveats before moving on to discuss selected implications of our findings in greater depth. First, the axial topographic preference we recovered for enucleated subjects in experimental series 2 remains unexplained. By itself, this issue does not challenge the conclusions of the paper, since there was clearly no evidence for magnetic orientation in the treated subjects. However, the fact that these mole-rats could orient topographically at all indicates that the nest-building behavior of sighted animals might have also been affected by topographical cues. Despite extensive considerations about potential reasons for the topographic bias, we were unable to find any promising explanations for the directional responses of the enucleated subjects. Although this obviously is an unsatisfying situation, I would argue that potential topographic biases in the sighted group are negligible, since they nevertheless expressed the well-documented species-specific south-eastern nest-building preference (Burda et al., 1990; Marhold et al., 1997; Thalau et al., 2006). Thus, we can conclude that our testing conditions were suitable to detect behavior guided by magnetic cues, or a lack thereof.

A methodological issue that can be raised for this as well as previously published studies on directional nesting in mole-rats is the lack of monitoring of the nest-construction process. Anecdotal observations by colleagues (R. Shirdhankar & E. P. Malkemper, pers. com.) indicate that once the animals have built a nest, they may repeatedly drag it along the edge of the arena. Thus, the position of the nest may be continuously shifted, creating issues for data scoring and interpretation. Unfortunately, this issue was noted after Chapter 2.2 had already been published. I may add, though, that when controlling for nest positions in experimental series 2, the mole-rats appeared to be calm and not in the process of changing the nests' orientation. Still, future studies should video-record the nest-building process to log both the initial position of a nest and the final one, in case a shift in orientation occurred. These two measures could then be compared to decide which one is more informative when it comes to magnetic preferences. This way, nest-building assays could become more transparent and perhaps even more robust.

A question that is almost universally brought up when publicly discussing magnetic orientation in mole-rat nest-building concerns the potential purpose of the population-level directional bias. In Ansell's mole-rats, the south-eastern bias has been replicated various times (e.g., Burda et al., 1990; Marhold et al., 1997; Thalau et al., 2006) and thus appears to be a constant innate component of the species' behavior rather than a learned response (compare Deutschlander et al., 2003; Painter et al., 2018). In other African mole-rats, directional nest-building dependent on magnetic field polarity has also been reported (western preference; *Heliophobius argenteocinereus* & *Fukomys mechowii* – Oliveriusová et al., 2012), but these findings have so far not been replicated and thus need to be interpreted with caution. One can hardly

imagine an adaptive benefit of innate nest-building preferences in an environment as artificial as a plane, circular arena and I would suggest that this stable preference is an evolutionary byproduct of a yet unidentified neurological trait. It is interesting that other rodents also express directional preferences for nest-building in similar situations (e.g., Malkemper et al., 2015) so that we might observe a phenomenon deeply rooted in rodent phylogeny. A better understanding of the evolutionary underpinnings and significance of this important model behavior might be gained by comparing nest-building responses in closely related species. For Ansell's mole-rats, these would include other Zambezian *Fukomys* lineages, such as Micklem's mole-rat, the Damaraland mole-rat, and the Mashona mole-rat.

Although the biological significance of directional nest-building remains obscure at the moment, the benefits of relying on a magnetic compass underground are obvious: In the absence of visual landmarks and other stimuli which might guide orientation, the geomagnetic field can serve as a stable navigational cue (Chapter 1.1.1). The spatial structure of burrow systems in diverse subterranean rodents, including *Fukomys*, are indeed suggestive of the geomagnetic field guiding tunneling behavior to a significant degree (Malewski et al., 2018). Recently, Finn (2020) suggested that Damaraland mole-rats also rely on magnetic cues during dispersal from their natal burrow. In the respective field study, dispersal routes from 88 individuals were analyzed and interpreted as evidence for magnetic orientation.

Although I consider the notion that magnetic cues guide dispersal in mole-rats as very plausible, I do not feel compelled by the presented data. Importantly, there is no evidence that the dispersal routes were indeed geomagnetically aligned. Finn (2020) assumes linear dispersal directions, calculated from the relative orientation of the natal burrow to the newly occupied one. Whether this was indeed the case remains unclear. Furthermore, a non-random orientation for dispersal was only shown for certain fractions of individuals, namely females dispersing more than 250 m and (if counting in a trend – $p = 0.06$) for males dispersing less than 250 m. This rather inconsistent pattern does not unambiguously support the conclusion that dispersing mole-rats are chiefly guided by magnetic cues. Unfortunately, the study makes little to no effort to test for influences of other factors, for instance local topography, on dispersal direction. Many dispersing mole-rats were crossing areas that were already occupied by conspecifics. Substrate-born odors from soil discharged from occupied burrow systems will likely guide dispersers in search for uncontested spaces or opposite-sex conspecifics (Leedale et al., 2021; Chapter 2.4). As suggested by the author himself, the study's approach should be repeated with biologger-equipped animals (Finn, 2021). Such devices would allow a far more detailed reconstruction of dispersal routes and could generate valuable information on mole-rat dispersal behavior, which remains one of the great enigmas of these animals' life history.

If such data would show a patterning aligned with geomagnetic parameters, this would convincingly demonstrate a role of magnetoreception during dispersal and thus corroborate the hypothesis of Finn (2021).

The main contribution of Chapter 2.2 arguably lays in narrowing down the search space for magnetoreceptors in a mammal. This is a crucial precondition for the further characterization of these enigmatic structures and their functional properties (see below). Although it is possible that additional receptors relevant to magnetoreception are distributed in other organs (compare e.g., Semm et al., 1980), the results of Chapter 2.2 suggest at least a notable involvement of ocular receptors in the mole-rat magnetic sense. If the cornea, as suggested, is indeed the seat of magnetoreception in mole-rats, the respective receptors would almost certainly be innervated by the ophthalmic branch of the trigeminal nerve (Marfurt & Del Toro, 1987; Al-Aqaba et al., 2010). This would be in line with neurological findings, suggesting that magnetic stimuli activate parts of the superior colliculus in mole-rats, that receive notable trigeminal input (Němec et al., 2001).

Given the mostly uniform structure of sensory organs in mammals, our findings predict that the polarity compass of other mammalian species are also dependent on corneal receptors. Because of the availability and widespread interest in bats as a model group in sensory biology, including magnetoreception (Wang et al., 2007), we proposed to search for corneal magnetoreceptors in chiropterans. Interestingly, since Chapter 2.2 has been published, experimental results exactly fitting our predictions were presented by Lindecke et al. (2021) for vespertilionid bats. In their study, the authors used oxybuprocaine hydrochloride to numb sensory nerve endings in the bat cornea. This local anesthetic that does not exert the off-target effects known from alternative agents such as lidocaine (e.g., Engels et al., 2018). A disadvantage compared to lidocaine lays in its effects abating within approximately 30 min, though (Lindecke et al., 2021). To test whether corneal anesthesia affected magnetoreception, Lindecke et al. (2021) translocated migrating bats, treated them with the anesthetic and quantified the direction they initially headed to after being released at an unknown site. Sham-control bats showed a highly significant preference to escape in a southern direction, while treated bats chose random routes. Thus, this study provides robust evidence for ocular, or more specifically corneal, magnetoreception in another mammalian clade and supports the inferences made in Chapter 2.2. Obviously, it would be interesting to utilize oxybuprocaine hydrochloride for nest-building assays in African mole-rats and hamsters to generate more robust evidence for corneal involvement in rodent magnetoreception.

If we, for the moment, assume that both rodents and bats make use of a light-independent magnetic compass seated in the cornea, this may indeed hint at a shared mechanism for magnetic field polarity-sensing in mammals. In fact, trigeminally-mediated corneal magnetoreception in mammals would align well with comparative data from other vertebrate groups: There is

evidence for the involvement of the ophthalmic branch of the trigeminal nerve in fish (homologous "superficial ophthalmic ramus" – Walker et al., 1997) and particularly in avian magnetoreception (Kishkinev & Chernetsov, 2015). Hence, one might hypothesize about an ancient shared pattern underlying the sensing of magnetic field polarity in vertebrates. If so, that would mirror the evolutionary inertia observed in other vertebrate sensory systems, the ground plan of which is generally conserved (Kardong, 2012). Given the few comparative data available at the moment regarding the physiology of vertebrate magnetoreception, this notion is without question conjectural but not implausible.

To convincingly homologize magnetoreceptive systems in vertebrates and among mammals, one would obviously need to characterize the respective receptor cells, which famously remain unidentified. Magnetoreceptor cells responding to field polarity are expected to contain magnetite crystals that align with the polarity axis of the ambient magnetic field (Begall et al., 2014). In the past, numerous attempts have been made to identify these receptors using the histological stain Prussian blue, which can be employed to mark iron-rich structures in tissue sections (see e.g., Treiber et al., 2012). In fact, Wegner et al. (2006) report Prussian blue-stained particles in the cornea of the Ansell's mole-rat and declared them to be candidate magnetoreceptors. However, the composition of these particles and their co-localization with neurofilaments was not tested by these researchers. By combining Prussian blue with immunostaining, Herold (2016) succeeded in also recovering ferric concretions in the mole-rat cornea but could not reaffirm that these particles are indeed innervated. Besides that, the validity of the findings by Wegner et al. (2006) can be challenged by the fact that Prussian blue is not a magnetite specific stain. Thus, other iron rich structures, such as macrophages that may invade the cornea during an acute inflammation, might have been detected (Chapter 2.2; Herold, 2016). Curiously, a very recent study critically examined the staining properties of Prussian blue in regard to biogenic magnetite and concludes that it is prone to yield false-negative results (Curdt et al., 2022 – but note that this article is in the preprint stage at the time of writing). For instance, it did not stain magnetotactic bacteria in positive control trials. Hence, Prussian blue appears to be completely unsuitable to detect magnetite-based receptor cells but remains useful to determine contamination of tissue samples with iron-rich dust (Curdt et al., 2022).

An alternative visualization technique for tissues in which magnetoreceptors are anticipated might be serial block-face scanning electron microscopy. Here, sequential tissue sections are imaged at a subcellular resolution of up to $\sim 5\ nm^2$ and are subsequently used to build a 3D digital clone of the tissue to be manipulated and surveyed (Peddie & Collinson, 2014; Courson et al., 2019). A complete Ansell's mole-rat cornea would be small enough to be visualized in its entirety via this technique, which has already been successfully applied to study corneal ultrastructure in laboratory mice (Courson et al., 2019; note that the larger mouse cornea had to

be cut into quadrants for study). However, the best candidate species to start searching for corneal magnetoreceptors this way or by leveraging alternative imaging techniques would arguably be blind mole-rats (*Nannospalax* sp./*Spalax* sp.). These rodents, like African mole-rats, possess a magnetic polarity compass and are readily available for laboratory research (Kimchi & Terkel, 2001). Different from their afrotropical counterparts, however, blind mole-rats lack normal functioning eyes. While the bathyergid eye displays the typical mammalian build-up, the blind mole-rat eye is embedded in subdermal tissue, atrophied, and no longer capable to enable vision (Cooper et al., 1993). The cornea is still a discernable component of the eye in blind mole-rats (Keleş et al. 2020) but to my best knowledge, its innervation has received no study so far. Given its subdermal location, one can expect that the density of nerve endings penetrating the cornea should be greatly reduced, as sensitivity to mechanic or thermal stimuli is no longer needed. Nevertheless, hypothetical magnetoreceptors should still be innervated. This highly derived condition might facilitate the identification of corneal magnetoreceptors in these subterranean rodents.

2.3 – Hearing sensitivity

Evoked auditory potentials from African mole-rats and coruros reveal disparity in subterranean rodent hearing

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Contributions:

- **Conception** – 90 %: I conceived the study, including its objectives and methodology, with input from SB.

- **Data collection** – 50%: Electrophysiological recording were made by me, AH, and LM with crucial guidance from PG. Thresholds were manually determined by AH, KRC, LM, and SB. The sound pressure level of vocalizations was measured by me and SB.

- **Data analyses** – 100%: I analyzed the dataset.

- **Writing the manuscript** – 90 %: I wrote the initial draft of the manuscript and revised it with input from all coauthors.

- **Revising the manuscript** – 90 %: I revised the manuscripts following the reviewer's comments together with input from all coauthors.

_____ _____

Signature of Ph.D. student Signature of supervisor

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RESEARCH ARTICLE

Evoked auditory potentials from African mole-rats and coruros reveal disparity in subterranean rodent hearing

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ABSTRACT

Hearing in subterranean rodents exhibits numerous peculiarities, including low sensitivity and restriction to a narrow range of comparatively low frequencies. Past studies provided two conflicting hypotheses explaining how these derived traits evolved: structural degeneration and adaptive specialization. To further elucidate this issue, we recorded auditory brainstem responses from three species of social subterranean rodents that differ in the degree of specialization to the underground habitat: the naked mole-rat (*Heterocephalus glaber*) and the Mashona mole-rat (*Fukomys darlingi*), which represent the ancient lineage of African mole-rats (Bathyergidae), and the coruro (*Spalacopus cyanus*), a South American rodent (Octodontidae) that adopted a subterranean lifestyle in more recent geological time. Additionally, we measured call amplitudes of social vocalizations to study auditory vocal coupling. We found elevated auditory thresholds and severe hearing range restrictions in the African mole-rats, with hearing in naked mole-rats tending to be more sensitive than in Mashona mole-rats, in which hearing notably deteriorated with increasing age. In contrast, hearing in coruros was similar to that of epigeic rodents, with its range extending into ultrasonic frequencies. However, as in the mole-rats, the coruros' region of best hearing was located at low frequencies close to 1 kHz. We argue that the auditory sensitivity of African mole-rats, although remarkably poor, has been underestimated by recent studies, whereas data on coruros conform to previous results. Considering the available evidence, we propose to be open to both degenerative and adaptive interpretations of hearing physiology in subterranean mammals, as each may provide convincing explanations for specific auditory traits observed.

KEY WORDS: Auditory brainstem response, Auditory threshold, Call amplitude, Naked mole-rat, Common mole-rat, *Spalacopus cyanus*

INTRODUCTION

Rodents have adopted subterranean lifestyles numerous times, with approximately 250 extant species from six families living predominately underground in self-maintained burrows (Begall et al., 2007). The subterranean realm poses several challenges to sensory physiology that must be overcome to meet the communicative and navigational demands of the animals. Although such limitations might be particularly obvious for visual communication, they are also relevant for hearing underground. The tunnel acoustics of the underground habitat differ in several aspects from surface conditions. Besides the obvious constraints of long-range and directional acoustic signaling within a burrow system, the subterranean realm is characterized by marked frequency-dependent differences in sound transmission. Frequencies higher than 1 kHz are notably attenuated, even over distances of just a few meters, while lower frequencies might be amplified (Heth et al., 1986; Lange et al., 2007; Okanoya et al., 2018). For instance, frequencies ranging between 200 and 800 Hz can experience a more than twofold sound pressure amplification in natural burrow systems of African mole-rats that live in tunnels varying between 4.5 and 9 cm in diameter (Lange et al., 2007). This phenomenon has been termed the stethoscope effect.

Facing these circumstances, subterranean rodents have adopted different strategies to communicate effectively underground. Most lineages are predominately represented by solitary burrowers, and few species (most of them within the African mole-rat family Bathyergidae and the mole-vole genus *Ellobius*) form social groups of varying size and complexity (Smorkatcheva and Kumaitova, 2014). Solitary species of several burrowing rodent taxa communicate with conspecifics in neighboring tunnels via seismic signals that are picked up by the somatosensory system, such as foot drumming (Narins et al., 1992) and head thumping (Hrouzková et al., 2018). However, this behavior is not known from social taxa. The latter communicate exclusively with conspecifics that they share a burrow system with and rely heavily on frequently exchanged vocal signals for that matter (Schleich et al., 2007; Bednářová et al., 2013; Barker et al., 2021). Both solitary and social species have evolved to exploit tunnel acoustics by shifting the pitch of their vocalizations into lower frequency ranges than what would be expected for rodents of their body size, often below 1 kHz (Capranica et al., 1974; Credner et al., 1997; Schleich and Busch, 2002; Devries and Sikes, 2008; but see Volodin et al., 2021).

The peculiar acoustic properties of their habitat in conjunction with the demands of social communication have created prolonged interest in the hearing capabilities of subterranean rodents. Two main trajectories have been proposed to characterize the evolution of hearing in these animals: degeneration and adaptive specialization (Burda et al., 1992; Burda, 2006). The degeneration hypothesis was popularized by Heffner and Heffner (1990, 1992, 1993), but it had already been foreshadowed by Fleischer (1973) in his morphological investigations. Heffner and Heffner (1990, 1992, 1993) employed a conditioned avoidance method to generate behavioral audiograms of their study species. They discovered unusually high auditory thresholds and narrow ranges of audible frequencies in subterranean rodent taxa compared with epigeic groups, as well as a consistent loss of the ability to localize short sound bursts. Although it was noted that all subterranean species displayed their greatest hearing sensitivity in the range of low-pitched sounds between 1 and 4 kHz, it was also pointed out that the respective thresholds at these

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frequencies were not lower than in surface-dwelling mammals (Heffner and Masterton, 1980; Heffner and Heffner, 1992). Analogous to the reduction of the visual system in animals living in light-deprived environments, Heffner and Heffner (1990) proposed that the subterranean realm would permit a degeneration of hearing by the absence of selective pressures maintaining it.

Contrary to this notion, other researchers put forward an adaptive interpretation of the patterns observed (Bruns et al., 1988; Burda et al., 1992). Proponents of this school diagnosed the lack of pinnae, the narrow and typically cerumen-filled auditory meatus, and the low efficiency of sound propagation in the middle ear as causes for the poor hearing sensitivity of subterranean mammals. However, these and other alterations of the ear in burrowing species do not necessarily represent degenerate traits (Burda et al., 1992). Respective authors have emphasized the low high-frequency cut-off as a stable and defining trait of subterranean rodent audiograms, non-randomly maintaining sensitivity to low-pitched, ecologically relevant frequencies. The overall reduction of hearing sensitivity was also interpreted to serve an adaptive purpose by countering the stethoscope effect and thereby preventing overstimulation of the ear during social encounters (Burda et al., 1992; Lange et al., 2007). Most studies on subterranean rodent hearing have since adopted the specialization hypothesis (Burda, 2006; Gessele et al., 2016) but arguments for hearing degeneration continue to be made (Mason et al., 2016).

Recently, Pyott et al. (2020) revived the discussion by presenting a comprehensive set of electrophysiological, molecular, and morphological data that could provide functional explanations for poor hearing in the two most intensively studied social subterranean rodent genera: *Heterocephalus* (naked mole-rats) and *Fukomys* (Northern common mole-rats). The authors argue that the functionality of the cochlear amplifier in these animals is strongly compromised, reducing their hearing sensitivity. This loss of function is hypothesized to derive from an abnormal structuring of the hair bundle stereocilia of the outer hair cells in bathyergids. The study demonstrated that hair bundle organization in these animals is remarkably distorted, whereas prestin-mediated hair cell motility is not confined (Pyott et al., 2020). Substitutions in genes that determine the structural integrity of hair bundles, and that are associated with hearing loss in humans, were indeed found to be accumulated in African mole-rats. Still, these substitutions show strong signatures of positive selection when compared with mice, guinea pigs and humans. Therefore, Pyott et al. (2020) interpret hearing alteration in bathyergids as adaptive, although the physiological effects of these mutations evoke the impression of degeneration.

For both bathyergid genera, Pyott et al. (2020) determined hearing thresholds that fluctuated around 60 dB SPL via the auditory brainstem response (ABR) method. Distortion product otoacoustic emissions (DPOAE), which depend on the motility of the outer hair cells and thus the functionality of the cochlear amplifier, were found to be absent in both species. Though largely consistent through the various methods applied in the study, the findings of Pyott et al. (2020) appear to be at odds with the elaborate vocal behavior of both naked and common mole-rats and with anecdotal observations in zoos and laboratories, which suggest more acute hearing (Hill et al., 1957; Ludwig and Collmar, 2009; Smith and Buffenstein, 2021; K.R.C. and S.B., personal observation). For the genus *Fukomys* in particular, the findings conflict with a significant body of work published over the last few decades (Kössl et al., 1996; Gerhardt et al., 2017; see Discussion).

In light of these discrepancies, we further investigated hearing in bathyergid mole-rats with an ABR approach, attempting to reproduce the results of Pyott et al. (2020) with naked mole-rats and *Fukomys* mole-rats. To further assess tempo and mode of the evolution of underground hearing, we also included the Chilean coruro (*Spalacopus cyanus*) in our study, which is the only fully subterranean octodontid rodent. Different from bathyergids, which might have invaded the subterranean realm in the Late Oligocene already (ca. 25 Mya; Bryja et al., 2018), coruros adopted their subterranean lifestyle far more recently in the Pliocene (ca. 3.5 Mya; Upham and Patterson, 2012). Behavioral audiograms and morphological observations on this species indicate well-developed hearing, similar in frequency range to that of diverse epigeic small mammals (Begall et al., 2004; Begall and Burda, 2006). In contrast, Pyott et al. (2020) presented evidence for a moderate accumulation of deleterious mutations in the octodontid stem lineage (however, no coruro sequence data were analyzed). This finding, together with comparative molecular data on other subterranean mammal groups (Davies et al., 2018; Pyott et al., 2020), argues for widespread hearing deficiencies in burrowers and would suggest more restricted auditory capabilities in coruros.

When extraordinarily high hearing thresholds are assumed for subterranean rodents, one would also expect that the animals vocalize using high volume (Okanoya et al., 2018). We therefore complemented our ABR recordings with measurements of the sound pressure levels of intraspecific contact calls in all three species under study.

MATERIALS AND METHODS

Subjects

We recorded ABRs from three species of social subterranean rodents, Mashona mole-rats [*Fukomys darlingi* (Thomas 1895), Nsanje population; *n*=9], naked mole-rats (*Heterocephalus glaber* Rüppell 1842; *n*=12) and coruros [*Spalacopus cyanus* (Molina 1782); *n*=12]. Individual subject data are summarized in Table 1. We classified individuals into three age groups based on established data on life history: juvenile [0–6 months (coruro)/0–12 months (Mashona mole-rat)], adult [6–48 months (coruro)/12–74 months (Mashona mole-rat)] and aged (older than adult range). All naked mole-rats were fully adult animals but of unknown exact age. None of the subjects showed apparent signs of hearing loss or behavioral disorders.

Mashona mole-rats were selected as substitutes to the Damaraland mole-rats (*Fukomys damarensis*, but see below) studied by Pyott et al. (2020), because the latter species is not available for laboratory studies in Europe. Our Mashona mole-rat laboratory strain derives from Nsanje, Southern Malawi. The animals are of comparable body size (130–200 g) to *F. damarensis*. Unpublished molecular data on this population support these animals as representatives of the species *F. darlingi* (although better known populations from Zimbabwe are dramatically smaller in body size; Bennett et al., 1994) and suggest that it forms the sister lineage to a taxon composed of *F. damarensis* and the Zambian *F. micklemi* clade (O. Mikula, personal communication), for which detailed information on hearing is already available (Gerhardt et al., 2017). We want to emphasize here that the animals used in the study by Pyott et al. (2020) derive from different captive lineages for which taxonomic identity has never been determined genetically or by aid of karyotypic methods (T. Park and R. Buffenstein, personal communication), the only ways to reliably identify *Fukomys* species in the absence of exact data on their geographic provenance (Van Daele et al., 2007). The classification of these mole-rats as *F. damarensis* is therefore questionable and needs to be verified.

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Table 1. Information on subjects participating in the study

Species	ID	Sex	Age group	No. of ABR sessions	Mass (g)
Fukomys darlingi	FD 0815	F	Aged	3	130
	FD 2229	M	Aged	3	158
	FD 3000	F	Aged	2	132
	FD 4105	F	Aged	2	130
	FD 4686	F	Aged	3	127
	FD 5361	M	Juvenile	3	59
	FD 5441	M	Juvenile	2	63
	FD 5450	F	Juvenile	1	42
	FD 9399	M	Aged	3	180
Heterocephalus glaber	HG 3105	F	Adult	1	40
	HG 5351	F	Adult	3	27
	HG 5400	M	Adult	1	49
	HG 5401*	M	Adult	2	53
	HG 5402	F	Adult	1	54
	HG 5403	F	Adult	1	43
	HG 5408*	F	Adult	2	42
	HG 5422*	M (?)	Adult	2	46
	HG 5423*	W (?)	Adult	1	27
	HG 5424*	M	Adult	1	56
	HG 67311*	M	Adult	1	32
	HG 7311*	M	Adult	1	37
Spalacopus cyanus	SC 0820**	F	Aged	2	105
	SC 2732	M	Aged	3	134
	SC 2760	M	Aged	3	126
	SC 2780	M	Aged	3	134
	SC 2797**	F	Adult	2	99
	SC 5343	M	Adult	3	127
	SC 5345	M	Adult	2	144
	SC 5346	F	Adult	3	106
	SC 5377**	M	Juvenile	3	69
	SC 7664	M	Aged	2	143
	SC 8732	F	Aged	3	96
	SC 9892	F	Adult	2	101

*Animals were tested with an additional 16 kHz step.
**Subjects were tested with an additional 36 kHz frequency step.

Subjects were housed at the University of Duisburg-Essen and were kept in a constantly heated room with a 12 h:12 h light:dark cycle, at 26±1°C constant temperature and 40–55% air humidity. All animals were socially housed and immediately returned to their home terraria after recovery from anesthesia. Enclosures were lined with wood shavings and enriched with clay pots, wooden and/or plastic tunnels, and animals were regularly offered nesting materials. Animals were kept on a staple diet of carrots and potatoes, supplemented with diverse vegetables and fruits, hay and seeds. Food was provided *ad libitum*; all studied species extract water from solids and do not drink free water.

Recordings of evoked auditory potentials

Subject preparation and monitoring

Experimental procedures were largely adopted from Gerhardt et al. (2017). ABR recordings were made between August 2020 and March 2021. Narcosis was achieved by intramuscular injection of ketamine and xylazine (mass-dependent dosage for *Fukomys* followed Garcia Montero et al. 2015, and was twice as high for *Spalacopus* and 50% higher for *Heterocephalus*), which are preferred anesthetics for ABR in small mammals (Smith and Mills, 1989; Ruebhausen et al., 2012). Whereas bathyergids close their eyes when anesthetized, coruros do not. Accordingly, their eyes were protected from desiccation by applying Vidisic® eye gel (Bausch & Lomb, Berlin, Germany). Over the time of the procedures, the subject's body temperature was maintained by a non-electric deltaphase isothermal heating pad (Braintree Scientific, Braintree, MA, USA) and repeatedly checked with a rectal electrode.

Auditory brainstem response

During testing, animals were staged in an aluminum cage (23.5×23.5×20 cm) placed within a custom-made anechoic chamber lined with foam (see Malkemper et al., 2015). All measurements as well as calibrations were performed within this chamber. A video camera installed into the chamber allowed us to monitor the animals during the tests. Brainstem potentials were recorded via subdermal electrodes (27 gauge, 13 mm, Rochester Electro-Medical, Lutz, FL, USA). The active electrode was positioned medially at the vertex of the animal, whereas the reference electrode was placed in a transverse orientation over the occipital region of the skull in close proximity to the brainstem (as in Gerhardt et al., 2017). The ground electrode was put to the subject's right thigh. Electrodes fed into a RA4LI low impedance headstage [Tucker Davis Technologies (TDT), Alachua, FL, USA] that was coupled with a Medusa RA4PA (TDT) preamplifier, both of which were positioned within the testing cage. The latter was connected to a TDT System 3 RZ6, which further amplified and digitalized recorded brainstem potentials and also generated the acoustic stimuli.

Stimulus presentation was achieved via an Arena Satellite speaker (frequency response 80 Hz to 54 kHz, used for stimuli between 200 Hz and 36 kHz; Tannoy, Coatbridge, UK) positioned approximately 20 cm from the left ear of the subject at an angle of 90 deg (the angle of sound incidence was 0 deg). Frequencies <200 Hz were emitted by a subwoofer (Punch HE Rockford Fosgate, Tempe, AZ, USA) that was positioned below the headstage level of the subject at the bottom of the anechoic chamber. Both speaker and subwoofer were operated via the RZ6. For calibration of speaker and subwoofer, a free-field microphone was employed (type 4939-C-002 with preamplifier 2669 C and conditioning amplifier Nexus 2692-A, Brüel & Kjær, Nærum, Denmark; frequency response 4 Hz to 100 kHz).

During calibration, the microphone was placed within the chamber. A dummy mimicking the volume of the respective study species was used to recreate the acoustic environment of the test situation. The microphone sensor was aligned to the position of a subject's left ear. The acoustic structure of the stimuli could be checked using a digital oscilloscope (Picoscope 4224, Pico Technology, St Neots, UK) that was jointed to the conditioning amplifier's output. Stimulus sound pressure level (SPL; reference: 20 µPa) was surveyed using the BioSig RZ software (v5.7.0, TDT), which was calibrated beforehand utilizing a Brüel & Kjær 4230 sound level calibrator with a ¼ inch microphone adaptor (B&K DB 0310). Deviations of up to 5 dB from the target sound pressure level were tolerated.

For each study species, we tested 15 different frequencies, which were preselected based on the hearing range determined by available studies on the respective species or congeneric relatives [naked mole rats: 0.03–12 kHz (16 kHz) (Heffner and Heffner, 1993; Okanoya et al., 2018), Mashona mole-rats: 0.03–16 kHz (Gerhardt et al., 2017), coruros: 0.03–32 kHz (36 kHz) (Begall et al., 2004); Table 2]. It became clear during the experiments that coruros exhibit good hearing at 32 kHz, so for three animals an additional 36 kHz step was included (noted in Table 1). Similarly, we decided to present a 16 kHz frequency step to seven naked

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Table 2. Species-specific frequencies (kHz) measured in the auditory brainstem response (ABR) set-up

Device	*Fukomys darlingi*	*Heterocephalus glaber*	*Spalacopus cyanus*
Subwoofer	0.03	0.03	0.03
	0.05	0.05	0.05
	0.1	0.1	0.1
Speaker	0.2	0.2	0.2
	0.4	0.5	0.5
	0.7	1	1
	1	2	1.3
	1.3	3	1.7
	1.7	3.5	2
	2	4	3
	4	4.5	6
	6	5	12
	8	6	16
	12	8	24
	16	12	32
		16*	36**

The device that generated the respective frequencies is listed.
*This condition was realized for seven subjects (see Table 1).
**This condition was realized for three subjects (see Table 1).

mole-rats because the duration of the anesthesia allowed us to do so without having to sedate them again. Nine frequency steps were tested in all species to allow for statistical comparisons (Table 2). Auditory stimuli were 5 ms long (1 ms rise/fall times, alternating starting phases) pure tones that were presented 12 times per second. Each frequency step comprised stimuli at nine different SPL levels that were presented with increasing intensity (0 to 80 dB, 10 dB steps). Tones were played 768 times at each SPL and the resulting recordings were averaged using the BioSig software.

All procedures were approved by the North Rhine-Westphalia State Environment Agency (permit number: 81-02.04.2019-A354).

Measurement of call amplitudes

We measured amplitudes of frequently occurring contact/greeting vocalizations, which aid in conspecific communication as general proxies for call loudness in the respective species: the naked mole-rat 'soft chirp', which is also known as the 'signature call' (n=22 from 3 individuals, mean peak frequency: ca. 3.5 kHz, cf. Okanoya et al., 2018; Barker et al., 2021), the Mashona mole-rat 'cheep' and 'cluck' vocalizations (n=20, from 2 individuals, mean peak frequencies: ca. 1.5–6.4 kHz, cf. Dvořáková et al., 2016) and the coruro 'cooing' vocalization (n=20, from 2 individuals, mean peak frequency: ca. 0.7 kHz, see Veitl et al., 2000).

Subjects were placed in pairs, or in the case of the naked mole-rat in groups of three individuals, in a separate glass terrarium. Call amplitudes (dB SPL) were measured with a PeakTeach® 5055 Sound Level Meter [mode: A (LO), fast adapting] that had been calibrated with a Type 4230 sound calibrator (94 dB, 1 kHZ, Brüel & Kjær). The sound level meter was moved freely by hand to maintain a close distance to the vocalizing animal. The sensor was on average positioned in a 90 deg angle at a distance of ca. 5–10 cm from the respective subject.

Threshold determination and statistics

Threshold estimation was performed by visual detection as is standard in the field (Gerhardt et al., 2017; Okanoya et al., 2018). Printouts of averaged ABR waveforms for a given frequency were assessed by three observers blind to the frequency condition. The mean estimate of the three observers was calculated and noted. Estimates were only scored when the difference between the assessed values was less than 15 dB.

All statistics were performed in RStudio (https://www.rstudio.com/). Data were checked for normal distribution employing the Shapiro–Wilk test and for homoscedasticity using the Levene test. Hearing thresholds were compared interspecifically for the nine frequencies on which all three species were tested (30 and 50 Hz, and 0.1, 0.2, 1, 2, 6, 12 and 16 kHz). Thresholds for naked mole-rats

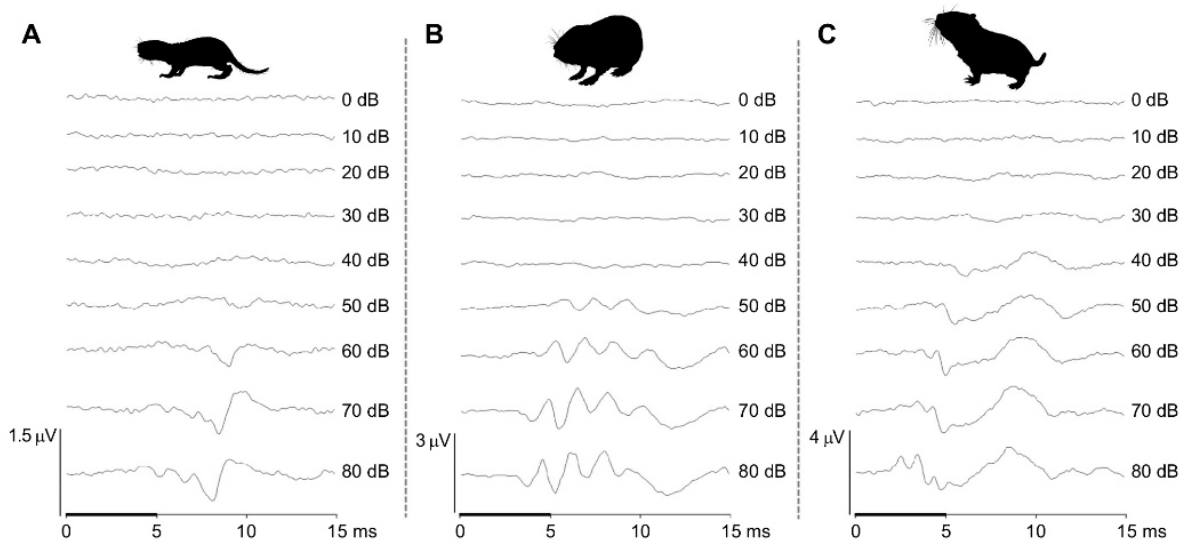

Fig. 1. Representative averaged (768×) auditory brainstem response (ABR) waveforms in the three tested subterranean rodent species. Auditory stimuli (pure tones) lasted 5 ms and are indicated in bold on the *x*-axes. (A) Naked mole-rat (*Heterocephalus glaber*, frequency shown=1 kHz); note the shallow waveform amplitude that is characteristic of this species. (B) Mashona mole-rat (*Fukomys darlingi*, frequency shown=1 kHz). (C) Coruro (*Spalacopus cyanus*, frequency shown=1.7 kHz). Silhouettes by Kai R. Caspar.

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and Mashona mole-rats were also compared at 4 kHz, a frequency at which we did not test coruros. Species-level comparisons of non-parametric data were calculated with the Kruskal–Wallis test followed by a pairwise Wilcoxon rank-sum test that employed Bonferroni correction to address for multiple comparisons. For parametric data, one-way ANOVA was used in combination with Tukey's HSD as a *post hoc* test.

To compare hearing sensitivity between age classes in coruros and Mashona mole-rats, we averaged threshold data for aged animals (n_{Coruro}=6, n_{MMR}=6), and pooled juvenile and young adult animals (n_{Coruro}=6, n_{MMR}=3). Subsequently, a Wilcoxon rank-sum test was run to compare the two groups.

RESULTS

Hearing sensitivity

Species-level ABR results are summarized in Table 3 and visualized in Fig. 2. Auditory thresholds of individual animals are listed in Table S1. The hearing range of the three studied species varied considerably at a sound intensity of 60 dB SPL, which is conventionally used as an intensity marker to denote the high- and low-frequency limits of hearing sensitivity in auditory studies (Heffner and Masterton, 1980). All species displayed a single more or less well-demarcated region of best hearing, where auditory thresholds were lowest.

The Mashona mole-rat had the most restricted hearing range, with a mean extent of 0.2–4 kHz. However, individual variation was considerable and related to age (see below). For frequencies where age-group specific average thresholds were located below 60 dB SPL, the mean (±s.d.) difference in sensitivity between aged and juvenile animals was 23.1±5.1 dB. In one aged individual, hearing thresholds were consistently located above 60 dB SPL, while one juvenile animal was still sensitive to frequencies of 6 kHz. On average, this species hears most acutely at 1 kHz, where mean hearing thresholds were found at 42.2±13.8 dB SPL. The lowest individual threshold in the sample was recorded at 1.3 kHz at 8.3 dB SPL in a juvenile animal.

In naked mole-rats, the mean hearing range extended from 0.2 to 6 kHz. The best hearing was found for frequencies between 1 and 3.5 kHz. In this range, thresholds were located at 39.6 and 43.1 dB SPL, respectively and therefore comparable to the ones of the Mashona mole-rat at a frequency of 1 kHz. The lowest recovered individual threshold was found for 3 kHz at 12 dB SPL.

The coruro had both the most acute hearing among the species in the sample and the greatest hearing range, which extended from 0.2 to 32 kHz. Sensitivity was greatest at frequencies between 1.3 and 2 kHz, where mean thresholds were recovered between 16.8 and 18.1 dB SPL. The lowest recorded threshold was 1.7 dB SPL and was found at a frequency of 1.3 kHz. Three coruros were tested on a 36 kHz step to better determine the high-frequency cut-off in this species. The mean sensitivity of these animals was 65±17.3 dB, with one juvenile animal responding at 45 dB SPL. However, mean thresholds did not differ between juvenile and adult coruros (see below).

Results from the statistical comparison of thresholds are summarized in Table S2. There were no significant differences in the hearing thresholds of the three species at frequencies of 30, 50 and 100 Hz (P>0.1). At all other tested frequencies, coruros differed significantly from the bathyergid species (P<0.05), exhibiting greater sensitivity. Hearing thresholds of naked mole-rats were consistently lower than those of Mashona mole-rats, but differences between the two species were only significant at 2 kHz (Tukey's HSD: P=0.017) and 4 kHz (Wilcoxon test: W=24.5, P=0.039).

The variation in hearing thresholds between age groups also differed between species. While coruro age classes did not differ in their hearing sensitivity (Wilcoxon test: W=116, P=0.885), younger Mashona mole-rats displayed significantly lower hearing thresholds than aged animals (W=167.5, P=0.023).

Table 3. Overview of recovered hearing thresholds of Mashona mole-rats (*Fukomys darlingi*), naked mole-rats (*Heterocephalus glaber*) and coruros (*Spalacopus cyanus*)

	Spalacopus cyanus (*n*=12)		*Fukomys darlingi* (*n*=9)		*Heterocephalus glaber* (*n*=12)	
Frequency (kHz)	Threshold (dB SPL)	s.d.	Threshold (dB SPL)	s.d.	Threshold (dB SPL)	s.d.
0.03	76.53	4.68	74.11	7.78	71.75	9.09
0.05	68.47	13.06	76.59	4.87	69.08	7.23
0.1	65.83	13.38	73.11	7.30	62.17	18.83
0.2	47.50	6.17	57.74	11.73	57.03	8.73
0.4			57.33	14.58		
0.5	35.83	8.83			49.56	13.74
0.7			49.78	13.60		
1	23.89	8.18	42.19	13.77	39.56	8.87
1.3	16.81	9.60	49.04	14.58		
1.7	18.06	7.65	54.44	15.37		
2	18.06	8.37	53.37	14.10	39.17	8.61
3	20.83	8.36			38.81	14.36
3.5					43.06	10.25
4			65.78	13.27	48.97	14.89
4.5					47.72	18.79
5					48.58	18.26
6	27.64	11.18	70.11	11.89	57.72	14.04
8			75.33	6.39	57.50	19.79
12	27.64	11.36	73.33	4.84	69.33	11.02
16	33.61	12.43	72.44	3.30	68.71	8.56
24	39.72	22.83				
32	43.19	18.26				
36	65.00	17.32				

Threshold values correspond to species means.

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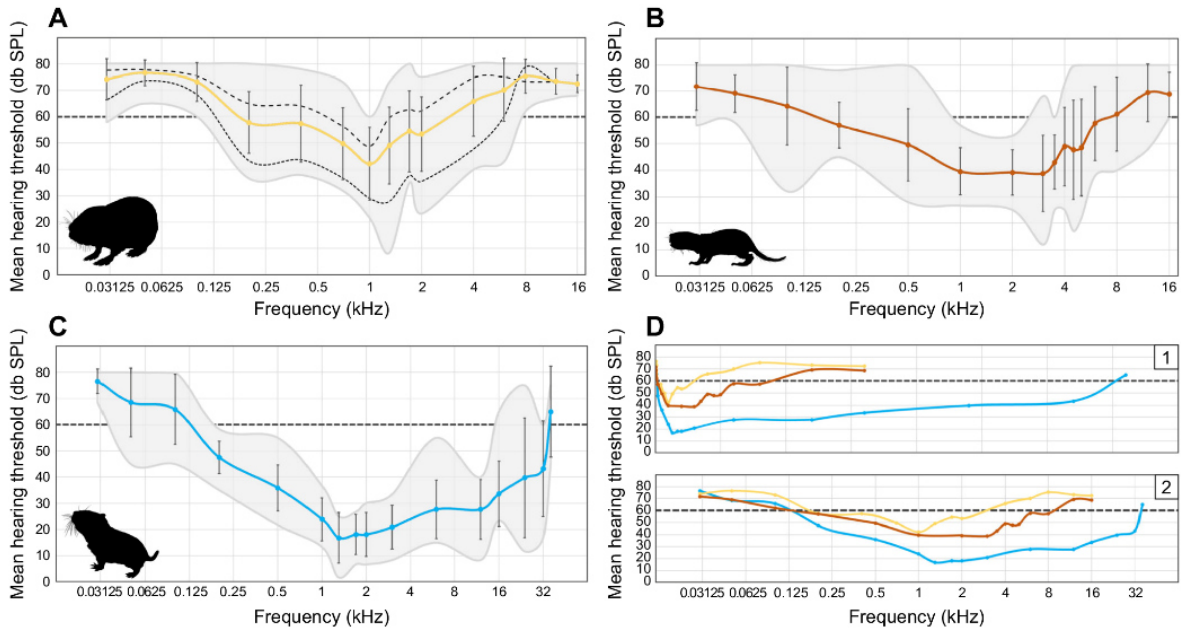

Fig. 2. Audiograms of subterranean rodents. Colored lines show mean hearing thresholds at the respective frequencies, error bars indicate standard deviations. The range of obtained threshold values is visualized by the grey overlay embedding the curves. Frequencies are plotted on $\log_2$ logarithmic scales if not indicated otherwise. (A) Mashona mole-rat (*Fukomys darlingi*). Mean hearing thresholds of aged (widely spaced dashed lines; *n*=6) and juvenile subjects (narrowly spaced dashed lines; *n*=3) are superimposed on the plot. (B) Naked-mole-rat (*Heterocephalus glaber*), *n*=12. (C) Coruro (*Spalacopus cyanus*), *n*=12. (D) Comparison of audiograms from the three species in a linear (1) and logarithmic plot (2).

Call amplitudes

Amplitudes of social vocalizations were similar overall between the three tested species (Fig. 3), despite the differences in body size and taxonomic affiliation. However, significant differences in call amplitudes still emerged (Kruskal–Wallis test: P=0.006, F=0.399). Median call amplitudes (±s.d.) were 52.6±3.3, 56.45±5.6 and 58.3 ±6.3 dB SPL for coruros, naked mole-rats and Mashona mole-rats, respectively. Pairwise comparisons showed that only coruros and naked mole-rats differed significantly in their call amplitudes (pairwise Wilcoxon test: P=0.0024; P>0.1 for other comparisons).

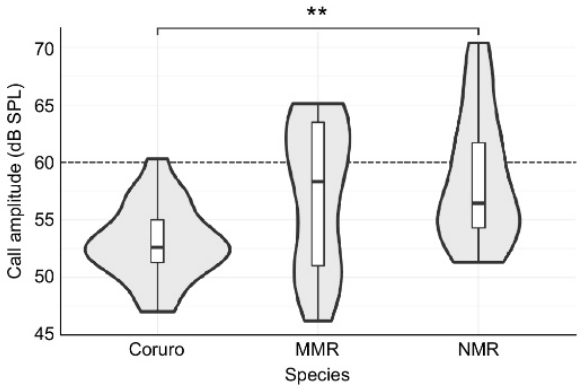

Fig. 3. Call amplitudes of subterranean rodents. MMR, Mashona mole-rat. NMR, naked mole-rat.

DISCUSSION

General discussion of results

ABRs showed marked differences in the hearing sensitivity of the three subterranean rodent species studied. The coruro differed from the two bathyergid species in displaying both a higher sensitivity, with mean thresholds below 20 dB SPL in the region of best hearing, and a far wider auditory range that reaches well into the ultrasonic domain. The ABR results for coruros are in good agreement with previously published behavioral audiograms (Begall et al., 2004). However, responses to frequencies above 20 kHz had not been tested in this species so far. Our results are the first to unambiguously demonstrate notable sensitivity to ultrasound in a hystricomorph subterranean rodent. Even at 36 kHz, one juvenile individual displayed a markedly low hearing threshold at 45 dB SPL, demonstrating that at least young coruros might have a hearing range that extends significantly further still. However, pronounced ultrasound sensitivity in coruros has already been suggested by earlier studies, for instance by cochlear frequency mapping (Begall and Burda, 2006). Nevertheless, at least for adults, the high-frequency cut-off at 44 kHz estimated by aid of this technique might need to be corrected to below 40 kHz. Besides that, it has been known that some coruro vocalizations include ultrasonic frequencies of pronounced intensity. In particular, juvenile coruros emit chirp calls that exhibit energy peaks within a frequency range of 17 to 31 kHz (Veitl et al., 2000). Although no other hystricomorph subterranean rodent is known to communicate in the ultrasonic range, several epigeic relatives of the coruro in the octodontid family are known to do so as well (*Octodon degus*: Long, 2009; *Octodontomys gliroides*: Pérez and Díaz, 2018). Sensitivity to and production of ultrasounds therefore likely

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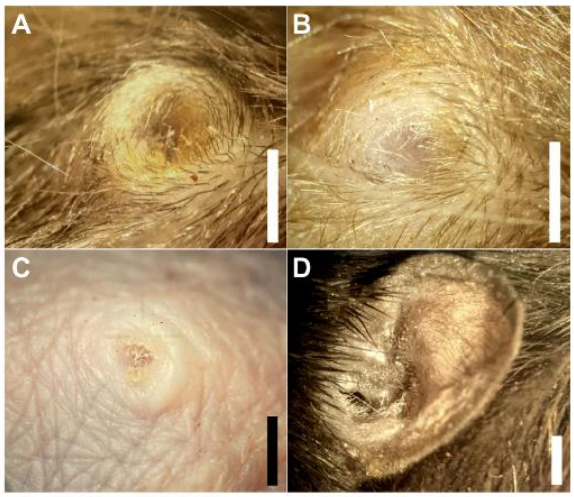

Fig. 4. The external ear in three genera of subterranean rodents. (A) *Fukomys* (adult, 4 years): the auditory meatus is narrow and lined by hair and accumulated cerumen. (B) *Fukomys* (immature, 5 months): note that cerumen is not yet obstructing the auditory meatus in the juvenile. (C) *Heterocephalus*: as in *Fukomys*, excess cerumen is well visible in the auditory meatus, which is again densely haired despite this species lacking body fur. (D) *Spalacopus*: a pinna is developed, and the auditory meatus is wide with only its entrance fringed by hair. Photographs were taken from cryopreserved specimens. Scale bars: 2 mm.

represents an ancestral trait that is still present in coruros despite their underground-dwelling habits. Accordingly, the range and sensitivity of hearing in coruros is not only greater than in other subterranean rodents (Heffner et al., 1994), but also approaches that of some fossorial and epigeic species with good low-frequency hearing such as the closely related degu (*Octodon degus*: Thomas and Tillein, 1997), the chinchilla (*Chinchilla lanigera*: Heffner and Heffner, 1991), Merriam's kangaroo rat (*Dipodomys merriami*: Heffner and Masterton, 1980), prairie dogs (*Cynomys* spp.: Heffner et al., 1994) and groundhogs (*Marmota monax*: Heffner et al., 2001). In line with this, the morphology of the coruro's middle ear exhibits only minor differences compared with epigeic caviomorph rodents, particularly the degu (Begall and Burda, 2006; Argyle and Mason, 2008). Gross morphological examination of coruros in our care also showed that these animals do not exhibit the narrow, cerumen-filled ear canals found in, for instance, bathyergids and spalacids (Fig. 4). Our results therefore corroborate the view that hearing in coruros has not undergone substantial changes in response to the invasion of the subterranean environment. Therefore, the few but presumably deleterious mutations in alleles relevant to audition that Pyott et al. (2020) described for the octodontoid lineage do not result in notable hearing impairment. Interestingly, the retainment of ultrasonic hearing sensitivity has recently been demonstrated for another geologically young subterranean rodent taxon, the Eurasian mole voles of the genus *Ellobius* (Buzan et al., 2008; Volodin et al., 2021).

Audiograms of naked mole-rats and Mashona mole-rats fit the established bathyergid pattern (Heffner and Heffner, 1993; Burda, 2006; Gerhardt et al., 2017) in displaying a pronounced restriction in audible frequencies and overall low sensitivity that did not fall below approximately 40 dB SPL, even in the regions of best hearing. The hearing curves of the two studied bathyergid species resembled each other in many respects and only diverged significantly in the 2 to 4 kHz region, where naked mole-rats exhibited more sensitive hearing. Just as in other *Fukomys* species (Müller and Burda, 1989; Gerhardt et al., 2017), the Mashona mole-rat's hearing is most responsive in a narrow frequency window at approximately 1 kHz and 40 dB SPL and its thresholds rise sharply at higher frequencies to sound pressure levels of above 60 dB at 4 kHz. Our results support the assumption that hearing sensitivity in the *Fukomys* genus is uniform despite pronounced differences in body mass between species (Gerhardt et al., 2017). In the naked mole-rat, lowest mean hearing thresholds are also located at approximately 40 dB SPL, but the region of best hearing extends from 1 to 3.5 kHz (see Okanoya et al., 2018 for similar findings) and the thresholds only approach 60 dB SPL at 6 kHz. Indeed, most calls of the remarkably vocal naked mole-rats show energy peaks in the frequency range of 2 to 4 kHz (Pepper et al., 1991; Okanoya et al., 2018). Surprisingly, both the peak and fundamental frequencies of most Mashona mole-rat calls (excluding mating calls) are centered at 2–5 kHz (Dvořáková et al., 2016) and therefore exceed the frequencies of best hearing in this species. However, the same is true for many vocalizations in congeneric species such as Ansell's mole-rat (*F. anselli*: Credner et al., 1997) and, to a lesser degree, the giant mole-rat (*F. mechowii*: Bednářová et al., 2013; cf. Gerhardt et al., 2017). Larynx size in these comparatively small-bodied mammals might constrain the production of loud low-frequency calls and could explain this discrepancy (Credner et al., 1997). Surprisingly, blind mole-rats of the genus *Nannospalax* are similar in body mass to some small-bodied *Fukomys* species but can produce lower-pitched vocalizations with peak frequencies around 0.5 kHz (Heth et al., 1986). However, blind mole-rats exhibit a Bulla thyreoidea that is not found in bathyergids, and which might act as a resonator (Credner, 1996). It remains unclear why the range of best hearing in the naked mole-rat is broader and includes higher frequencies than that in *Fukomys* (Fig. 2D). The extended total hearing range in naked mole-rats compared with *Fukomys* cannot be deduced from the morphology of the middle ear ossicles and the bony labyrinth. The structure of the incudo–mallear complex as well as the less developed cochlear coiling in the naked mole-rat have been proposed previously to indicate worse audition in this species than is found in other bathyergids (Mason et al., 2016).

Despite stark differences, there are also important similarities between all three studied taxa. Bathyergids and coruros converge in that the region of best hearing is located between or at least includes the frequency range 1 and 2 kHz. In that regard, the data are in agreement with previous audiograms of subterranean rodents (Heffner and Heffner, 1993; Begall et al., 2004; Burda, 2006), which all showed highest sensitivities in that unusually low frequency range and a more restricted range of best hearing than many other small mammals (Heffner and Masterton, 1980; Heffner et al., 1994). These peculiar peak sensitivities correspond well with the tunnel acoustics of the subterranean environment (Lange et al., 2007; Okanoya et al., 2018).

We recovered significant differences in hearing thresholds between juvenile and aged animals in the Mashona mole-rat, but not between coruro age groups. On the one hand, this discrepancy could relate to the fact that age differences in the Mashona mole-rats were far more pronounced than in the coruros and therefore do not necessarily point to varying influences of age on hearing sensitivity in the two groups. On the other hand, different from coruros, African mole-rats accumulate cerumen in their ear canals, which is expected to gradually worsen auditory performance in older individuals (Fig. 4; compare Kössl et al., 1996). We suggest that such a cerumen plug contributes importantly to the observed

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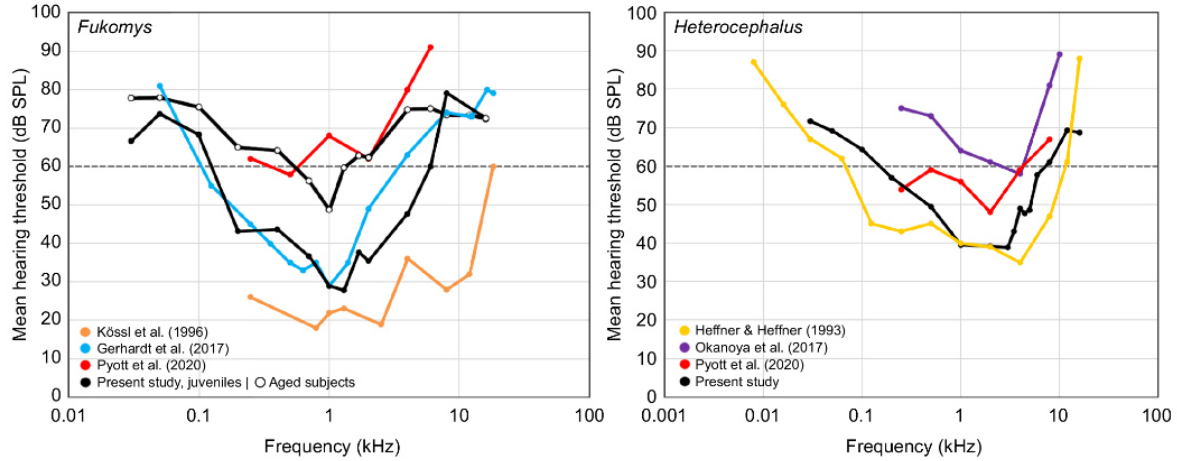

Fig. 5. Hearing curves for northern common mole-rats (*Fukomys*) and naked mole-rats (*Heterocephalus*) from various studies and obtained with different methodologies. Names in brackets refer to the respective species of *Fukomys* tested. Pyott et al. (2020) – ABR [*F. damarensis* (?)]. Gerhardt et al. (2017) – ABR (*F. anselli*). Kössl et al. (1996) – distortion product otoacoustic emissions (DPOAE) (*F. anselli*). Okanoya et al. (2018) – ABR. Heffner and Heffner (1993) – behavioral audiogram. Present study – ABR (*F. darlingi*), differentiated into aged and juvenile age groups. Dotted lines indicate 60 dB SPL.

differences between Mashona mole-rat age groups. Different from adults, we observed no cerumen at the opening of the auditory meatus in juvenile *Fukomys* (Fig. 4). However, we cannot infer to what extent this difference among age classes is connected to cerumen accumulation instead of to impairments of the organ of Corti that progress as senescence advances (Yamasoba et al., 2013). The hearing curve that we recovered for juvenile *Fukomys* is very similar to the ones described by Gerhardt et al. (2017), who tested mole-rats that were up to 175 weeks (3.35 years) old (Fig. 5). Hence, we expect that in animals up to this age, cerumen plugs do not impair hearing. Although demographic data on wild *Fukomys* are sparse, there is preliminary evidence that few free-ranging individuals actually reach such an age (Schmidt et al., 2013). Hence, we tentatively suggest that the thresholds recovered for juveniles are more representative for the majority of animals in the wild than the ones we describe for aged subjects.

Fitting its higher hearing sensitivity, calls in the coruro were fainter than in the bathyergids, but the loudness of measured vocalizations was still surprisingly similar in the three species, given their different taxonomic affinities and body sizes. Expectedly, call amplitudes were located well in the range of species-specific hearing that we recovered in the ABRs for the respective peak frequencies. However, the median call amplitudes of bathyergids are, if at all, barely loud enough to be audible if hearing sensitivities recovered in other studies, especially those in Pyott et al. (2020), are considered. Along with other inconsistencies across papers on hearing in bathyergids, this issue requires further discussion.

How strongly impaired is hearing in African mole-rats?

The acuity and physiology of hearing in the African mole-rats of the family Bathyergidae have been studied intensively, but with striking differences in specific outcomes. All reports agree that both hearing sensitivity and range are extremely restricted in bathyergids compared with epigeic rodents, but it is not yet clear which physiological traits cause this difference. Our results on Mashona mole-rats agree with previous electrophysiological hearing studies by Müller and Burda (1989) and Gerhardt et al. (2017) on congeneric species (Fig. 5). However, we recovered in part drastically lower sensitivities than suggested by behavioral audiograms for *Fukomys* (Brückmann and Burda, 1997).

Behavioral audiograms typically (but not always) yield thresholds approximately 5–15 dB lower than ABRs, which has been explained as a side effect of anesthesia (Smith and Mills, 1989; Ramsier and Dominy, 2010; Sisneros et al., 2016; Gerhardt et al., 2017). However, differences in outcome between the two methods are supposed to be most pronounced at low frequencies and are expected to diminish or even revert with increasing frequency (Ramsier and Dominy, 2010; Sisneros et al., 2016). This is not a consistent pattern in bathyergids (Gerhardt et al., 2017). The only available behavioral audiogram for *Fukomys* by Brückmann and Burda (1997) reports good hearing at high frequencies beyond 10 kHz with thresholds located 30 dB to more than 40 dB lower than those recovered in electrophysiological studies, including this one, in that tonal range. Gerhardt et al. (2017) already pointed out critical methodological issues with this study that could explain these inconsistencies and which we do not reiterate here. Replication of these results is required to eventually establish a reliable behavioral audiogram for *Fukomys*. Instead, our ABR results for naked mole-rats show good overall agreement with the established behavioral audiogram (Heffner and Heffner, 1993; Fig. 5). If we compare the alignment of the two curves with audiograms of other species obtained with these two methods, they fit well into the spectrum of agreement (Ramsier and Dominy, 2010).

On first glance, our data do also not appear to align with the results of a DPOAE study on Ansell's mole-rats (*Fukomys anselli*), which recorded responses to frequencies as high as 18 kHz and low peak sensitivities below 20 dB SPL (Kössl et al., 1996; see Fig. 5). Here, the recovered hearing sensitivity showed a similar range to the behavioral audiogram for common mole-rats, but was on average approximately 10 dB greater (Brückmann and Burda, 1997). Gerhardt et al. (2017) explained the discrepancies between the results of Kössl et al. (1996) and ABR-derived data by the fact that DPOAE measurements required the mole-rat auditory meatus to be cleaned and widened. Otherwise, the faint otoacoustic emission signals could not be picked up. With cleaning, hair and accumulated cerumen was removed, which attenuates incoming sounds and

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likely acts as a low-pass filter, preventing high frequencies to penetrate deeper into the ear (Kössl et al., 1996). Similarly obstructed ear canals have been observed in spalacid blind mole-rats and, to a lesser degree, also in naked mole-rats (Burda et al., 1992; Mason et al., 2016). The DPOAE results are therefore in line with the notion that the poor hearing of common mole-rats is significantly influenced by the sealed auditory meatus and therefore do not contradict the ABR data reported by us and other previously mentioned authors.

In contrast to Kössl et al. (1996), Pyott et al. (2020) found markedly higher thresholds in ABR set-ups for naked mole-rats and *Fukomys* mole-rats than we did, and failed to obtain DPOAE signals from either species (see also Okanoya et al., 2018 for even higher ABR thresholds in *Heterocephalus*; Fig. 4). The *Fukomys* mole-rats studied by Pyott et al. (2020) displayed thresholds barely falling below 60 dB SPL. If the latter results are representative, *Fukomys* mole-rats would, even when considering the threshold elevation caused by ABR measurements, be barely able to hear many of their social vocalizations, which, as we show, are mostly fainter. In light of the results of Kössl et al. (1996), it is remarkable that Pyott et al. (2020) found no evidence for DPOAE in *Heterocephalus* or *Fukomys*. A lack of DPOAE would imply that the cochlear amplifier, which is dependent on the motility of the outer hair cells in the organ of Corti and their linkage to the tectorial membrane by stereocilia, is non-functional (Pyott et al., 2020). Although we concur with the assumption that stereocilial defects are likely involved in the poor hearing of bathyergids and perhaps also other burrowing rodents (cf. Raphael et al., 1991), we doubt that the cochlear amplifier in these animals is non-functional and that their hearing thresholds are generally as high as reported by Pyott et al. (2020).

A major issue of this hypothesis is that it cannot explain the findings of Kössl et al. (1996), who worked with Ansell's mole-rats (*Fukomys anselli*). Although Pyott et al. (2020) do not present molecular data on Ansell's mole-rats, the study shows that relevant mutations potentially affecting the cochlear amplifier are shared by all *Fukomys* species and do not vary among congeners. It is extremely unlikely that critical mutations reversed solely in the Ansell's mole-rat lineage. Particularly, because Pyott et al. (2020) report no sign of such a reversal in Micklem's mole-rat (*Fukomys micklemi*), a recently diverging sister species of *F. anselli* (Van Daele et al., 2007). Pyott et al. (2020) were aware of the study by Kössl et al. (1996) but reported no obstruction of the auditory meatus by cerumen and hairs in the two bathyergid genera studied. Accordingly, they did not clean the ear canals of their subjects. Clean ear canals in adult bathyergids would be surprising in light of both the observations by other authors (Burda, 2006; Mason et al., 2016; see Fig. 4) and the high thresholds that the same study recovered for these animals in the ABR set-up (Fig. 5). Pyott et al. (2020) studied 5-year-old mole-rats, in which some cerumen accumulation must be expected. Thus, undetected remnants of cerumen sealing the auditory meatus could potentially explain the lack of DPOAEs in the tested species. Pyott et al. (2020) propose that the differences between Ansell's mole-rat and their tested *Fukomys* species, which were classified as Damaraland mole-rats, could result from hearing specializations in the former. These would include greater hearing sensitivity in conjunction with a region of increased hair cell density and, therefore, frequency representation in the apical regions of the cochlea. However, hair cell densities follow the same pattern in the Damaraland mole-rat and are indeed uniformly expressed in *Fukomys* species as well as in the sister genus *Cryptomys* (Lange, 2006). As shown by Gerhardt et al. (2017), Ansell's mole-rats do not hear significantly better than congeneric species, which is also suggested by our results. Instead, the mole-rats tested by Pyott et al. (2020) exhibit remarkably poor hearing, which is roughly comparable to that in our aged subject group, despite them being significantly younger (5 years versus >10 years, see Fig. 5). Therefore, the respective data do not appear to be generally representative of *Fukomys*.

We want to emphasize a procedural difference between our approach and the works of Pyott et al. (2020) and Okanoya et al. (2018), which could explain the varying outcomes on hearing sensitivity in *Heterocephalus* and *Fukomys*. Both studies used dosages of anesthetics that far exceeded those we employed here: 80 mg kg^{-1} ketamine and 20 mg kg^{-1} xylazine (Pyott et al., 2020) and 35–50 mg kg^{-1} ketamine and 8 mg kg^{-1} xylazine (Okanoya et al., 2018), compared with 6 mg kg^{-1} ketamine and 2.5 mg kg^{-1} xylazine for *Fukomys* and 9 mg kg^{-1} ketamine and 3.4 mg kg^{-1} xylazine for *Heterocephalus*. The dosages used by Pyott et al. (2020) and Okanoya et al. (2018) were comparable to or even higher than those applied to murine rodents to record ABR (Cederholm et al., 2012; Ruebhausen et al., 2012), although these have an elevated metabolic rate compared with bathyergids (Šumbera, 2019). We are unaware of studies that compared the effects of varying ketamine/xylazine volumes on ABR outcomes in small mammals but believe that such extreme differences in dosage could have contributed significantly to the high hearing thresholds communicated in the aforementioned studies. Kössl et al. (1996) found that ketamine dosages above 50 mg kg^{-1} also have a diminishing effect on DPOAE in Ansell's mole-rats. However, even at significantly higher dosages (90 mg kg^{-1}) comparable to the ones applied by Pyott et al. (2020), DPOAE were still detectable, so the differences in anesthesia protocols cannot explain why the latter study found no evidence at all for DPOAE in bathyergids (Kössl et al., 1996).

It should also be noted that the morphology of the organ of Corti in animals from the *Fukomys* laboratory strain used by Pyott et al. (2020) is aberrant, as these animals exhibit supernumerary outer hair cells (see Lange, 2006 for a comparison with wild-caught *F. damarensis*) and other unusual features relating to hearing physiology (Barone et al., 2019; Pyott et al., 2020). It therefore remains unclear whether the results of Pyott et al. (2020) on *Fukomys* in both the DPOAE and ABR set-ups might have been biased by pathologies.

To conclude, although Pyott et al. (2020) advanced the field in many regards and provide convincing arguments that the insensitive hearing in bathyergids relates at least in part to hair bundle defects, methodological issues, conflicting data from other studies, and anecdotal reports from diverse settings and localities (Ludwig and Collmar, 2009; Smith and Buffenstein, 2021) suggest that their auditory performance is not as poor as reported by these authors. Whether the cochlear amplifier is indeed non-functional in bathyergids needs to be clarified by future studies and can be doubted in light of the findings by Kössl et al. (1996).

Implications for the evolution of hearing in burrowing rodents

Comparisons between coruros and more ancient burrowing groups such as African mole-rats or blind mole-rats are valuable to infer how changes in hearing physiology relate to the ecological transition to the subterranean realm. Can our results advance the debate on whether hearing evolution in subterranean rodents follows adaptive or degenerative paths?

The hearing range and sensitivity of the coruro appear to be very similar to those of epigeic caviomorph rodents, which implies that a

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subterranean lifestyle per se does not produce selection pressures that induce a quick adaptation to the underground environment. Neither the low high-frequency cut-offs nor high hearing thresholds known from African mole-rats, blind mole-rats and pocket gophers (Heffner and Heffner, 1993) are found in coruros. However, compared with non-burrowing caviomorph rodents such as the chinchilla and the guinea pig (Heffner et al., 1971; Heffner and Heffner, 1991), the coruro displays a more restricted range of best hearing that is strongly shifted towards lower frequencies. This difference could represent a fast-evolving hearing adaptation of subterranean rodents as it appears restricted to and is present in all obligate underground-dwelling species studied so far (Burda et al., 1992; Heffner and Heffner, 1993). In fossorial rodents, such as ground squirrels, a similar shift and restriction of the region of best hearing is not evident (Heffner et al., 1994, 2001). However, as little is known about hearing sensitivity in epigeic octodontids, we cannot be sure how strongly the coruro really diverges from its ancestral family pattern.

Despite its subterranean and highly social lifestyle, hearing thresholds in coruros remain low. This is at odds with the hypothesis that the reduction of peak hearing sensitivity observed in most subterranean rodents is an adaptation to protect the ear from overstimulation by low-frequency vocalizations amplified in the burrow environment (Burda et al., 1992; Lange et al., 2007). There are further arguments against this notion. First, subterranean rodents communicate predominantly over short distances within their burrow systems (see Amaya et al., 2016 for an exception). The social bathyergids, for instance, almost exclusively vocalize when conspecifics are immediately close by and in tactile range, precluding sound amplification from affecting at least the main addressee of the vocal signal. Besides that, prolonged social encounters mostly occur in the nest chamber of the burrow system, which, owing to its shape and bedding, is expected to exhibit acoustic properties very different from those of tunnels. As already remarked by Mason (2013), this hypothesis also conflicts with the fact that subterranean rodents have lost, or in the case of the coruro (Begall and Burda, 2006), severely reduced, one of the two mammalian middle ear muscles, which otherwise could protect the ear from overstimulation (but see Burda et al., 1992 and Mason, 2006 for the possibility that middle ear muscles are maintained in epigeic groups to allow the ear to better adapt to high frequencies – an obsolete capacity underground). It is also difficult to argue for an adaptive value of high thresholds in the low-frequency region because the sensitivity of epigeic mammals in respective frequency ranges does often not notably differ (Heffner and Heffner, 1993) or is even higher than in subterranean groups (Heffner and Masterton, 1980). Instead, it appears that an ancestral hearing sensitivity is retained in the low-frequency range, while thresholds gradually increase towards higher frequencies, which is compliant with tunnel acoustics (Lange et al., 2007; Okanoya et al., 2018).

Yet, the coruro, mole-voles (Volodin et al., 2021) and, to a lesser extent, fossorial ground squirrels (Heffner et al., 1994; Jackson et al., 1997; Heffner et al., 2001) demonstrate that the loss of high-frequency hearing in underground environments does not evolve fast. The example of the mole-voles illustrates that even if the middle ear is optimized to process low frequencies, ultrasound vocalizations can still constitute an important aspect of intraspecific communication in subterranean rodents (Lange et al., 2004; Volodin et al., 2021). It is therefore doubtful that the extreme hearing range restriction in groups such as bathyergids and spalacids represents a trade-off to enable responsiveness to low frequencies underground. The delayed loss of high-frequency sensitivity could therefore be interpreted in favor of the degeneration model of hearing evolution in subterranean mammals.

An obstruction of the outer ear canal by hair and cerumen, as observed in diverse subterranean mammals, will notably contribute to poor hearing (Fig. 4; see previous section). However, the question of whether this trait serves an adaptive function is not resolved. Burda (2006) suggested that partially sealed auditory meatus represents an adaptation to prevent debris from entering, particularly when pinnae are absent. However, the occurrence of that character among subterranean mammals is not universal and can fluctuate even between closely related groups. For instance, the ear canals in the European mole (*Talpa europaea*) are typically unobstructed, whereas they are filled with cerumen in American mole genera (Mason, 2006). In groups sensitive to ultrasound, a sealed auditory meatus would be surprising, as it likely constitutes a substantial low-pass filter (Kössl et al., 1996). Indeed, we did not observe cerumen plugs in coruros (Fig. 4) and do not expect them to be found in the pinna-less mole-voles that communicate in the ultrasound range as well. Therefore, the sealing of the auditory meatus and its effect on hearing could represent a burrowing adaptation or reflect a neutrally selected deregulation of ear secretion in this peculiar habitat. In any case, it is difficult to argue that it evolved as an adaptive trait to facilitate hearing underground.

Besides all these factors, it is crucial to consider the genetic underpinnings of hearing in burrowing rodents. Some studies have reported positive selection for loci involved in hearing and outer hair cell hair bundle integrity in subterranean mammals (for instance ADGRV1 and USH1C; Davies et al., 2018; Pyott et al., 2020). Respective alleles have been speculated to benefit low-frequency hearing (Pyott et al., 2020). However, it remains unclear how that is realized, particularly because there is no plausible mechanism for hair bundle defects granting such an advantage. Counterintuitively, disintegration or even the absence of hair bundles is most frequent in the apical regions of the cochlea in spalacids and bathyergids, where low frequencies are processed (Raphael et al., 1991; Pyott et al., 2020).

From a proximate perspective, hair bundle defects and obstructed ear canals can explain important aspects of the poor hearing in various subterranean rodents, but why these traits arose in an evolutionary context remains elusive. Until the influence of candidate genes affecting hearing in burrowing mammals is better characterized, it will be difficult to determine whether specific derived hearing traits in these animals are due to adaptation, degeneration or perhaps even pleiotropy. Future research should consider including more epigeic species as a comparison to burrowing relatives in order to clarify the potential adaptive value of specific alleles. Interestingly, the hearing gene mutations listed by Pyott et al. (2020) are not restricted to subterranean mammals, but were also found to be drastically accumulated in African cane-rats (*Thryonomys* spp.), which are closely related to bathyergids. Different from mole-rats, these large-bodied rodents are fully epigeic and only occasionally dig shallow burrows in areas lacking the dense vegetation they prefer to hide in (Kingdon, 1974; erroneously denoted as fossorial by Pyott et al., 2020).

While awaiting further data on the interplay of genetics and hearing physiology, we suggest being open to both adaptive and degenerative interpretations of specific auditory traits in subterranean rodents. Given the evidence laid out above, we would argue that the elevated hearing thresholds and loss of high-frequency hearing found in these animals reflect a lack of selective pressures to maintain sensory characteristics that evolved in epigeic ancestors. In contrast, the consistent low-frequency shift of the area

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of best hearing that is already observable in geologically young lineages such as the coruro, is likely an adaptation to the peculiar acoustics of the underground realm. Ultimately, crucial questions about the hearing of burrowing rodents remain unresolved despite ongoing research efforts. Building on recent multidisciplinary approaches (Pyott et al., 2020), these issues need to be addressed by combining behavioral, physiological and genetic data to obtain a holistic picture of how and why these animals perceive the world in the way they do.

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References

Amaya, J. P., Areta, J. I., Valentinuzzi, V. S. and Zufiaurre, E. (2016). Form and function of long-range vocalizations in a Neotropical fossorial rodent: the Anillaco tuco-tuco (*Ctenomys* sp.). *PeerJ* **4**, e2559. doi:10.7717/peerj.2559

Argyle, E. C. and Mason, M. J. (2008). Middle ear structures of *Octodon degus* (Rodentia: Octodontidae), in comparison with those of subterranean caviomorphs. *J. Mammal.* **89**, 1447-1455. doi:10.1644/07-MAMM-A-401.1

Barker, A. J., Veviurko, G., Bennett, N. C., Hart, D. W., Mograby, L. and Lewin, G. R. (2021). Cultural transmission of vocal dialect in the naked mole-rat. *Science* **371**, 503-507. doi:10.1126/science.abc6588

Barone, C. M., Douma, S., Reijntjes, D. O. J., Browe, B. M., Köppl, C., Klump, G., Park, T. J. and Pyott, S. J. (2019). Altered cochlear innervation in developing and mature naked and Damaraland mole rats. *J. Comp. Neurol.* **527**, 2302-2316. doi:10.1002/cne.24682

Bednářová, R., Hrouzková-Knotková, E., Burda, H., Sedláček, F. and Šumbera, R. (2013). Vocalizations of the giant mole-rat (*Fukomys mechowii*), a subterranean rodent with the richest vocal repertoire. *Bioacoustics* **22**, 87-107. doi:10.1080/09524622.2012.712749

Begall, S. and Burda, H. (2006). Acoustic communication and burrow acoustics are reflected in the ear morphology of the coruro (*Spalacopus cyanus*, Octodontidae), a social fossorial rodent. *J. Morphol.* **267**, 382-390. doi:10.1002/jmor.10411

Begall, S., Burda, H. and Schneider, B. (2004). Hearing in coruros (*Spalacopus cyanus*): special audiogram features of a subterranean rodent. *J. Comp. Physiol. A* **190**, 963-969. doi:10.1007/s00359-004-0552-1

Begall, S., Burda, H. and Schleich, C. E. (2007). Subterranean rodents: news from underground. In *Subterranean Rodents: News from Underground* (ed. S. Begall, H. Burda and C. E. Schleich), pp. 3-9. Berlin, Heidelberg: Springer.

Bennett, N. C., Jarvis, J. U. M. and Cotterill, F. P. D. (1994). The colony structure and reproductive biology of the Afrotropical Mashona mole-rat, *Cryptomys darlingi*. *J. Zool.* **234**, 477-487. doi:10.1111/j.1469-7998.1994.tb04861.x

Brückmann, G. and Burda, H. (1997). Hearing in blind subterranean Zambian mole-rats (*Cryptomys* sp.): collective behavioural audiogram in a highly social rodent. *J. Comp. Physiol. A* **181**, 83-88. doi:10.1007/s003590050095

Bruns, V., Müller, M., Hofer, W., Heth, G. and Nevo, E. (1988). Inner ear structure electrophysiological audiograms of the subterranean mole rat, *Spalax ehrenbergi*. *Hear. Res.* **33**, 1-9. doi:10.1016/0378-5955(88)90017-2

Bryja, J., Konvičková, H., Bryjová, A., Mikula, O., Makundi, R., Chitaukali, W. N. and Šumbera, R. (2018). Differentiation underground: range-wide multilocus genetic structure of the silvery mole-rat does not support current taxonomy based on mitochondrial sequences. *Mamm. Biol.* **93**, 82-92. doi:10.1016/j.mambio.2018.08.006

Burda, H. (2006). Ear and eye in subterranean mole-rats, *Fukomys anselli* (Bathyergidae) and *Spalax ehrenbergi* (Spalacidae): progressive specialisation or regressive degeneration? *Anim. Biol.* **56**, 475-486. doi:10.1163/157075606778967847

Burda, H., Bruns, V. and Hickman, G. C. (1992). The ear in subterranean Insectivora and Rodentia in comparison with ground-dwelling representatives. I. Sound conducting system of the middle ear. *J. Morphol.* **214**, 49-61. doi:10.1002/jmor.1052140104

Buzan, E. V., Krystufek, B., Hänfling, B. and Hutchinson, W. F. (2008). Mitochondrial phylogeny of Arvicolinae using comprehensive taxonomic sampling yields new insights. *Biol. J. Linn. Soc.* **94**, 825-835. doi:10.1111/j.1095-8312.2008.01024.x

Capranica, R. R., Nevo, E. and Moffat, A. J. M. (1974). Vocal repertoire of a subterranean rodent (*Spalax*). *J. Acoust. Soc. Am.* **55**, 481-481. doi:10.1121/1.3438017

Cederholm, J. M. E., Froud, K. E., Wong, A. C. Y., Ko, M., Ryan, A. F. and Housley, G. D. (2012). Differential actions of isoflurane and ketamine-based anaesthetics on cochlear function in the mouse. *Hear. Res.* **292**, 71-79. doi:10.1016/j.heares.2012.08.010

Credner, S. (1996). Akustische Kommunikation unter der Erde: Vokalisations-Charakteristiken von unterirdisch und sozial lebenden Graumullen (*Cryptomys* sp., Bathyergidae). *Doctoral dissertation*, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany.

Credner, S., Burda, H. and Ludescher, F. (1997). Acoustic communication underground: vocalization characteristics in subterranean social mole-rats (*Cryptomys* sp., Bathyergidae). *J. Comp. Physiol. A* **180**, 245-255. doi:10.1007/s003590050045

Davies, K. T. J., Bennett, N. C., Faulkes, C. G. and Rossiter, S. J. (2018). Limited evidence for parallel molecular adaptations associated with the subterranean niche in mammals: a comparative study of three superorders. *Mol. Biol. Evol.* **35**, 2544-2559. doi:10.1093/molbev/msy161

Devries, M. S. and Sikes, R. S. (2008). Vocalisations of a North American subterranean rodent *Geomys breviceps*. *Bioacoustics* **18**, 1-15. doi:10.1080/09524622.2008.9753587

Dvořáková, V., Hrouzková, E. and Šumbera, R. (2016). Vocal repertoire of the social Mashona mole-rat (*Fukomys darlingi*) and how it compares with other mole-rats. *Bioacoustics* **25**, 253-266. doi:10.1080/09524622.2016.1141117

Fleischer, G. (1973). Studien am Skelett des Gehörorgans der Säugetiere, einschließlich des Menschen. *Säugetierkundliche Mitt.* **21**, 131-239.

Garcia Montero, A., Burda, H. and Begall, S. (2015). Chemical restraint of African mole-rats (*Fukomys* sp.) with a combination of ketamine and xylazine. *Vet. Anaesth. Analg.* **42**, 187-191. doi:10.1111/vaa.12180

Gerhardt, P., Henning, Y., Begall, S. and Malkemper, E. P. (2017). Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency cut-offs. *J. Exp. Biol.* **220**, 4747. doi:10.1242/jeb.175190

Gessele, N., Garcia-Pino, E., Omerbašić, D., Park, T. J. and Koch, U. (2016). Structural changes and lack of HCN1 channels in the binaural auditory brainstem of the naked mole-rat (*Heterocephalus glaber*). *PLoS ONE* **11**, e0146428. doi:10.1371/journal.pone.0146428

Heffner, R. S. and Heffner, H. E. (1990). Vestigial hearing in a fossorial mammal, the pocket gopher (*Geomys bursarius*). *Hear. Res.* **46**, 239-252. doi:10.1016/0378-5955(90)90005-A

Heffner, R. S. and Heffner, H. E. (1991). Behavioral hearing range of the chinchilla. *Hear. Res.* **52**, 13-16. doi:10.1016/0378-5955(91)90183-A

Heffner, R. S. and Heffner, H. E. (1992). Hearing and sound localization in blind mole rats (*Spalax ehrenbergi*). *Hear. Res.* **62**, 206-216. doi:10.1016/0378-5955(92)90188-S

Heffner, R. S. and Heffner, H. E. (1993). Degenerate hearing and sound localization in naked mole rats (*Heterocephalus glaber*), with an overview of central auditory structures. *J. Comp. Neurol.* **331**, 418-433. doi:10.1002/cne.903310311

Heffner, H. and Masterton, B. (1980). Hearing in Glires: domestic rabbit, cotton rat, feral house mouse, and kangaroo rat. *J. Acoust. Soc. Am.* **68**, 1584-1599. doi:10.1121/1.385213

Heffner, R., Heffner, H. and Masterton, B. (1971). Behavioral measurements of absolute and frequency-difference thresholds in guinea pig. *J. Acoust. Soc. Am.* **49**, 1888-1895. doi:10.1121/1.1912596

Heffner, R. S., Heffner, H. E., Contos, C. and Kearns, D. (1994). Hearing in prairie dogs: transition between surface and subterranean rodents. *Hear. Res.* **73**, 185-189. doi:10.1016/0378-5955(94)90233-X

Heffner, R. S., Koay, G. and Heffner, H. E. (2001). Audiograms of five species of rodents: implications for the evolution of hearing and the perception of pitch. *Hear. Res.* **157**, 138-152. doi:10.1016/S0378-5955(01)00298-2

Heth, G., Frankenberg, E. and Nevo, E. (1986). Adaptive optimal sound for vocal communication in tunnels of a subterranean mammal (*Spalax ehrenbergi*). *Experientia* **42**, 1287-1289. doi:10.1007/BF01946426

Hill, W. C. O., Porter, A., Bloom, R. T., Seago, J. and Southwick, M. D. (1957). Field and laboratory studies on the naked mole rat, *Heterocephalus glaber*. *Proc. Zool. Soc. Lond.* **128**, 455-514. doi:10.1111/j.1096-3642.1957.tb00272.x

Hrouzková, E., Šklíba, J., Pleštilová, L., Hua, L., Meheretu, Y., Sillero-Zubiri, C. and Šumbera, R. (2018). Seismic communication in spalacids: signals in the giant root-rat and *Gansu zokor*. *Hystrix. Ital. J. Mammal.* **29**, 243-245.

Jackson, L. L., Heffner, H. E. and Heffner, R. S. (1997). Audiogram of the fox squirrel (*Sciurus niger*). *J. Comp. Psychol.* **111**, 100-104. doi:10.1037/0735-7036.111.1.100

Kingdon, J. (1974). *East African Mammals. An Atlas of Evolution in Africa Vol. II, Part B (Hares and Rodents)*, Chicago: The University of Chicago Press.
Kössl, M., Frank, G., Burda, H. and Müller, M. (1996). Acoustic distortion products from the cochlea of the blind African mole rat, *Cryptomys* spec. *J. Comp. Physiol. A* **178**, 427-434. doi:10.1007/BF00193979
Lange, S. (2006). Sinnesökologie afrikanischer Sandgräber (Bathyergidae) am Beispiel von Hör- und Geruchssinn. *Doctoral dissertation, Universität Duisburg-Essen*, Essen, Germany.
Lange, S., Stalleicken, J. and Burda, H. (2004). Functional morphology of the ear in fossorial rodents, *Microtus arvalis* and *Arvicola terrestris*. *J. Morphol.* **262**, 770-779. doi:10.1002/jmor.10277
Lange, S., Burda, H., Wegner, R. E., Dammann, P., Begall, S. and Kawalika, M. (2007). Living in a "stethoscope": burrow-acoustics promote auditory specializations in subterranean rodents. *Naturwissenschaften* **94**, 134-138. doi:10.1007/s00114-006-0168-0
Long, C. V. (2009). Pups of the degu (*Octodon degus*) include ultrasonic frequencies in care-eliciting calls. *Proc. Inst. Acoust.* **31**, 237-244.
Ludwig, W. and Collmar, M. (2009). Erfahrungsbericht zur Haltung von Nacktmullen (*Heterocephalus glaber*) im Zoo Dresden. *Der Zoologische Garten* **78**, 61-74. doi:10.1016/j.zoolgart.2008.09.003
Malkemper, E. P., Topinka, V. and Burda, H. (2015). A behavioral audiogram of the red fox (*Vulpes vulpes*). *Hear. Res.* **320**, 30-37. doi:10.1016/j.heares.2014.12.001
Mason, M. J. (2006). Evolution of the middle ear apparatus in talpid moles. *J. Morphol.* **267**, 678-695. doi:10.1002/jmor.10430
Mason, M. J. (2013). Of mice, moles and guinea pigs: functional morphology of the middle ear in living mammals. *Hear. Res.* **301**, 4-18. doi:10.1016/j.heares.2012.10.004
Mason, M. J., Cornwall, H. L. and Smith, E. S. J. (2016). Ear structures of the naked mole-rat, *Heterocephalus glaber*, and its relatives (Rodentia: Bathyergidae). *PLoS ONE* **11**, e0167079. doi:10.1371/journal.pone.0167079
Müller, M. and Burda, H. (1989). Restricted hearing range in a subterranean rodent, *Cryptomys hottentotus*. *Naturwissenschaften* **76**, 134-135. doi:10.1007/BF00366611
Narins, P. M., Reichman, O. J., Jarvis, J. U. M. and Lewis, E. R. (1992). Seismic signal transmission between burrows of the Cape mole-rat, *Georychus capensis*. *J. Comp. Physiol. A* **170**, 13-21. doi:10.1007/BF00190397
Okanoya, K., Yosida, S., Barone, C. M., Applegate, D. T., Brittan-Powell, E. F., Dooling, R. J. and Park, T. J. (2018). Auditory-vocal coupling in the naked mole-rat, a mammal with poor auditory thresholds. *J. Comp. Physiol. A* **204**, 905-914. doi:10.1007/s00359-018-1287-8
Pepper, J. W., Braude, S., Lacey, E. and Sherman, P. W. (1991). Vocalizations of the naked mole-rat. In *The Biology of the Naked Mole-Rat* (ed. P. W. Sherman, J. U. M. Jarvis and R. D. Alexander), pp. 243-274. Princeton: Princeton University Press.
Pérez, M. J. and Díaz, M. M. (2018). *Octodontomys gliroides* (Rodentia: Octodontidae). *Mamm. Species* **50**, 74-83. doi:10.1093/mspecies/sey010
Pyott, S. J., van Tuinen, M., Screven, L. A., Schrode, K. M., Bai, J.-P., Barone, C. M., Price, S. D., Lysakowski, A., Sanderford, M., Kumar, S. et al. (2020). Functional, morphological, and evolutionary characterization of hearing in subterranean, eusocial African mole-rats. *Curr. Biol.* **30**, 4329-4341.e4. doi:10.1016/j.cub.2020.08.035
Ramsier, M. A. and Dominy, N. J. (2010). A comparison of auditory brainstem responses and behavioral estimates of hearing sensitivity in *Lemur catta* and *Nycticebus coucang*. *Am. J. Primatol.* **72**, 217-233. doi:10.1002/ajp.20780
Raphael, Y., Lenoir, M., Wroblewski, R. and Pujol, R. (1991). The sensory epithelium and its innervation in the mole rat cochlea. *J. Comp. Neurol.* **314**, 367-382. doi:10.1002/cne.903140211
Ruebhausen, M. R., Brozoski, T. J. and Bauer, C. A. (2012). A comparison of the effects of isoflurane and ketamine anesthesia on auditory brainstem response (ABR) thresholds in rats. *Hear. Res.* **287**, 25-29. doi:10.1016/j.heares.2012.04.005
Schleich, C. and Busch, C. (2002). Acoustic signals of a solitary subterranean rodent *Ctenomys talarum* (Rodentia: Ctenomyidae): physical characteristics and behavioural correlates. *J. Ethol.* **20**, 123-131. doi:10.1007/s10164-002-0064-9
Schleich, C. E., Veitl, S., Knotková, E. and Begall, S. (2007). Acoustic communication in subterranean rodents. In *Subterranean Rodents: News from Underground* (ed. S. Begall, H. Burda and C. E. Schleich), pp. 113-127. Berlin, Heidelberg: Springer.
Schmidt, C. M., Jarvis, J. U. M. and Bennett, N. C. (2013). The long-lived queen: reproduction and longevity in female eusocial Damaraland mole-rats (*Fukomys damarensis*). *Afr. Zool.* **48**, 193-196. doi:10.1080/15627020.2013.11407583
Sisneros, J. A., Popper, A. N., Hawkins, A. D. and Fay, R. R. (2016). Auditory evoked potential audiograms compared with behavioral audiograms in aquatic animals. In *The Effects of Noise on Aquatic Life II* (ed. A. Popper and A. Hawkins), pp. 1049-1056. New York: Springer.
Smith, M. and Buffenstein, R. (2021). Managed care of naked mole-rats. In *The Extraordinary Biology of the Naked Mole-Rat* (ed. R. Buffenstein T. J. Park and M. M. Holmes), pp. 381-407. New York: Springer.
Smith, D. I. and Mills, J. H. (1989). Anesthesia effects: auditory brain-stem response. *Electroencephalogr. Clini. Neurophysiol.* **72**, 422-428. doi:10.1016/0013-4694(89)90047-3
Smorkatcheva, A. V. and Kumaitova, A. R. (2014). Delayed dispersal in the Zaisan mole vole (*Ellobius tancrei*): helping or extended parental investment? *J. Ethol.* **32**, 53-61. doi:10.1007/s10164-013-0392-y
Šumbera, R. (2019). Thermal biology of a strictly subterranean mammalian family, the African mole-rats (Bathyergidae, Rodentia) – a review. *J. Therm. Biol.* **79**, 166-189. doi:10.1016/j.jtherbio.2018.11.003
Thomas, H. and Tillein, J. (1997). Functional organization of the auditory cortex in a native Chilean rodent (*Octodon degus*). *Biol. Res.* **30**, 137-148.
Upham, N. S. and Patterson, B. D. (2012). Diversification and biogeography of the Neotropical caviomorph lineage Octodontoidea (Rodentia: Hystricognathi). *Mol. Phylogenet. Evol.* **63**, 417-429. doi:10.1016/j.ympev.2012.01.020
Van Daele, P. A. A. G., Verheyen, E., Brunain, M. and Adriaens, D. (2007). Cytochrome b sequence analysis reveals differential molecular evolution in African mole-rats of the chromosomally hyperdiverse genus *Fukomys* (Bathyergidae, Rodentia) from the Zambezian region. *Mol. Phylogenet. Evol.* **45**, 142-157. doi:10.1016/j.ympev.2007.04.008
Veitl, S., Begall, S. and Burda, H. (2000). Ecological determinants of vocalisation parameters: the case of the coruro *Spalacopus cyanus* (Octodontidae), a fossorial social rodent. *Bioacoustics* **11**, 129-148. doi:10.1080/09524622.2000.9753455
Volodin, I. A., Dymskaya, M. M., Smorkatcheva, A. V. and Volodina, E. V. (2021). Ultrasound from underground: cryptic communication in subterranean wild-living and captive northern mole voles (*Ellobius talpinus*). *Bioacoustics*. doi:10.1080/09524622.2021.1960191
Yamasoba, T., Lin, F. R., Someya, S., Kashio, A., Sakamoto, T. and Kondo, K. (2013). Current concepts in age-related hearing loss: epidemiology and mechanistic pathways. *Hear. Res.* **303**, 30-38. doi:10.1016/j.heares.2013.01.021

Supplementary Material

Supplementary Table 1: Individual-level auditory thresholds (in dB SPL) for three subterranean rodent species. Threshold values are rounded to 1 dB. Frequencies are provided in kHz. See Table 1 for further information on each subject.

Naked mole-rat (*Heterocephalus glaber*)

Frequency	HG1 3105	HG1 67311	HG2 5400	HG2 7311	HG3 5401	HG3 5402	HG3 5403	HG3 5408	HG3 5422	HG3 5423	HG3 5424	HG5 5351
0.03	60	80	77	73	77	80	80	57	70	70	57	80
0.05	63	80	75	62	65	70	75	68	63	58	70	80
0.1	32	72	80	80	58	72	58	48	75	57	60	80
0.2	48	55	63	57	48	57	60	78	45	62	53	58
0.5	47	52	38	55	50	50	63	80	28	32	53	47
1	40	45	40	43	30	48	40	57	35	27	43	27
2	27	40	50	25	53	43	35	45	32	42	43	35
3	23	12	40	45	47	52	37	68	35	40	40	27
3.5	33	38	52	25	60	40	57	50	37	38	40	47
4	28	47	52	43	63	40	47	73	50	25	48	72
4.5	27	55	58	30	55	47	17	80	55	47	30	72
5	22	43	62	25	48	37	53	80	47	43	43	80
6	45	43	60	55	60	38	80	80	75	52	53	52
8	40	55	73	73	62	40	67	80	47	57	58	80
12	55	80	78	77	75	80	65	60	48	62	72	80
16	/	75	/	80	62	/	/	63	63	78	60	/
18	/	/	/	/	62	/	/	/	65	/	/	/

Coruro (*Spalacopus cyanus*)

Frequency	SC1 2797	SC1 7664	SC2 2732	SC2 2760	SC2 2780	SC2 9892	SC3 5343	SC3 5346	Scae 5377	SCae 8732	SCh 0820	SCh 5345
0.03	80	80	75	80	70	80	70	75	68	80	80	80
0.05	80	80	80	57	72	72	50	75	55	77	80	45
0.1	57	67	45	77	48	75	73	80	47	65	77	80
0.2	42	50	50	43	50	45	50	35	47	55	45	58
0.5	47	30	28	37	33	22	55	33	33	43	35	33
1	35	15	18	37	18	23	27	20	22	13	22	37
1.3	8	37	7	13	20	2	15	23	22	20	10	25
1.7	25	10	7	23	25	25	8	12	12	22	22	27
2	15	32	12	17	17	13	30	7	15	8	23	28
3	33	28	20	8	20	25	13	8	22	15	25	32
6	32	28	15	30	33	30	55	33	23	15	22	15
12	15	45	8	30	33	20	40	25	18	22	35	40
16	23	45	28	38	30	23	65	27	30	43	23	27
24	12	55	22	17	32	17	60	22	52	73	42	75
32	33	78	33	47	52	30	60	18	38	60	17	52
36	75	/	/	/	/	/	/	/	45	/	75	/

Supplementary Table 1 (continued)

Mashona mole-rat (*Fukomys darlingi*)

Frequency	FD1 5450	FD1 9399	FD2 2229	FD2 5361	FD2 5441	FD3 30000	FD4 0815	FD5 4105	FD5 4686
0.03	58	75	77	75	67	75	80	80	80
0.05	65	80	78	78	78	77	78	75	80
0.1	60	80	70	80	65	80	78	75	70
0.2	45	60	57	37	48	80	63	57	73
0.4	48	73	52	45	38	78	52	55	75
0.7	32	48	50	45	33	73	57	50	60
1	33	53	37	22	32	60	58	27	58
1.3	38	70	47	8	37	73	55	45	68
1.7	38	65	45	35	40	62	72	53	80
2	33	75	65	23	50	72	45	57	60
4	43	60	77	40	60	80	78	77	77
6	43	77	80	60	77	77	70	72	75
8	80	80	70	80	77	77	80	72	62

Supplementary Table 2: Results of statistical comparisons (ANOVA, Kruskal-Wallis test, paired Wilcoxon test, Tukey's honest significant differences (THSD)) of hearing thresholds in coruros, Mashona mole-rats (MMR) and naked mole-rats (NMR). Frequencies are provided in kHz. Significant *p*-values are indicated in bold.

Frequency	Coruro / MMR	Coruro / NMR	MMR / NMR
0.03	Kruskal-Wallis chi-squared = 1.8857, $p = 0.3895$		
0.05	Kruskal-Wallis chi-squared = 4.5134, $p = 0.1047$		
0.1	ANOVA: F-value = 1.503 $p = 0.238$		
0.2	THSD, $\mathbf{p = 0.041}$	THSD, $\mathbf{p = 0.047}$	THSD, $p = 0.983$
1	THSD, $\mathbf{p < 0.001}$	THSD, $\mathbf{p = 0.002}$	THSD, $p = 0.828$
2	THSD, $\mathbf{p < 0.001}$	THSD, $\mathbf{p < 0.001}$	THSD, $p = \mathbf{0.017}$
4	NA	NA	Wilcoxon Test, $\mathbf{p = 0.039}$
6	Wilcoxon Test, $\mathbf{p < 0.001}$	Wilcoxon Test, $\mathbf{p < 0.001}$	Wilcoxon Test, $p = 0.238$
12	Wilcoxon Test, $\mathbf{p < 0.001}$	Wilcoxon Test, $\mathbf{p < 0.001}$	Wilcoxon Test, $p = 1$
16	THSD, $\mathbf{p < 0.001}$	THSD, $\mathbf{p < 0.001}$	THSD, $p = 0.701$

Discussion

For this manuscript, it is important to address methodological flaws in low-frequency ABR stimulus design that were overlooked during the study's preparation and which were pointed out to us by Pim van Dijk and Geoffrey A. Manley who visited our working group in early spring 2022.

We used tones with a total length of 5 ms, irrespective of stimulus frequency to evoke brainstem responses. However, this rigid stimulus design brings problems for low-frequency tones. With decreasing frequency, the time to complete a full wave cycle naturally increases. If a stimulus is 5 ms long (here including 1 ms rise-fall time for the amplitude of the wave), the lowest frequency, for which a wave cycle can be completed is 200 Hz. However, our study also employed stimuli with a lower frequency than that, namely at 100 Hz, 50 Hz and 30 Hz. Hence, sound waves for these three frequency steps were curtailed. It is, however, unclear whether a full wave cycle is needed to elicit an ABR signal. For instance, Gerhardt et al. (2017) assume that it suffices to have stimuli that include just a single maximum or minimum amplitude of the respective sound wave. When following this approach, 50 Hz would be recovered as the minimum viable frequency to test. Therefore, at least our 30 Hz stimuli were strongly distorted and the validity of the respective measurements is questionable.

But there are additional issues. The rise-fall time of the stimulus should be at least as long as one wave cycle at the respective frequency to permit it to exhibit a constant sound pressure level. This necessitates an increase in rise-fall times with decreasing stimulus frequencies, as was done, for instance, by Manley & Kraus (2010), and compromises the quality of < 1kHz stimuli in our study as well as in the one by Gerhardt et al. (2017). Finally, as a technical necessity, each stimulus frequency emission is accompanied by a certain spectrum of frequencies adjacent to the desired frequency. At low frequencies < 200 Hz, spectra may be too broad and noisy to make reliable ABR recordings at the chosen narrow intervals (30 Hz, 50 Hz, 100 Hz), because not only hair cells responding to the desired frequency (e.g., 100 Hz) will be stimulated by the spectrum, but also those tuned to slightly higher or lower frequencies (e.g., 50/200 Hz; van Dijk & Manley, pers. com.). As responses to higher frequencies must be expected to be more acute than to lower ones within this part of the hearing spectrum, it is likely that we were unintentionally measuring the sensitivity of untargeted receptors. Accordingly, the low-frequency sensitivity of a subject might be overestimated. At higher frequencies, which were measured at greater frequency intervals, this issue is far less pressing.

Given all these criticisms, the reliability of our low-frequency measurements may appear doubtful. However, there are also indications that the related measurement distortions were not critical. E. Pascal Malkemper was kind enough to share data on recordings made for a previous ABR study on *Fukomys* hearing sensitivity that used the exact same set-up as we did here (Gerhardt et al., 2017). Originally, the authors measured low frequency (< 800 Hz) responses for

stimulus lengths matching the ones of Chapter 2.3. However, to comply to a reviewer's request, they also tested two subjects (one individual of the species *F. anselli* and *F. micklemi* each) with longer stimuli that encompassed three complete wave cycles (50 Hz: 60 ms, 125 Hz: 24 ms, etc.). The hearing thresholds recovered by employing these different stimuli were rather similar and surprisingly lower when longer stimuli were used (Fig. 6, E. P. Malkemper, pers. com.). These findings suggest that the low-frequency hearing sensitivity of our subjects has not been notably overestimated due to flawed stimulus design, at least not for frequencies of ≥ 50 Hz. Still, critique regarding our methodology surely is warranted due to the aforementioned shortcomings and I want to transparently acknowledge the study's deficits in that particular regard.

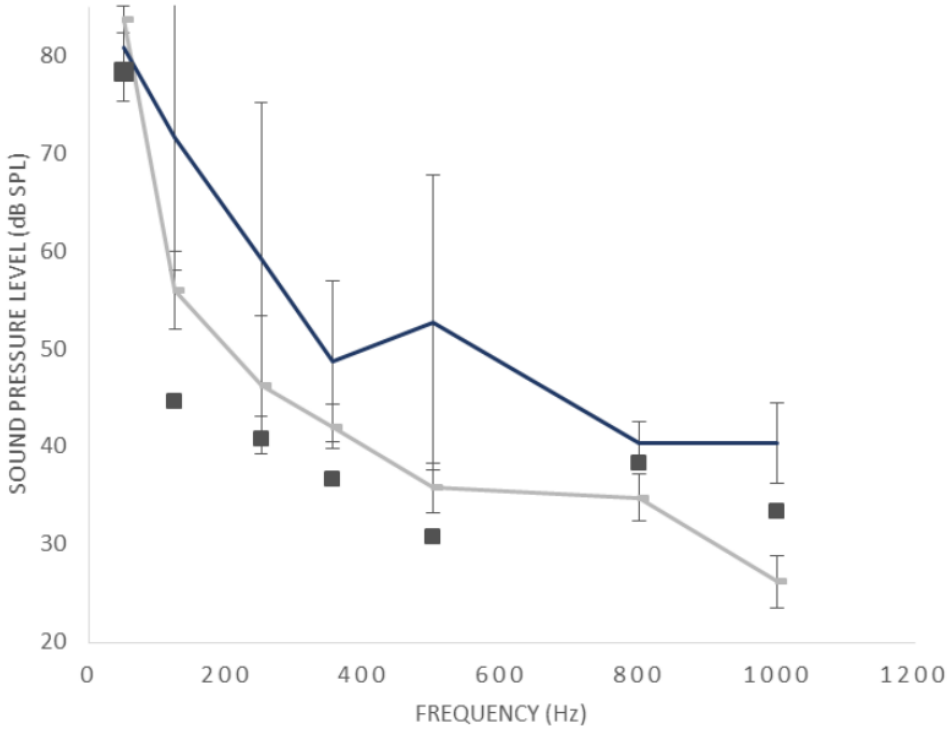

Figure 6: Low-frequency ABR hearing thresholds of Ansell's mole-rat (*Fukomys anselli*) test cohorts from Gerhardt et al. (2017). Light line: Young subjects (n = 5; on average 2 years old) tested with short acoustic stimuli (5 ms). Dark line: Results from a retest of the latter subjects two years later at an average age of 4 years. Squares: Young subject (n = 1) tested with longer stimuli that encompassed three complete wave cycles (see main text). Figure by E. P. Malkemper.

Insightful comments by Pim van Dijk and Geoff Manley not only had me reflect on the methodology of Chapter 2.3, but also made me aware of additional inconsistencies in the arguments and findings presented in the comprehensive study by Pyott et al. (2020) that aimed to unravel causes of bathyergid hearing and stimulated us to revisit this topic as well. A key finding of Pyott et al. (2020) is the apparent absence of distortion product otoacoustic emissions (DPOAE; evoked emissions from the cochlea that derive from sound stimulus-induced hair cell motility) in these animals. As discussed in Chapter 2.3, results reported by Kössl et al. (1996) contradict this

assumption and the administration of high dosages of anesthetic might have contributed to Pyott et al. (2020) not measuring DPOAE in mole-rats. But there are further, even more decisive arguments that methodological issues rather than an actual lack of DPOAE explain the findings communicated by that study.

As detailed in Chapters 1.1.1 and 2.3, Pyott et al. (2020) argue that the distorted or locally missing stereocilial bundles of outer hair cells that they describe for the bathyergid cochlea cannot sustain cochlear amplification. When my colleagues and I worked on Chapter 2.3, we were also convinced that a lack of outer hair cell-induced tectorial membrane displacement could be a plausible cause of the apparent lack of DPOAE in bathyergids. However, as our working group exclusively focuses on mammals, it escaped our notice that auditory studies on non-mammalian species disprove this reasoning. It can be assumed that most tetrapods exhibit DPOAE responses (as well as spontaneous otoacoustic emissions), as implied by studies in frogs, lizards, and birds (Taschenberger et al., 1995; van Dijk et al., 2002). Yet, the presence of distinct motile outer hair cells functionally coupled to a tectorial membrane is a feature unique to the organ of Corti in the mammalian ear (Kardong, 2012). In fact, even species which lack a tectorial membrane or equivalent structures in their hearing apparatus exhibit DPOAE that mirror the ones of mammals in various key aspects (Taschenberger et al. 1995). Instead, motile hair cells and/or stereocilial bundles (the sole motile region of the hair cell in non-mammals) alone appear to be fully sufficient for DPOAE generation and patterning in vertebrates (Taschenberger et al., 1995; Manley, 2001). Hair cell and hair bundle motility must be present in African mole-rats, since prestin is normally expressed in the OHCs (Jia & He, 2005; Pyott et al., 2020). Curiously, even insect ears can generate DPOAE, although lacking hair cells altogether (Kössl et al., 2008). Given all that, we would expect that the bathyergid ear, even if lacking a functional coupling of outer hair cells and tectorial membrane, can generate DPOAE. It should be pointed out, however, that such a dissociation of these structures was not reported from the cochleae of diverse bathyergid species studied previously (Lange, 2006). So why did Pyott et al. (2020) did not detect DPOAE signals?

When discussing the methodology of Pyott et al. (2020) together with Manley and van Dijk, we noticed a key difference to that of Kössl et al. (1996) which was unfortunately overlooked in the paper included in Chapter 2.3. In the former study, the DPOAE probe with the microphone and recorder was only superficially inserted into the ear of the bathyergid subjects (2 – 4 mm) and was not additionally sealed. Given that, it is not surprising that DPOAE recordings were made on a remarkably high noise floor of 5 – 15 dB SPL, which should greatly impair picking up these rather faint otoacoustic emissions. Instead, Kössl et al. (1996), inserted the tube deeper (6 – 10 mm) into the external ear canal and sealed it with tooth paste. Here, the noise levels were constantly below -10 dB SPL. Particularly in *Fukomys*, the ear canal has a distinct bend form which may complicate DPOAE recordings and necessitates a deep insertion of the recording probe (Kössl

et al., 1996). I figure that this rather simple methodological problem may explain why Pyott et al. (2020) were unable to pick up DPOAE. To finally settle this issue, a study on bathyergid hearing precisely replicating the methodology of Kössl et al. (1996) is needed. In combination with their anesthesia protocol likely affecting the results of their ABRs (Chapter 2.3), these shortcomings render their findings on hearing sensitivity and cochlear functionality doubtful. To conclude on the same note as Chapter 2.3, the hearing of bathyergids is unquestionably poor, but surely better than assumed by Pyott et al. (2020). There is one final point that I want to address here and that touches on possible evolutionary scenarios for hearing alterations underground. Chapter 2.3 argues that the stethoscope effect is an unlikely selective driver for elevated hearing thresholds in subterranean rodents. However, I was made aware of an additional argument that might provide support for this idea, namely that low-frequency environmental noise, amplified in tunnels, would otherwise overstimulate the ear. Potential sources of such noise are storms, rainfall, or megaherbivores crossing respective areas (compare Mason, 2013). Needless to say, it has never been measured how these factors might influence the noise level in underground tunnels (let alone across soil types and environments), making this claim speculative. Our finding that hearing acuity in coruros remains high despite of the subterranean lifestyle might argue against this notion. Yet, it should be noted that in some other geologically young fossorial rodents, such as prairie dogs (*Cynomys* sp.), a threshold elevation is evident although other hearing traits, for instance decent high-frequency sensitivity and best hearing at frequencies > 1 kHz, appear plesiomorphic (Heffner et al., 1994; Jackson et al., 1997). Curiously, both low-frequency and high-frequency (> 8 kHz) hearing thresholds are markedly elevated in prairie dogs. Does this relate to a need to counter low-frequency background noise in tunnels? The lack of data moves me to take an agnostic stance on this question.

2.4 – Olfactory communication

Perioral secretions enable complex social signaling in cooperatively-breeding mole-rats (genus *Fukomys*)

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Contributions:

- **Conception** – 70 %: I conceived the study with notable input from SB and PS.
- **Data collection** – 50%: I collected histological, morphological, and behavioral data together with DI, KK, TZ, SZ, and SB. GC-MS data were generated by PZ.
- **Data analyses** – 70 %: I analyzed all datasets, except for GC-MS data, which were analyzed by PS.
- **Writing the manuscript** – 85 %: I wrote the initial draft of the manuscript and revised it with input from all coauthors.

_____ _____

Signature of Ph.D. student Signature of supervisor

Perioral secretions enable complex social signaling in African mole-rats (genus *Fukomys*)

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Abstract

Subterranean common mole-rats of the genus *Fukomys* (family Bathyergidae) live in large cooperatively-breeding families. Odor cues have been hypothesized to importantly mediate social behaviors in the underground ecotope, but only little is known about the role of olfactory signaling in burrowing mammals. Here we characterize the so far neglected perioral glands of *Fukomys* and other African mole-rats as an important source of olfactory social information. Histology demonstrates these structures to be derived sebaceous glands that are developed regardless of sex and reproductive status. However, gland activity is higher in *Fukomys* males, leading to sexually dimorphic patterns of stain and clotting of the facial pelage. Behavioral assays revealed that conspecifics prefer male but not female perioral swabs over scent samples from the back fur and that male sebum causes similar attraction as anogenital scent, a known source of social information in *Fukomys*. Finally, we assessed volatile compounds in the perioral sebum of the giant mole-rat (*Fukomys mechowii*) via GCxGC-MS-based metabolomic profiling. Volatiles displayed pronounced sex-specific signatures but also allowed to differentiate between intrasexual reproductive status groups. These different lines of evidence suggest that mole-rat perioral glands provide complex odor signals that play a crucial role in social communication.

Key words: olfaction, sebaceous gland, sebum, Bathyergidae, odor preference

Introduction

The strictly subterranean, tooth-digging Northern common mole-rats of the genus *Fukomys* (family Bathyergidae – African mole-rats) have become a model group to study social dynamics in cooperatively-breeding small mammals (Burda, 1990; Burland et al., 2002; Patzenhauerová et al., 2013; Zöttl et al., 2016; Torrents-Ticó et al., 2018). These sub-Saharan hystricomorph rodents live in family groups organized around a single reproductive pair that occupy and maintain

extensive burrow systems (Patzenhauerová et al., 2013). Dispersal of offspring is delayed so that juveniles may stay with their parents well into adulthood, creating cohesive family groups that typically comprise around 10 members (Burda et al., 2000; Bennett & Jarvis, 2004; Torrents-Ticó et al., 2018). Social dynamics in wild *Fukomys* have been studied most intensively in the Damaraland mole-rat (*Fukomys damarensis*) of the Kalahari Desert, but are assumed to be largely uniform among congeneric species (Burda et al., 2000; Torrents-Ticó et al., 2018). Dispersal in Damaraland mole-rats is sex-biased, with males dispersing at higher rates and across longer distances than females do (Torrents-Ticó et al., 2018; Mynhardt et al., 2021). Females will typically establish new family groups by digging their own burrow system and will live solitarily until a mate arrives – at times for several years (Thorley et al., 2021). Males, however, are more likely to invade established family groups and challenge the same-sex breeder there (Young & Bennett, 2013; Torrents-Ticó et al., 2018; Mynhardt et al., 2021). This creates asymmetrical reproductive competition, which is reflected in pronounced male-biased sexual dimorphism in most *Fukomys* species (Caspar et al., 2021a).

The peculiar social system of *Fukomys* mole-rats is hypothesized to be crucially maintained by olfactory signals. In line with that, comparative genomic evidence points to excellent olfactory capacities in these animals (Stathopoulos et al., 2014). Behavioral experiments have demonstrated that group members can individually identify each other based on olfactory cues, such as anogenital scent (Heth et al., 2002). This allows the discrimination of familiar from foreign individuals and enables the strict incest taboo found among *Fukomys* families (Burda, 1995). Without regular contact to each other, however, family members will at some point seize to recognize their relatives (ca. 18 days in *F. anselli* – Burda, 1995; > 4 months in *F. mechowii* – Bappert & Burda, 2005). Interestingly, mole-rats can still differentiate such estranged siblings from total foreigners based on scent cues, which might indicate that body odors convey information about relatedness in these rodents (Heth et al., 2004). A recent study also demonstrated that *Fukomys* can distinguish between groups and single foreign conspecifics as well as identify the sex of the latter based on soil-born scents (Leedale et al., 2021). This further supports an important role of odors for social communication and implies that the search for mates in dispersing mole-rats could be guided by olfactory stimuli.

A yet unappreciated source of scent signals in *Fukomys* mole-rats are their perioral secretions, which stain the cheek region adjacent to the procumbent extrabuccal incisors of the animals. Perioral stains ("mentum" – Macholán et al., 1998) have been noted in many *Fukomys* species (*F. amatus*: Macholán et al., 1998; *F. anselli*: Begall et al., 2021; *F. damarensis*: Bennett & Jarvis, 2004; *F. darlingi*: De Graaff, 1964; *F. mechowii*: Peters, 1881; *F. micklemi*: pers. obs.; *F. vandewoestijneae*: Van Daele et al., 2013; but note that data on basal-branching species from the Northern hemisphere are missing).

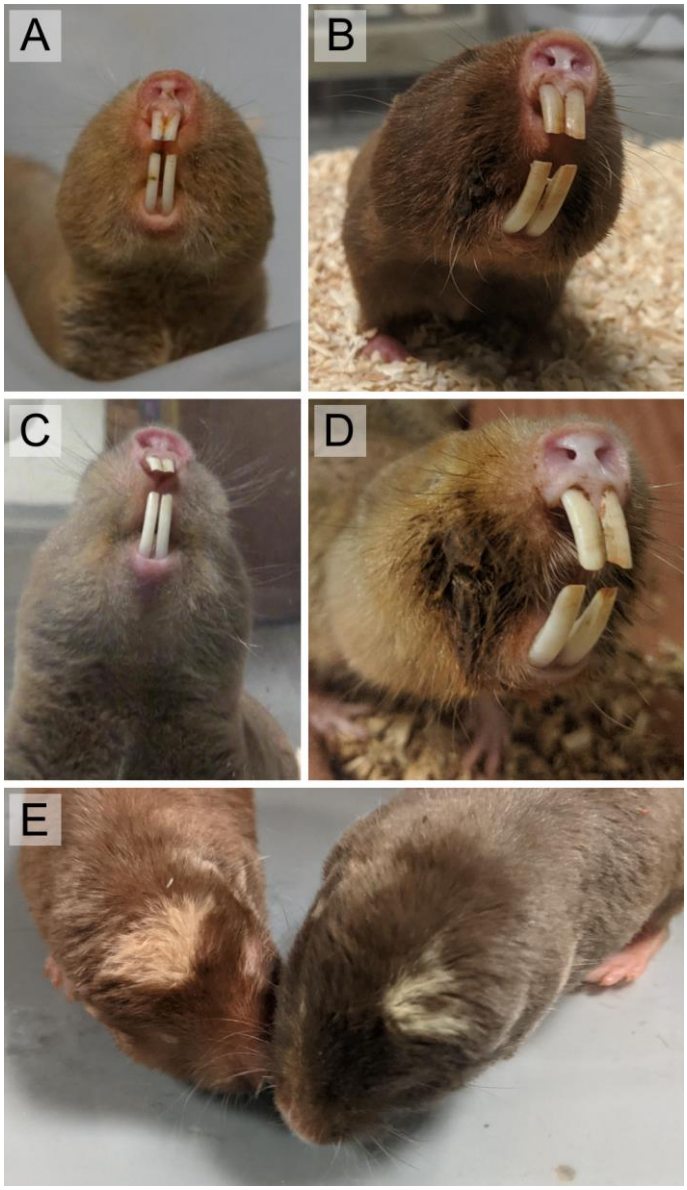

Figure 1: Sex-specific expression of perioral secretions in adults of different species of Northern common mole-rats (*Fukomys*) and relevant social behaviors. Note the stronger expression and clotting of the fur in males. A: Female Micklem's mole-rat (*Fukomys micklemi*) B: Male Micklem's mole-rat. C: Giant mole-rat female (*Fukomys mechowii*). D: Giant mole-rat male. The perioral secretions in this individual are particularly pronounced. E: Facial nuzzling in a freshly mated pair of Mashona mole-rats (*Fukomys darlingi*). The female (left) is sniffing the perioral region of the male. Photos: Kai. R. Caspar.

Typically, the stain is dark brown with a reddish to yellowish tinge and is restricted to the perioral region (Fig. 1A-D). The secretion is dry and solid, with a texture and consistency comparable to candle wax (pers. obs.). In the sister lineage to *Fukomys*, the Southern African mole-rat genus *Cryptomys*, yellow perioral stains have been reported (Fagir et al., 2021), but those are more inconspicuous than in the former (K. Finn, pers. com.). In other bathyergids, no noticeable facial stains appear to be present but it is known that many if not all rodent species exhibit perioral glands that aid in olfactory communication. These structures have been described in various taxa

of hystricomorph, sciuromorph, and myomorph rodents, including the subterranean blind mole-rats of Eurasia (Quay, 1965; Sokolov, 1982), and are especially well-studied in squirrels (Brady & Armitage, 1999). Indeed, such perioral glands have already been sketchily described in the naked mole-rat (Quay, 1965; Kimani, 2013), another social bathyergid.

Although perioral secretions in *Fukomys* have been known for centuries and are a striking component of the animals' appearance (Peters, 1881) they have attracted only little attention from researchers so far and their biological significance has remained enigmatic. De Graaff (1964) proposed that the stain is derived from specific food items, but as observations from the wild were accumulating and once *Fukomys* had been established in captivity, it became obvious that the stains are endogenous secretions and unrelated to general food intake.

While there is no evidence that *Fukomys* use perioral secretions to actively scent mark their environment, they could play an important role in social communication. Both *Cryptomys* and *Fukomys* engage in conspicuous reciprocal cheek nuzzling in various social situations (Fig. 1E). This behavior often initiates copulation (see e.g., Bennett, 1989 – *Cryptomys hottentotus*; Scharff et al., 1999 – *Fukomys mechowii*) but is also observed when unfamiliar individuals, regardless of sex, meet for the first time (Burda, 1989 – *Fukomys anselli*). In the mating context, it typically precedes anogenital inspection (Scharff et al., 1999). Obviously, perioral scents are of interest to both partners during these interactions, suggesting a role in social and particularly sexual signaling.

Several studies hypothesized that only certain status groups of mole-rats display visible perioral secretions. In particular, it has been proposed that just reproductive males and thus one animal per family, would exhibit perioral stains. Although this idea has never explicitly been tested, various field studies report to rely this character to identify breeding males (*F. anselli*: de Bruin et al., 2012; *F. damarensis*: Mynhardt et al., 2021; Maswanganye et al., 1999; *F. mechowii*: Lövy et al., 2013). The restriction of this feature to breeding males would suggest a role in both intra- and intersexual signaling and might imply an involvement in sexual suppression of subordinate males. However, there is no consensus about whether perioral secretions are indeed specific to male breeders. For instance, Kawalika (2004) reported that in Zambian giant mole-rats (*F. mechowii*) perioral stains are displayed by both sexes irrespective of reproductive status. On the other hand, Caspar et al. (2021a) noted anecdotally that the degree of expression in perioral secretions of captive Ansell's mole-rats (*F. anselli*) is sex-specific but non-dependent on breeding status, with males in general displaying more intense stains than females (see also Caspar et al., 2021b for *F. mechowii*). In any case, a restriction of secretion to particular status groups in mole-rat communities might have important implications for their social function.

Here, we aim to characterize the occurrence, chemical composition, and biological significance of perioral secretions in mole-rats of the genus *Fukomys* by the aid of morphological and histological observations, behavioral assays, and chemical analyses.

Materials and Methods

All statistics were performed in R (R Core Team, 2021).

Histology of the mouth corner integument in African mole-rats (*Fukomys* spp. & *Heterocephalus glaber*)

We sampled the perioral integument of the mouth corners in 13 *Fukomys* mole-rats, comprising three species (*F. anselli*, *F. mechowii*, and *F. micklemi*) as well as both sexes and intrasexual status groups (breeder vs. non-breeder), to gain insights about the morphology of glands producing perioral secretions in these animals (see Suppl. Tab. 1, for further data on sampled specimens). For comparison, we also included samples from four naked mole-rats (*Heterocephalus glaber*), another cooperatively-breeding species of bathyergid in which perioral glands but no visible secretions have been reported so far. All animals were adults deriving from the Department of General Zoology in Essen and were sacrificed for other research projects. No animals were sacrificed primarily to obtain perioral samples. Hence, the representation of species, sexes and reproductive status groups was imbalanced. Ultimately, we recovered perioral glands in the tissue samples of all of the four examined individuals of *F. mechowii* and *H. glaber*, respectively, in three out of six *F. anselli* and in none of the three *F. micklemi* (Suppl. Tab. 1). Given that we recovered glands across status groups and sexes in both genera, we are convinced that the apparent lack of glands in some individuals reflects issues with tissue sampling rather than their absence in the respective animals.

Skin was excised and prepared for standard histological sectioning and staining. *Fukomys* mouth angles were shaved with a handheld trimmer (Isis GT420; Aesculap, Suhl, Germany) before sampling. Tissue samples were fixed in 4% buffered paraformaldehyde for 24 h at 8 °C, subsequently transferred to 1 x DPBS buffer (PAN-Biotech; Aidenbach, Germany), and stored at the same temperature until being automatically dehydrated (Tissue Processor TP12 - RWW Medizintechnik; Hallerndorf, Germany) and embedded into paraffin (EG1150 H embedder – Leica Biosystems; Deer Park, USA). Embedded samples were manually sectioned (thickness: 5 µm) on a Microm HM 340 rotary microtome (Microm International; Walldorf, Germany), transferred to a digital precise water bath (Witeg WB-11; Wertheim am Main, Germany) warmed to 40 °C and subsequently mounted on glass slides. Tissue sections were stained using a standard hematoxylin-eosin manual staining protocol. ROTI®Histokitt II (Carl Roth; Karlsruhe, Germany)

was used as a xylol-based cover medium for the tissue sections. Samples were examined and photographed on a VHX-600 digital light microscope (Keyence; Osaka, Japan).

We used ImageJ (Schneider et al., 2012) to take quantitative measurements of glandular cell sizes from the micrographs. We measured the area of medially sectioned mature non-pyknotic cells in perioral glands from Ansell's mole-rats (n_{males} = 2, $n_{females}$ = 1) and naked mole-rats (n_{males} = 1, $n_{females}$ = 3). Subsequently, we tested whether there are sex differences as well as species differences in this variable for perioral glands. For Ansell's mole-rats, we also checked for differences in cell size between perioral glands and ordinary, hair follicle-associated sebaceous glands. We compared cell sizes between gland types by means of the two-sided t-test and calculated Cohen's D as a measure of effect size. To explore effects of species and sex on perioral gland cell size we computed a linear mixed effect model using the *lmer()* function from the lme4 package (Bates et al., 2015) of the following form: $log_{10}(cell\ size) \sim species + sex : species$. The individual ID was additionally included as a random factor and we calculated η^2 as a measure of a coefficient's effect size. Normality of data as well as of model residuals was checked with the Shapiro-Wilk test.

Occurrence of perioral staining among sexes and status groups of *Fukomys*

The degree of expression of perioral stains in two *Fukomys* species, the giant mole-rat (*F. mechowii*) and Micklem's mole-rat (*F. micklemi*) was studied to test the influence of selected biological variables. The two species represent distantly related congeneric lineages (Ingram et al., 2004).

We examined mole-rats with monitored life histories living in the laboratories of the Department of General Zoology, University of Duisburg-Essen, Essen, and the Department of Zoology, University of South Bohemia, České Budějovice (Suppl. Tab. 2). All mole-rats were housed in social groups with food provided ad libitum.

Giant mole-rats derive from animals caught in the Zambian Ndola region and exhibit the diagnostic karyotype of 2*n* = 40. We included 30 males (14 reproductive ones, 16 non-reproductive ones) and 68 females (14 reproductive ones, 54 non-reproductive ones) of giant mole-rats, resulting in a total sample of *n* = 98. The imbalance among the sexes and the two female status groups is a result of the strongly female-biased sex-ratio of neonates found in this species (Caspar et al., 2021b), which also affected our sampling efforts for the mass spectrometric analyses (see below). The Micklem's mole-rat lab lineage derives from animals caught at Kataba in Western Zambia, the type locality of the species (Chubb, 1909), and are characterized by a karyotype of 2*n* = 60. In this species, we studied 40 males (19 reproductive ones, 21 non-reproductive ones) and 32 females (20 reproductive ones, 12 non-reproductive ones), thus comprising a total sample of *n* = 72.

For documentation of the perioral stains, animals were briefly separated from their group, weighed, and photographed. Based on these photographs the degree of expression was scored on a species-specific qualitative scale from 1 (no visible secretion) to 4 (excessive secretion). Classifying criteria are enumerated in Table 1 and stain categories are visualized in Figure 2.

Table 1: Classifying criteria for perioral stain patterns for *Fukomys mechowii* and *F. micklemi* (see also Figure 2 for visualization).

Perioral secretion pattern	*Fukomys mechowii*	*Fukomys micklemi*
1	No visible secretion	No visible secretion
2	Darkening of pelage immediate to the corners of the mouth, often with small lateral circular extensions	Darkening of pelage immediate to the corners of the mouth
3	Secretions visibly clot the pelage in the corners of the mouth and extend dorsally towards the periphery of the mystacial vibrissal field	Secretions visibly clot fur in the corners of the mouth and notably extend from them in lateral orientation.
4	Extensive wax-like secretions clotting large portions of the face and extending well into the mystacial vibrissal field	Extensive fur clotting in the corners of the mouth that extends to the periphery or into the mystacial vibrissal field.

For each of the two species, we separately calculated cumulative link models for ordinal regression (*clm()* function of the ordinal package – Christensen, 2019; logit link function) to estimate the effects of biological variables on the expression of perioral stains. We used a two-step approach to the models: First, we used reproductive status, sex and the interactions between these two factors as model predictors to answer the question whether perioral stains are a sex-specific status signal (model I: *stain pattern ~ sex * sex : reproductive status*). Subsequently, we tested whether body mass (in g, log-transformed) and individual age (in months, log-transformed) predicts this trait within the sexes (model II: *stain pattern ~ sex : log_{10}(age) + sex: log_{10}(body mass)*).

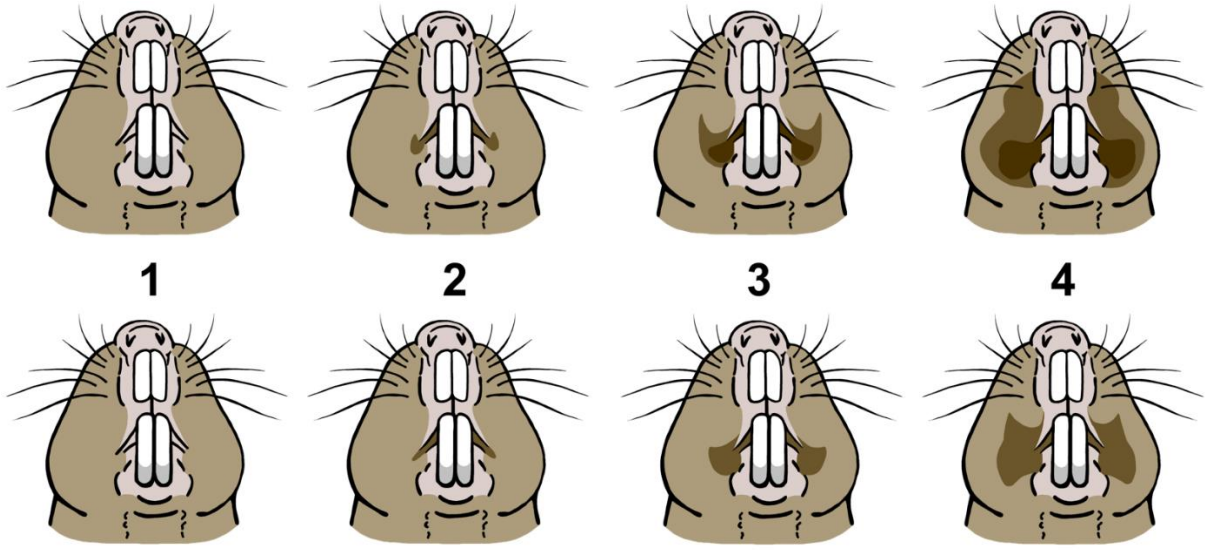

Figure 2: Visualization of qualitatively distinguished perioral stain patterns in *Fukomys mechowii* (top) and *Fukomys micklemi* (bottom). Compare Table 1 for a list of scoring criteria. Figure by Kai. R. Caspar.

Olfactory preference tests

We ran olfactory preference assays to assess the relative informative value of mole-rat perioral secretions compared to other bodily scents. Adult (at least 17 months old) Micklem's mole-rats (*F. micklemi*) participated in this part of the study, because the species expresses notable perioral stains while being of small size and thus easily manipulated for testing. The olfactory preference assay was designed as a two-choice set up, in which test subjects were presented with odorous swabs taken from different body regions of the same foreign conspecific donor animal with visible perioral secretions (see below). One option was invariably constituted by swabs taken from the perioral area, while the second one either derived from the donors' dorsal pelage or perineal area. While dorsum samples acted as a simple control to simulate the presence of a foreign conspecific, anogenital smear is known to convey complex social information in *Fukomys* (Heth et al., 2002; Hagemeyer et al., 2004; Heth et al., 2004).

Odorous smear from the donor animals was collected with moistened commercial cotton swabs that were gently rubbed against the respective body region. For perioral and dorsum samples, this was done until a discoloration of the tip became visible. During the procedure, one experimenter would briefly fixate the donor animals while a second one collected the samples. The swabs were rolled out on the surface of glass cuvette lids (10.2 cm x 8.5 cm x 1.1 cm) which were subsequently used to present the odors to the test subjects (analogous to Heth et al., 2002). During this procedure as well as during the set-up of the two-choice assay, the respective experimenters wore gloves to avoid olfactory contamination of the equipment.

Test subjects were individually taken from their home cages and brought to a darkroom in which the assay set up was deployed. To allow the experimenter to operate, the room was illuminated by an LED table lamp emitting monochromatic red light (Parathom R50 80.337 E14 Red 617 nm, 6 W, Osram; Munich, Germany), which is invisible to African mole-rats (Kott et al., 2010). The animals were placed in a terrarium (50 cm x 38 cm x 30 cm) in which they were presented with the two glass plates carrying the odors of the donor animal. Glass plates were positioned equidistant from the center along the long-axis of the terrarium with a 5 cm distance to the walls and were fixed with tape on the underside to remain in place. The position (left vs. right) at which the different odor types were presented was randomized. Test animals were observed exploring the set-up for three minutes after being placed into the center of the terrarium. All experiments were recorded (SONY HDR-CX505 camcorder) and behaviors were quantified based on these recordings. Interest in the presented odors was approximated by the time spent sniffing at the respective glass plate. Sniffing was defined as the animal lowering and moving its head over the glass plate accompanied by visible movement of the rhinarium. Besides sniffing time, the latency until first sniffing for either glass plate and the number of sniffing events was quantified. However, later on these measures were deemed to be uninformative and not considered for further analyses, since the animals were for the most part alternating between the two presented options. Experimental runs in which total sniffing time was < 5 s were discarded for later analyses, leaving us with 66 valid runs in total (Suppl. Tab. 3). The researcher quantifying the sniffing responses was blinded regarding the identity of the offered scent samples.

Animals were tested in three situations. The sample sizes itemized for sex are shown in brackets:

1) *Dorsal vs. perioral secretion, male donor* (n_{males} = 13, n_{females} = 15): Mole-rats could choose between swabs from the dorsal pelage and perioral region of a foreign male conspecific.

2) *Dorsal vs. perioral secretion, female donor* (n_{males} = 12, n_{females} = 12): Mole-rats could choose between swabs from the dorsal pelage and perioral region of a foreign female conspecific.

3) *Anogenital vs. perioral secretion, male donor* (n_{males} = 13, n_{females} = 11): Mole-rats could choose between swabs from the perineum and perioral region of a foreign male conspecific. Males rather than female donors were exclusively chosen because greater interest in male secretions was found in previous runs comparing perioral and dorsal samples.

Deviations in the sample compositions for the different test situations derive from changes in the lab population caused by deaths and animals being transferred to other institutions. If possible, individual animals were tested across all three situations. A maximum of one experimental run per animal per day was performed. After each run, the set up was cleaned with water and mild detergent.

Differences in sniffing time for perioral compared to dorsum and anogenital samples were statistically assessed for each of the three test situations. Data were checked for normality using the Shapiro-Wilk test. Parametric datasets were analyzed by employing the paired t-test and calculating Cohen's d as a measure of effect size, non-parametric ones by using the paired Wilcoxon signed rank test and Wilcoxon r to indicate effect sizes. Responses of males and females were compared for all test situations. However, sex differences were not found to be significant and thus data from males and females were pooled for all analyses to increase statistical power. Total sniffing times were compared across the three test situations by means of the Kruskal-Wallis test.

Metabolomic profiling

We collected perioral secretions from giant mole-rats (*F. mechowii*) to identify volatile organic compounds which might act in social communication via two-dimensional comprehensive gas chromatography with mass detection (GCxGC-MS). Samples from 26 animals were analyzed (Suppl. Tab. 4; secretions from four further animals were used to calibrate the GCxGC-MS). *F. mechowii* was selected for this aspect of the study since secretions are expressed in particularly great quantities compared to congeneric species. In addition, we applied the same methodology to analyze volatiles from small amounts of hay and cereals to consider potential diet-related contamination of secretions. Samples were collected from manually restrained live animals. Perioral secretions glue the hair in the cheek region together, so that clotted hairs could be swiftly cut and manually collected in Eppendorf tubes. Subsequently, samples were stored at -20 °C until analysis. The dynamic headspace method was used to sample the secretion compounds. The sampling process was carried out automatically using a multi-purpose sampler device (MPS, Gerstel, Germany). The sebum samples were placed in 10 ml glass vials and incubated for 5 minutes at 50 °C before a flow of nitrogen of 20 ml/min was used for continuous volatile extraction. The extraction was carried out for 10 minutes. The volatiles were sorbed on a Tenax sorbent packed in a glass tube (Tenax® TA, Gerstel, Germany) at 20 °C and subsequently released in a thermal desorption unit (TDU) at 295 °C into a programed temperature vaporizer (PTV) inlet precooled to a -30 °C where the volatiles were captured on a glass wool. The PTV inlet unit was then fast heated up to 300 °C and the analytes were introduced into a gas chromatograph. The volatiles were then analyzed employing the GCxGC-MS (Pegasus 4D, Leco Corporation, USA). A combination of non-polar and polar separation columns was used for the separation: Primary column: Rxi-5sil MS (28 m x 0.25 mm ID, Restek, Australia); Secondary column BPX-50 (1.6 m x 0.1 mm ID, SGE, Australia). Other parameters were set as follows: splitless mode, constant He flow 1 ml/min, modulation time 3 s (hot pulse 0.9 s), modulation temperature offset with respect to the secondary oven 15 °C. The temperature program applied on the primary oven: 35 °C (hold 1

min), then increase (8 °C/min) to 320 °C (hold 2 min). The temperature offset applied on the secondary column was +5 °C. Transferline temperature was held at 250 °C. The mass detector was equipped with an EI ion source and TOF analyzer enabling a unit mass resolution. The scanned mass range was 29 – 700 m/z. The ion source chamber was held at 280 °C. LECO's ChromaTOF v4.5 was employed to control the instrument and for data processing. Selected compounds were identified by automatically matching their mass spectra with a library of mass spectra (NIST MS 2.2, USA).

To prepare the bioinformatic analysis of the GCxGC-MS data, we first generated histograms of all samples and blanks. The resulting distribution was bi-modal with compounds that occurred only in samples (green line in Figure 6A) and those that occurred in both samples and blanks (intersection). To decide which compounds are true positive metabolites, we used the mixtools package (Gentleman et al., 2004) which calculates the posterior probability ($p < 0.05$) for the identity to either of the two peaks within the mixture of two overlapping normal distributions. Next, we applied a normalization based upon quantiles, which normalizes a matrix of peak areas (i.e. intensities) with the function *normalize.quantiles* of the preprocessCore package (Crawley, 2007). To explore potential sources of variation in our data, we used sparse partial least squares discriminant analysis (sPLS-DA) within the mixOmics package (Rohart et al., 2017) for the fact that it has satisfying predictive performances with large datasets. To extract *p*-values of differentially abundant compounds, we used the power law global error model (PLGEM – Pavelka et al., 2004) which is an efficient tool to calculate differentiation within large data sets (e.g., proteomes, transcriptomes, metabolomes) with distributions that deviate from normality (see methods in Kuntova et al., 2018) for more details). We used ggplot2 (Wickham 2016) to visualize differentially abundant compounds.

Ethics statement

Ethical review and approval for the behavioral assays was not required since animal housing (approved by permit no. 32-2- 1180-71/328 Veterinary Office of the City of Essen) as well as all experiments complied with the corresponding animal testing regulations and were approved by the animal welfare officer in charge. No ethical permissions were necessary. All behavioral tests as well as handling protocols conformed to the relevant ethical standards and did not harm the animals.

Results

Histology of the cheek region in African mole-rats

We found large, specialized sebaceous glands in the mouth corner regions of both sexes and irrespective of reproductive status in *Fukomys* (Fig. 2) as well as *Heterocephalus* (Suppl. Fig. 1). The exocrine glands in question are strongly branched, multilobated structures (Fig. 2A). Their wide secretory ducts open directly onto the skin surface (Fig. 2B; Suppl. Fig. 1). Although we were unable to measure their full extent, these glands form fields covering an area of at least several square millimeters in both species. As all sebaceous glands, they show a holocrine secretion pattern, releasing lysed cell masses into their glandular ducts (Fig. 3C). When stained using HE-solution, the glands appear well demarcated and in a light violet color. Individual gland cells are large (see below), contain well visible nuclei, and increase in size from their formation site in the peripheral layer to the center of a lobule. Perioral glands are embedded in fibrous connective tissue permeated by skeletal muscles. Otherwise, the histology of the cheek region was unremarkable and our observations aligned with those of earlier studies on bathyergid skin (Pleštilová et al., 2020; Kimani, 2013; Hesselmann, 2010; Sokolov, 1982). Regular sebaceous glands (Fig. 3F), but no other types of skin glands, were also recovered in both genera. In *Fukomys*, most hairs are arranged in follicle compounds, which are associated with one to several small, globular sebaceous glands (compare Hesselmann, 2010). In *Heterocephalus*, body hair is almost completely reduced so that regular sebaceous glands are only found in association with the vibrissae (compare Sokolov, 1982).

Apart from deviations in morphology, cell size between perioral and regular sebaceous glands differed significantly in *Fukomys anselli*. Cells from regular sebaceous glands had a median area of 135.4 μm^2 (n = 69; SD: 33.71) in the studied sections, while it was 314.7 μm^2 (n = 279; SD: 92.16) for perioral gland cells (t = 26.105, $p < 10^{-15}$; d = 2.23). There were no significant differences between the sexes in the size of the cells constituting ordinary sebaceous glands (t = 0.218, p = 0.831; d = 0.09).

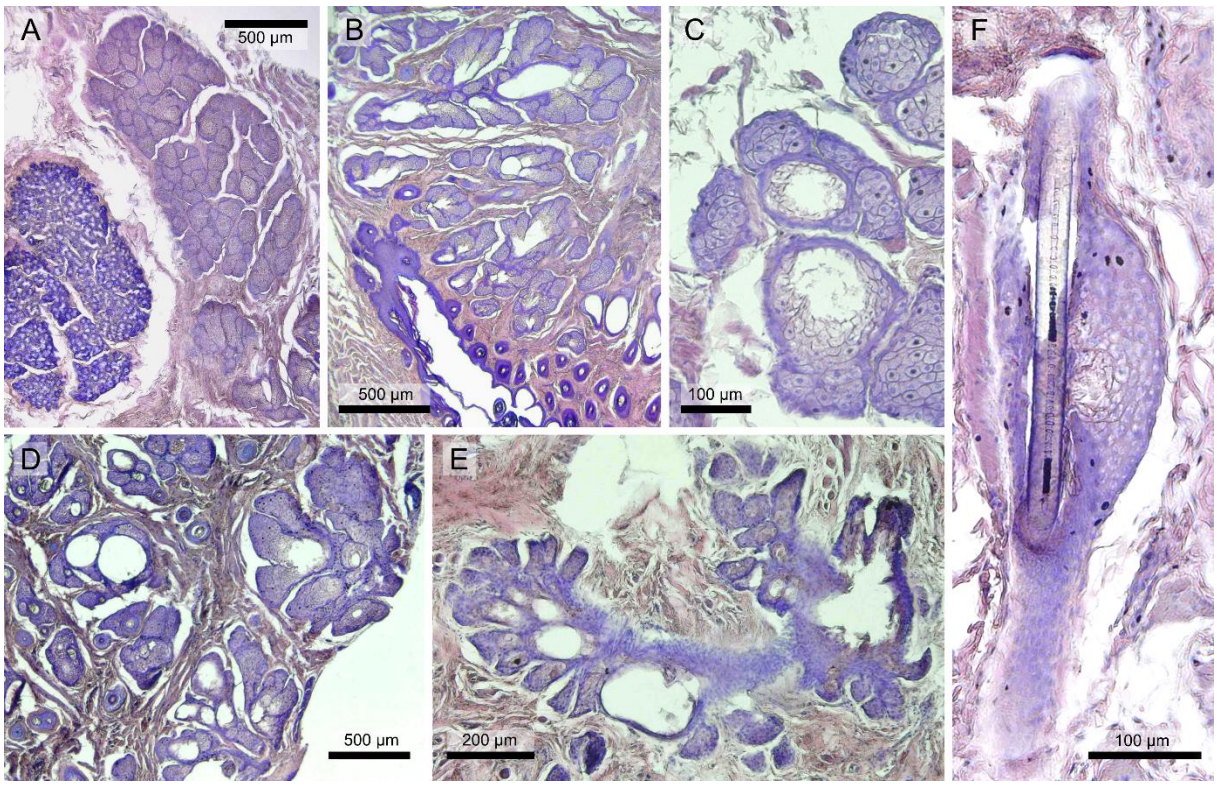

Figure 3: Perioral glands and regular sebaceous glands in the mouth corners of Northern common mole-rats (*Fukomys*). A: Perioral gland lobe with visible acini (right) situated deep in the dermis next to an oral mucus gland (left) in a male *Fukomys anselli*. B: Perioral gland field in a male *Fukomys anselli*. Note the wide lumina of the excretory ducts, which open directly onto the skin surface. C: Acini of perioral glands in a male *Fukomys anselli*. Note the lysis of pyknotic cells that are shed into the excretory ducts. D: Perioral gland field in a female *Fukomys anselli*. There were no obvious differences in perioral gland morphology between the sexes. E: Perioral gland in a male *Fukomys mechowii*. F: Regular sebaceous gland in *Fukomys anselli*. Note the simple globular morphology and association with a hair follicle.

Table 2: Statistical key figures for the linear mixed effect model on perioral gland cell sizes in *Fukomys anselli* and *Heterocephalus glaber.*

Coefficient	Estimate	Std. Error	*p*-value
Species (*H. glaber*)	-0.193	0.084	0.107
Species (*F. anselli*) : Sex (male)	0.010	0.089	0.919
Species (*H. glaber*) : Sex (male)	0.277	0.084	**0.047**

Results of the linear mixed effect model on perioral gland cell sizes in bathyergids are summarized in Table 2. The regression model revealed perioral gland cell size not to differ notably between *Fukomys* and *Heterocephalus* (t = -2.293, p = 0.107; η^2 = 0.37). However, we found a

significant sex difference in cell size in *Heterocephalus* (t = 3.291, p = 0.047; η^2 = 0.55), with the male exhibiting larger cells (n = 92; median: 365.5 µm²; SD: 113.76) than the females (n = 222; median: 192.1 µm²; SD: 72.76). In *Fukomys*, no such dimorphism was recovered (t = 0.111, p = 0.919; $\eta^2 < 0.01$).

Occurrence of perioral staining among sexes and status groups of *Fukomys*

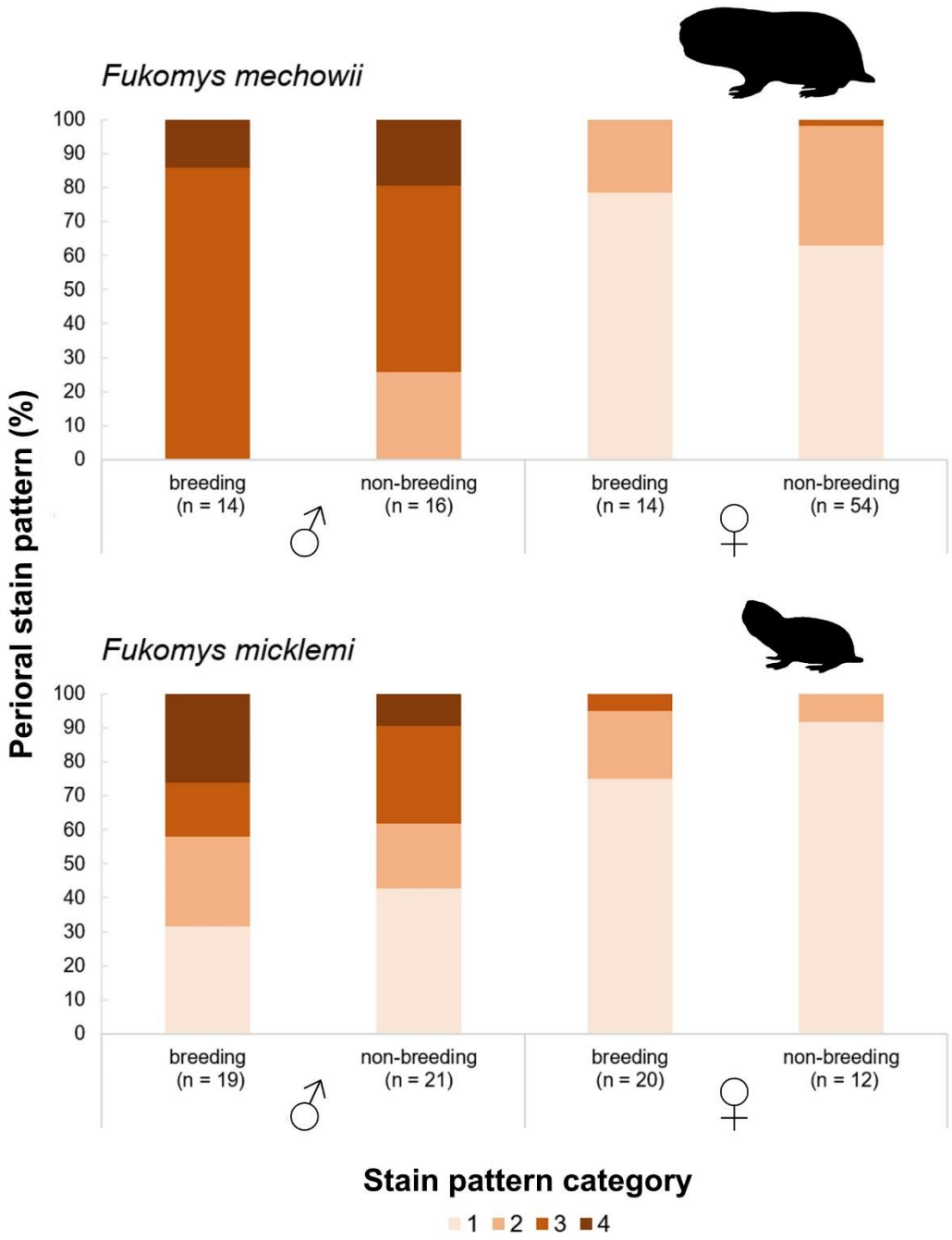

Figure 4: Distribution of perioral stain patterns across sexes and reproductive status groups in two species of Northern common mole-rats (*Fukomys*).

We found perioral stains to be highly sex-specific but not related to reproductive status in both studied species. Patterns of sex and status-dependent perioral stain expressions in *Fukomys* are shown in Figure 4 and are itemized in Supplementary Table 3. Males tended to show a greater development of stains than females and excessive perioral secretions (category 4) were exclusively found among the males of both species (Fig. 4). Secretion was found to be exaggerated and more strongly sexually dimorphic in *Fukomys mechowii* compared to *Fukomys micklemi* (Fig. 4). The results of the ordinal regression analyses on stain expression are provided in Table 3. In our initial models, we found that in both species sex is a good to excellent predictor of perioral stain intensity, with males showing a more pronounced expression than females (Table 3, $p < 0.02$). However, reproductive status does not influence stains in either sex or species (Table 3, $p > 0.2$). The subsequent models tested whether individual age or body mass might intrasexually influence the expression of stains. We found that these factors had no significant effects on these traits in either sex in *F. micklemi*, while we found age to be a significant predictor of stain expression in male *F. mechowii* ($p < 0.001$) exclusively (Table 4). Note that we only sampled adult individuals here and did not systematically study when stains formed during ontogeny. Our anecdotal observations suggest that stains manifest at an age between 12 and 18 months. The youngest individual in which we noticed perioral stain was a 7-month-old *F. mechowii* female, which was sampled for the characterization of volatile compounds (Suppl. Tab. 4).

Table 3 (continues on following page): Results from ordinal regression models on perioral stain expression in two species of *Fukomys*. For each species, two models were calculated. Model I tested for effects of sex and reproductive status on perioral stain expression, while Model II did so for intrasexual effects of age and body mass.

Fukomys mechowii (n = 98)			
Model I			
Coefficient	**Estimate**	**Std. Error**	***p*-value**
Sex (m)	5.745	1.185	**< 0.001**
Sex (f) : Status (repro)	-0.784	0.708	0.269
Sex (m) : Status (repro)	0.805	0.821	0.327
Model II			
Sex (f) : Age	-0.208	0.375	0.580
Sex (m) : Age	1.892	0.807	**0.019**
Sex (f) : Mass	-0.153	0.755	0.840
Sex (m) : Mass	-0.5187	0.815	0.524

Fukomys micklemi (*n* = 72)			
Model I			
Coefficient	**Estimate**	**Std. Error**	***p*-value**
Sex (m)	2.805	1.124	**0.013**
Sex (f) : Status (repro)	1.260	1.159	0.277
Sex (m) : Status (repro)	0.521	0.586	0.374
Model II			
Sex (f) : Age	1.330	0.969	0.170
Sex (m) : Age	0.416	0.475	0.382
Sex (f) : Mass	-0.598	1.476	0.685
Sex (m) : Mass	0.739	1.378	0.592

Olfactory preference tests

Micklem's mole-rats showed great interest in male but not female perioral swabs, although individual differences in responses were pronounced (Fig. 5). The animals showed a highly significant preference (paired Wilcoxon test; $V = 353$, $p < 0.0001$, $r = 0.76$) for odor derived from male perioral secretions (median sniffing time: 23.17 s; SD: 26.3) over swabs from the dorsum (median sniffing time: 12.45 s; SD: 14.9) irrespective of sex. There was one dropout run for this condition (final sample: $n_{males} = 13$, $n_{females} = 14$). There was a pronounced difference in the median sniffing time of female test animals (43.6 s) compared to males (14.1 s) for the perioral swabs, but it nevertheless failed to be significant (Wilcoxon test; $W = 63$, $p = 0.19$, $r = 0.26$). In fact, significant sex differences were recovered in none of the three experimental conditions. In contrast to male-derived samples, interest in female perioral swabs (median sniffing time: 10.25 s; SD: 13.0) was not different from that for swabs taken from the dorsal pelage (median sniffing time: 11.79 s; SD: 14.6) of donor animals (Wilcoxon test; $V = 58$, $p = 0.39$, $r = 0.21$). Additionally, we noticed the largest number of dropouts for this condition, with 6 out of the total 10 dropouts being observed here. This left us with 18 valid runs ($n_{males} = 7$, $n_{females} = 11$).

Given this sex-specific difference, we continued with testing preferences for male perioral secretions compared to anogenital smear, which is a known source of social information in mole-rats. There was no significant difference in sniffing times between male perioral swabs (median sniffing time: 24.1 s; SD: 15.24) and the perineal swabs (median sniffing time: 18.05 s; SD: 15.19) examined by the mole-rats (t-Test; $t = 1.38$, $p = 0.18$, $d = 0.24$). There were three dropout runs in this condition (final sample: $n_{males} = 10$, $n_{females} = 11$).

The Kruskal-Wallis test did not indicate significant differences in the total time spent sniffing at both the two odor samples, across the three test situations (Kruskal-Wallis $X^2 = 3.93$, $p = 0.14$).

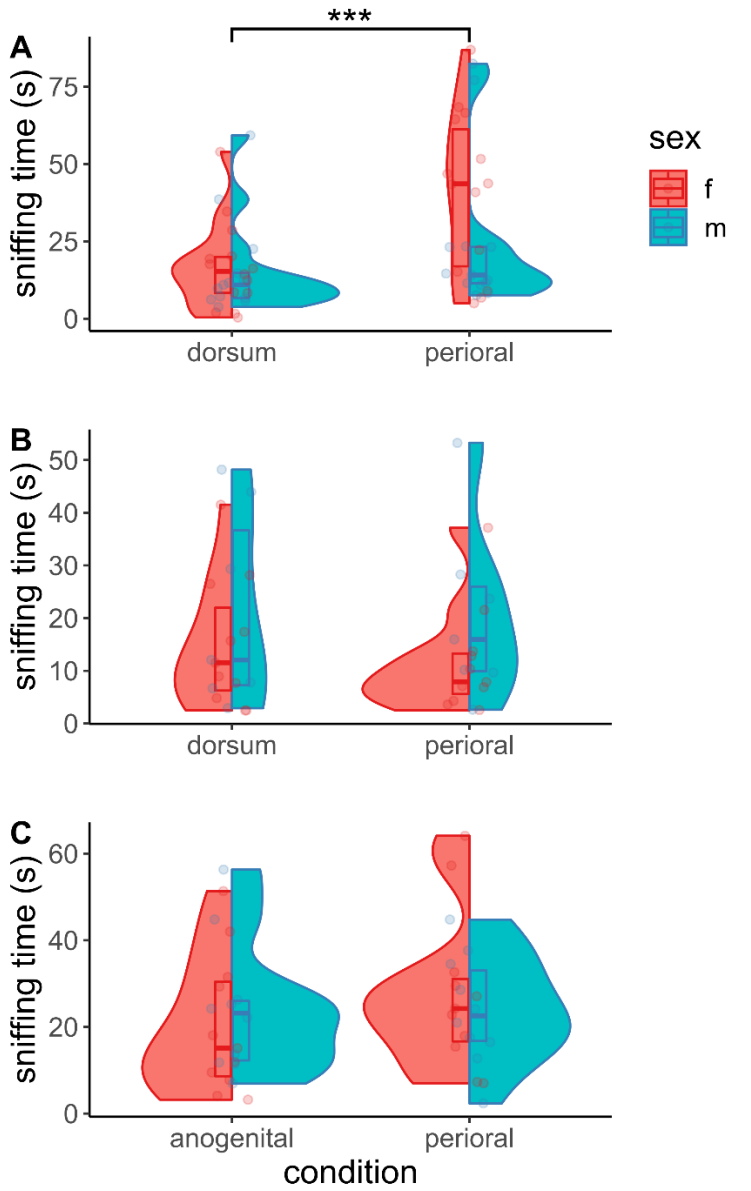

Figure 5: Interest of Micklem's mole-rats (*Fukomys micklemi*) in swabs of selected body odors from foreign conspecifics. A: Dorsal pelage vs. mal perioral sebum. B: Dorsal pelage vs. female perioral sebum. C: Anogenital secretion vs. perioral sebum. Only condition (A) yielded significant preferences for one of the offered options.

Metabolomic profiling

GCxGC-MS profiling of perioral secretions in *Fukomys mechowii* yielded a total of 765 volatile metabolites. However, some metabolites were also partially present in blanks. So, we employed Gaussian modeling to extract posterior *p*-values and used only those samples that were

significantly not-belonging to blanks or to a group of false positives, thus yielding a total of 443 'true' positive samples (all data under the green Gaussian curve in Fig. 6A; Suppl. Tab. 5).

To explore whether GCxGC-MS profiles reflect sex, we employed a supervised form of discriminant analysis, sPLS-DA, that relies on the class membership of each observation. The sPLS-DA model, based on significant components, accounted for 15 % (component 1) and 9 % (component 2) of the data variance (Fig. 6B). We used the Area Under the Curve (AUC) to provide evidence that the discrimination is perfect in both dimensions (AUC1 vs. AUC2) thus yielding AUC = 0.916 and $p = 0.0004$ for component 1, and AUC = 1, $p = 0.00002$ for component 2. This analysis thus indicates a strong sexual dimorphism in perioral sebum volatiles. Our AUC approach also revealed that component 2 is more informative than comp. 1 when looking at separations based on the reproductive status within each sex (Fig. 6C)**.** Female non-breeders are most different from the remaining status groups (AUC = 0.99, $p = 0.0001$) but all others could also be reliably differentiated (female breeders – AUC = 0.96, $p = 0.004$; male breeders – AUC = 0.88, $p = 0.0025$; male non-breeders – AUC = 0.88, $p = 0.037$). It should be pointed out that male non-breeders cluster completely within the odor space of breeders, while the differentiation among female status groups appears far more pronounced (Fig. 6C). Yet, each separation is significant on comp. 2 and thus volatile profiles may have the potential to signal reproductive status.

To further test the hypothesis that males and females have different profiles of volatiles we used PLGEM models of differential expression/abundance to extract levels of sexual dimorphism. A volcano plot (Fig. 6D) visualizes the striking differences between the sexes, which are already detectable at the bottom of this highly symmetrical plot. However, strictly statistically speaking, when fold difference is set to 2 and *p*-value to $p < 0.05$, a total of just 28 compounds are sexually dimorphic with 7 compounds being male-biased and 21 ones being female-biased. Next, we asked whether those compounds that are sexually dimorphic are also highly abundant. Thus, we recalculated signal intensities to abundances (0 - 100%). In Figure 6E, we clearly see that those compounds that are most abundant (> 0.29%) are least likely to be sex-biased, or in other words sexual dimorphism is expressed by many compounds with rather smaller abundances while the species-specific odor-space comprises non-dimorphic compounds which are highly abundant.

The comparison of volatiles between perioral sebum, hay, and cereals demonstrated a similar number of identified compounds in the food items and the secretions (Suppl. Fig. 2; Suppl. Tab. 6). Yet only ~10 % ($n = 68$) of the total compound diversity was shared between all the three sets and even fewer being exclusively shared between sebum and hay ($n = 2$) as well as sebum and cereals ($n = 3$). We can thus exclude notable biases due to food intake for our chemical analysis.

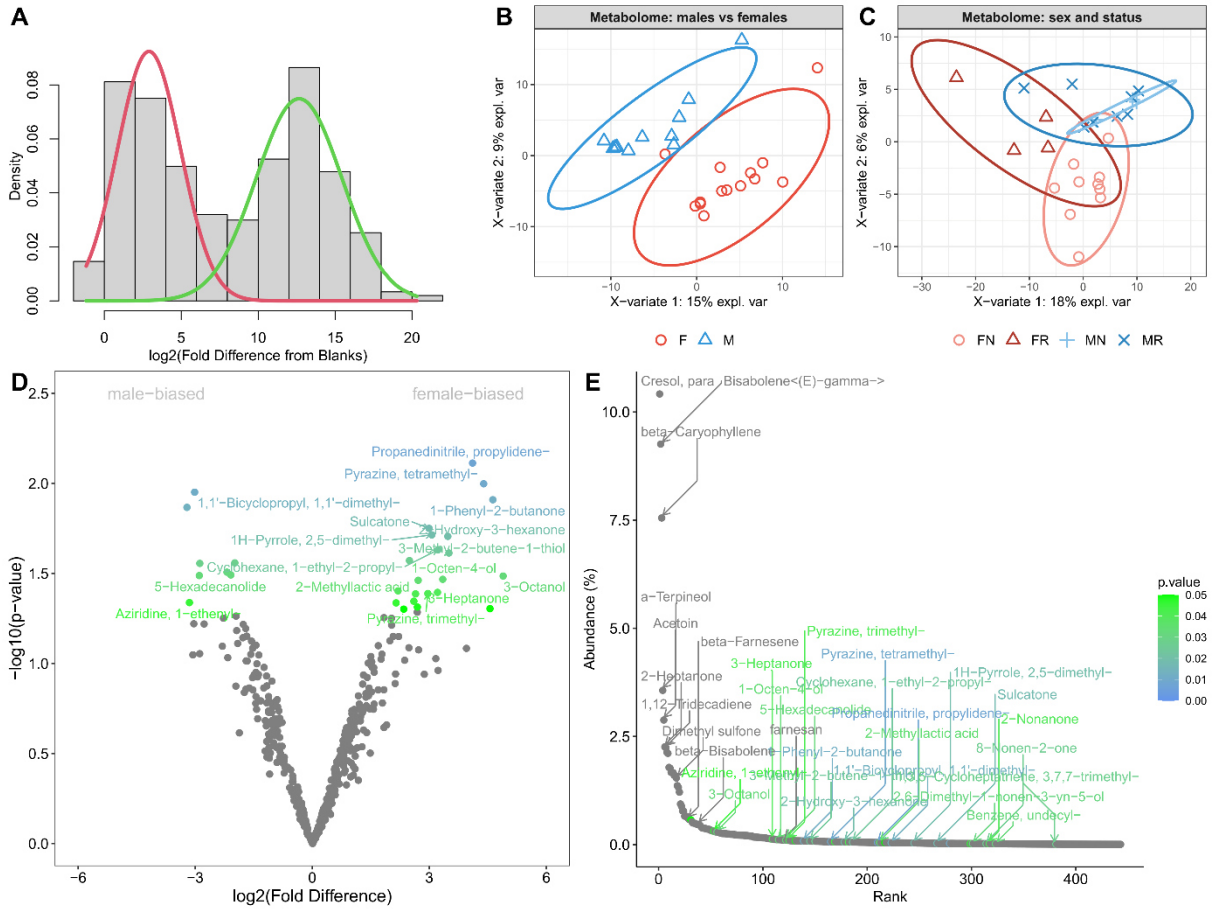

Figure 6: GCxGC-MS/MS analysis of volatile metabolomes from perioral secretions in *Fukomys mechowii*. A: The distribution of fold differences between samples and blanks is binomial while Gaussian modelling served to separate true (green line) from false positives. B: Sparse partial least-squares discriminant analysis revealed perfect discrimination between males (M) and females (F) and similarly reproductive status (R: reproductive, N: non-reproductive) is detectable in individuals of either sex (C). D: Volcano plot showing the distribution of female-biased and male-biased compounds. E: Abundance plot illustrating that most abundant volatiles are less likely to be sexually dimorphic. Colors are scaled from green ($p < 0.05$) to blue in D and E.

Discussion

Histology of perioral glands

This study is the first to report the presence and histology of enlarged and complex perioral glands in male as well as female *Fukomys*. For *Heterocephalus*, they had been previously discussed in passing by Quay (1965) and Kimani (2013). The anatomy of the perioral glands complies to a pattern reported from all hystricomorph rodent taxa studied so far (*Capromys pilorides*, *Hystrix indica*, *Myocastor coypus* – Quay, 1965; Sokolov, 1982), in that exclusively sebaceous glands and no sudoriferous components constitute the structures. However, to our best knowledge,

secretions of perioral glands in all these taxa, or in fact any other rodent, do not permanently clot the fur in a way similar to *Fukomys*, suggesting their condition to be exceptional. Interestingly, the mouth corners are devoid of comparable secretions in *Heterocephalus*.

The finding of sexually dimorphic perioral gland cell sizes in *Heterocephalus* was surprising, as this species displays remarkable monomorphism in other morphological and physiological traits (e.g., Jarvis 1991). Sex differences in gland cell size could indicate that specific secretion variables might deviate between male and female *Heterocephalus*. Yet, similar differences are not evident in *Fukomys*, although gland activity is markedly higher in males than in females of this genus (Fig. 4, see below). In any case, our findings must be interpreted with caution, as the number of sampled individuals is low for both genera and only includes a single *Heterocephalus* male as well as one *Fukomys* female. Although *Heterocephalus* has been extensively studied in regard to its social organization and communication (Buffenstein et al., 2021), chemical signaling in these animals remains essentially unknown, so that perioral secretions might represent a promising subject for future research.

Apart from the conspicuous perioral glands we describe here, no integumental scent glands are documented in *Fukomys* so far. However, it is known that various hystricomorph rodents, including *Heterocephalus*, possess specialized sebaceous skin glands in the anal area (Sokolov, 1982; Kimani, 2013). Tullberg (1899) even described such anal glands in the Southern common mole-rats of the genus *Cryptomys* (= "*Georychus coecutiens*"), providing additional indication for their presence in *Fukomys*. Secretions from these glands might well underlie the great social significance of anogenital scents in *Fukomys* (Heth et al., 2002, 2004) and can be expected to complement olfactory signaling via perioral secretions.

Occurrence of perioral staining among sexes and status groups of *Fukomys*

We found perioral stains to be strongly sexually dimorphic but not affected by reproductive status in either of the two studied *Fukomys* species. Thus, although both sexes possess well developed perioral glands, the quantity of secretion is typically far greater in males. This might suggest that sexual hormones affect perioral secretion patterns.

Indeed, it has been shown that the activity of regular sebaceous glands in rodents is stimulated by androgens and inhibited by estrogens (Thody & Shuster, 1989). Androgens have also been demonstrated to increase the size and activity of the supracaudal sebaceous gland of the guinea pig (*Cavia porcellus*), another hystricomorph species (Martan, 1962). The specialized perioral sebaceous glands of *Fukomys* might similarly respond to these hormones, giving rise to the observed sex differences in fur stain at the mouth corners. Our analyses demonstrate that reproductive status does not notably influence the expression of perioral stain in adults of either sex. Hence, we challenge the assumption that this is a characteristic trait of breeding males

(Maswanganye et al., 1999; de Bruin et al., 2012; Lövy et al., 2013; Mynhardt et al., 2021). But if adult male breeders and non-breeders essentially display equally noticeable perioral secretions, why do field studies often report it from just one animal per colony?

A simple explanation might lay in the dispersal behavior of wild *Fukomys*. At the time when perioral secretions start to be notably developed, typically at an age between 12 and 18 months, many male non-breeders have already dispersed from their natal family. Long-term field studies on *F. damarensis* indicate that male dispersal happens at a mean age of 12 months already, thus limiting the time that a non-breeder with fully developed perioral secretions might be captured in its natal colony (Torrents-Ticó et al., 2018). However, the time of dispersal is highly variable and it is thus not unlikely that fully adult sons may be captured along with their fathers when multiple groups are sampled. This complicates the identification of the breeding male based on perioral secretions alone and calls for a careful diagnosis of breeding status that takes other traits into account. Besides perioral stain, various studies report that male breeding status can be assessed by palpation of the testes, which are assumed to be larger in breeding males (e.g., Mynhardt et al., 2021). However, available data on whether relative testes mass is greater in reproductive compared to non-reproductive *Fukomys* males are conflicting (de Bruin et al., 2012; Garcia Montero et al., 2016). In case of doubt, a promising indicator might instead be the width of the upper incisors. Observations on captive mole-rat families suggest that incisor width is a good relative age marker, particularly in males (Burda 2022; pers. obs.). For Zaisan mole voles (*Ellobius tancrei*), a subterranean murid species with bathyergid-like extrabuccal incisors, it has already been demonstrated that incisor width can act as an age marker well into adulthood in both sexes, before values for different age cohorts will eventually converge (Kuprina & Smorkatcheva, 2019). At what age incisor width becomes uninformative to differentiate breeding males from younger non-breeders in *Fukomys* remains to be determined.

Olfactory preference tests

As indicated by non-significant differences in total sniffing time across experimental conditions, the mole-rats' interest in foreign conspecific odors was comparable across the three test situations. However, dependent on the offered scents, subjects would allocate the time spent sniffing to one of the two options. Male perioral secretions were found to be preferred by conspecifics over scent samples taken from the same individual's dorsum and to exhibit a comparable attractiveness to anogenital odor. Anogenital smear evidently conveys rich social information in *Fukomys* (Heth et al., 2002; Heth et al., 2004) and exceeds other body scents in its quality to effectively signal individual identity (Hagemeyer et al., 2004). The equivalence of perioral and anogenital swabs in the preference assay thus suggests an important communicative role for male perioral secretions in *Fukomys*. For female perioral swabs, we did not find a

significant preference over samples taken from the back pelage. This could indicate a difference in the perceived informative value of female perioral odor or might simply be the result of less secretions being produced by females, resulting in a fainter scent.

A possible biasing factor for our behavioral assays might be that we have not considered the reproductive status of neither the donor nor the test subjects although our subsequently generated results on perioral metabolomics suggest that mole-rats could be able to differentiate these status groups based on sebum volatiles and perhaps adopt their behavior accordingly (see below). However, as we quantified the relative informative value of odor sample pairs derived from the same respective individuals, we would not expect this issue to be a notable confounding factor here (see also Bappert et al., 2012). Nevertheless, the reproductive status of scent-sampled individuals should definitely be considered in future studies on these animals.

In any case, these results from the assays further indicate an asymmetric signaling function for perioral odors, with males investing more in the quantity of secretions to convey socially relevant signals to conspecific receivers than females do to evoke more notable responses. The observation that females spent notably longer examining male secretions than the opposite sex did, might suggest that male perioral sebum has a particular role in intersexual communication.

Metabolomic profiling

Our analyses of volatile compounds detected via GCxGC-MS demonstrate notable individual variation and striking sexual dimorphism in the volatile chemical composition of perioral secretions in *Fukomys,* while they provide additional evidence for reproductive status-dependent signaling in both sexes. However, greater sample sizes are needed to robustly confirm this pattern and to confidently identify compounds that differentiate between intrasexual reproductive status groups, particularly male ones. Although sexual signatures were highly significant, it should be pointed out that the majority of compounds, in particular the ones occurring in the highest concentrations, are found among both sexes and can thus be expected to signal species identity. Highly sex-specific compounds represented only a fraction of the diversity and quantity of detected volatiles. Therefore, the proportional mixture of several compounds conveys a sexual signal in *Fukomys* perioral secretions, a pattern also known from the scent glands of various other rodents (Schulte et al., 1994).

Our GCxGC-MS approach used a method which compares mass spectra and retention indices of the detected compounds from perioral secretions to those in an existing library to identify volatiles. Compound names are thus just the most likely estimates. However, some of these compounds have been intensively studied and represent ‘good matches’ even without using external standards. Many of the metabolites that we detected were previously characterized in

various other organisms including bacteria, plants and other animal species. This suggests that important fractions of the recovered compound diversity are not of endogenous origin but are produced by microbes colonizing the perioral sebum. This aligns well with the finding that sebaceous secretions in other mammals also house rich microbiota (compare Leclaire et al., 2014). The notably small overlap in compounds between sampled food items and sebum demonstrates that food matter did not noteworthy bias our analyses. Nevertheless, besides compounds of endogenous and microbial origin, odorants presented in the perioral area might also derive from yet other sources. For instance, the sebum might act as a hydrophobic sponge that adsorbs additional odoriferous compounds from urine or feces that are transferred to the perioral region during (auto)coprophagy or anogenital autogrooming. Future studies should aim to clarify the exact origins of odorous compounds from the perioral sebum and what information mole-rats can deduct from them. The most abundant volatile in both sexes was para-Cresol which conveys a typical 'pig smell' and is also secreted by male elephants during musth (Rasmussen & Perrin 1999). We also detected two variants of Bisabolenes (Bisabolene<(E)-gamma-> and beta) to be highly abundant. These sesquiterpenes are produced by many plants as well as by fungi and also function as pheromones in insects (Aldrich et al., 1993). Caryophyllene (3rd most abundant compound) is a natural sesqui-terpene present for example in cannabis and hops which has a high affinity to the CB2 receptor in mice, with strong anti-inflammatory effects (Alberti et al., 2017). Similarly, 2-Heptanone is a ketone that stimulates alarm reactions in insects, while it evokes anxiety reactions in mice and rats, even without involvement of the vomeronasal organ (Gutiérrez-García et al., 2018). This compound was also found to be abundant in both sexes.

Hence, it is clear that multiple sources contribute to the general mole-rat perioral odor space. This pattern was mirrored by sex-biased metabolites. For example, tetramethyl-Pyrazine is a bacterial metabolite and is significantly female-biased in our data. Likewise, 1-Phenyl-2-butanone is significantly female-biased, a compound that was previously detected in defensive secretions of various invertebrates including millipedes (Makarov et al., 2010). Sulcatone is also female-biased and represents a ubiquitous eukaryote metabolite. 5-Hexadecanolide is significantly male-biased in our data and, interestingly, is known to act as a pheromone in the queens of the Oriental hornet (*Vespa orientalis* – Raina & Singh 1996). 1-Ethenylaziridine is also male-biased and has previously been detected in various bacterial species (Filipiak et al., 2012).

An influence of reproductive and/or social status on volatile compounds of sebaceous gland secretions, as we observed in giant mole-rats, has also been demonstrated in a number of other social mammals, including rodents (Pohorecky et al., 2008), primates (Setchell et al., 2010), and carnivorans (Leclaire et al., 2014). If it is present, however, a status-dependent differentiation of odors appears to be more typical for males than for females. Respective odor profiles have been hypothesized to provide an honest signal of rank and physiological condition to potential

competitors (Setchell et al., 2010) and thus might aid in reducing tension and aggression. Sex and status-dependent signals from the perioral glands might serve this role in *Fukomys* families as well, facilitating the identification and social evaluation of both group members and foreign individuals that might enter an established family (compare Bappert et al., 2012).

Whether group-specific differences are adaptive or not, they might proximately be determined by the hormonal status of a respective individual. It has been demonstrated that sex hormone levels as well as pregnancy and lactation can affect commensal microbial communities and do regulate body odor via this path (e.g., Setchell et al., 2010; Pohorecky et al., 2008; Kean et al., 2011). To which extent these factors might explain differentiation in *Fukomys* perioral volatile diversity remains to be determined.

Given that perioral nuzzling is typically initiating copulation (e.g., Scharff et al., 1999), it might be tempting to speculate that certain volatiles act as sex pheromones in mole-rats. Indeed, some of the compounds we detected have been suggested to represent sexual pheromones in mice, for instance 2-Heptanone (Thoss et al., 2019). However, contrary to expectation, these molecules show no sexually dimorphic expression pattern in giant mole-rats. Apart from that, it should be noted that the vomeronasal system, which typically responds to pheromones, appears to be of little relevance in *Fukomys* and other African mole-rats. The bathyergid vomeronasal organ is growth-deficient (Dennis et al., 2019; Jastrow et al., 1998) and its vomeronasal receptor repertoire is small. The latter is typical for subterranean rodents in general (Jiao et al., 2019). However, there are still structural indications for the bathyergid vomeronasal organ being functional (Dennis et al., 2019) and even if that is not the case, pheromones may also be perceived by the primary olfactory epithelium (Wang et al., 2007), so that a pheromone function for perioral compounds cannot be excluded.

Synopsis and Conclusion

We have shown that Northern common mole-rats (as well as naked mole-rats) of both sexes possess complex and structurally derived sebaceous glands situated in their mouth corners. These perioral glands show a sex-specific secretion activity, which is higher in males and not affected by reproductive status. Conspecifics of both sexes, but particularly females, are notably responsive to male perioral secretion, which suggest them to serve in social communication. Finally, metabolic profiling revealed that the composition of volatile compounds of perioral sebum is sexually dimorphic and also allows the differentiation of female and male breeders and non-breeders. These results suggest that perioral secretions convey important sex-specific social infor-mation in both sexes but that perioral signaling is asymmetrical, as males invest more into the quantity of perioral secretions. It appears possible that while female secretions might be

exclusively perceived during close contact (i.e. facial nuzzling) the greater quantities of male secretion enable a more potent olfactory signal that might play an important role in attracting and courting mates. It is tempting to speculate that scents derived from the perioral glands are passively deposited onto the soil during tooth digging. The characteristic waxy consistency of the secretion might aid in prolonging the longevity of the scent signal (compare Scordato et al., 2007). This way, for instance, male tenants could effectively signal their presence and perhaps their physical condition in a burrow system to same-sex intruders that might challenge their position (Torrents-Ticó et al., 2018; Mynhardt et al., 2021).

The perioral secretions of *Fukomys* can be added to a long list of sexually dimorphic traits in these monogamous, cooperatively-breeding rodents, all pointing to a notable role of male intrasexual competition within their social system (Caspar et al., 2021a). Yet, it is intriguing to note that not all populations of *Fukomys* seem to express conspicuous perioral stains and that there is pronounced intrasexual variation. The physiological causes and behavioral implications of this variability will need to be clarified.

Several further new questions on olfactory signaling in common mole-rats and other bathyergids arise from this research. For instance, it remains to be determined how perioral and anal gland derived scents complement each other in the mediation of social behaviors and whether traces of perioral sebum can indeed act as lasting scent marks in burrow systems. It is to hope that such work on the conspicuous perioral secretions of social mole-rats will not only clarify how these animals effectively communicate underground, but also stimulate research on the understudied mouth corner glands of other rodent species.

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Author contributions

KRC, SB and PS designed the study; DI, KK, KRC, and SB collected behavioral and morphological data, KRC, SZ & TZ performed histology and quantified respective data, PZ conducted the GCxGC-MS experiments, KRC & PS analyzed and curated the data, KRC & PS wrote the manuscript with input from all remaining authors. All authors reviewed the manuscript.

Data availability

The paper and its accompanying supplementary information contain all data discussed in the study.

Ethics declarations

The authors declare no competing interests.

Supplementary Files

Supplementary Figure 1: Histology of perioral glands in the naked mole-rat (*Heterocephalus glaber*). A: male. B: female.

Supplementary Figure 2: A: Gaussian modeling of cereal (here denoted as "food") and hay volatiles derived from the mole-rats' regular food, only true positives under the green line were selected. B: Intersection plot showing that a similar number of compounds is found in hay plus food and in mole-rats while only ~10 % (n = 68) is shared between all the three sets.

Supplementary Table 1: Information on histologically examined mole-rats.

Supplementary Table 2: Information on mole-rats examined to score perioral stain patterns.

Supplementary Table 3: Results from olfactory preference tests in Micklems mole-rats (*Fukomys micklemi*).

Supplementary Table 4: Information on giant mole-rats (*Fukomys mechowii*) sampled for GCxGC-MS.

Supplementary Table 5: Information on volatiles derived from giant mole-rat perioral sebum.

Supplementary Table 6: Information on volatiles derived from the mole-rats' hay and cereal food.

References

Alberti, T. B., Barbosa, W. L. R., Vieira, J. L. F., Raposo, N. R. B., & Dutra, R. C. (2017). (−)-β-caryophyllene, a CB2 receptor-selective phytocannabinoid, suppresses motor paralysis and neuroinflammation in a murine model of multiple sclerosis. *International Journal of Molecular Sciences, 18*(4)**,** 691.

Aldrich, J. R., Numata, H., Borges, M., Bin, F., Waite, G. K., & Lusby, W. R. (1993). Artifacts and pheromone blends from *Nezara* spp. and other stink bugs (Heteroptera: Pentatomidae). *Zeitschrift für Naturforschung C, 48***,** 73–79.

Bappert, M. T., Burda, H., & Begall, S. (2012). To mate or not to mate? Mate preference and fidelity in monogamous Ansell's mole-rats, *Fukomys anselli*, Bathyergidae. *Journal of Vertebrate Biology*, *61*(1), 71–83.

Bappert, M., & Burda, H. (2005). Forgotten siblings or how the long memory of the eusocial giant mole-rat *Cryptomys mechowi* (Rodentia: Bathyergidae) serves to maintain incest avoidance. *Mammalian Biology*, *70*(Suppl.), 5.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.

Begall, S., Burda, H., & Caspar, K. R. (2021). *Fukomys anselli* (Rodentia: Bathyergidae). *Mammalian Species*, *53*(1012), 160–173.

Bennett, N. C. (1989). The social structure and reproductive biology of the common mole-rat, *Cryptomys h. hottentotus* and remarks on the trends in reproduction and sociality in the family Bathyergidae. *Journal of Zoology*, *219*(1), 45–59.

Bennett, N. C., & Jarvis, J. U. (2004). *Cryptomys damarensis*. *Mammalian Species*, *2004*(756), 1–5.

Brady, K. M., & Armitage, K. B. (1999). Scent-marking in the yellow-bellied marmot (*Marmota flaviventris*). *Ethology Ecology & Evolution*, *11*(1), 35–47.

Buffenstein, R., Park, T. J., & Holmes, M. (2021). *The Extraordinary Biology of the Naked Mole-Rat*. Springer.

Burda, H. (1989). Reproductive biology (behaviour, breeding, and postnatal development) in subterranean mole-rats, *Cryptomys hottentotus* (Bathyergidae). *Zeitschrift für Säugetierkunde*, *54*(6), 360–376.

Burda, H. (1990). Constraints of pregnancy and evolution of sociality in mole-rats with special reference to reproductive and social patterns in *Cryptomys hottentotus* (Bathyergidae, Rodentia). *Journal of Zoological Systematics and Evolutionary Research*, *28*(1), 26–39.

Burda, H. (1995). Individual recognition and incest avoidance in eusocial common mole-rats rather than reproductive suppression by parents. *Experientia*, *51*, 411–413.

Burda, H. (2022). Zambian mole-rats: 33 years on the scene and what we still do not know and how we could learn it. *Frontiers in Ecology and Evolution*, *10*, 866709.

Burda, H., Honeycutt, R. L., Begall, S., Locker-Grütjen, O., & Scharff, A. (2000). Are naked and common mole-rats eusocial and if so, why? *Behavioral Ecology and Sociobiology*, *47*(5), 293–303.

Burland, T. M., Bennett, N. C., Jarvis, J. U., & Faulkes, C. G. (2002). Eusociality in African mole-rats: new insights from patterns of genetic relatedness in the Damaraland mole-rat (*Cryptomys damarensis*). *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1495), 1025–1030.

Caspar, K. R., Müller, J., & Begall, S. (2021a). Effects of sex and breeding status on skull morphology in cooperatively breeding Ansell's mole-rats and an appraisal of sexual dimorphism in the Bathyergidae. *Frontiers in Ecology and Evolution*, *9*, 638754.

Caspar, K. R., Burda, H., & Begall, S. (2021b). *Fukomys mechowii* (Rodentia: Bathyergidae). *Mammalian Species*, *53*(1011), 145–159.

Christensen, R. H. B. (2019). Ordinal-regression models for ordinal data. R package version 12-10. Available at: https://cran.r-project.org/package=ordinal (accessed February 27, 2022).

Chubb, E. C. (1909). On mammals from the Upper Zambezi River. *Annals and Magazine of Natural History*, *3*(13), 33-36.

de Bruin, P. R., Viljoen, H., Sichilima, A. M., & Bennett, N. C. (2012). Socially induced infertility in Ansell's mole-rat: are there depressed hormone levels in non-reproductive males and females? *Journal of Zoology*, *286*(1), 15–21.

de Graaff, G. (1964). A systematic revision of the Bathyergidae (Rodentia) of Southern Africa. Ph.D. dissertation, University of Pretoria, Pretoria, South Africa.

Fagir, D. M., Bennett, N. C., Ueckermann, E. A., Howard, A., & Hart, D. W. (2021). Ectoparasitic community of the Mahali mole-rat, *Cryptomys hottentotus mahali*: potential host for vectors of medical importance in South Africa. *Parasites & Vectors*, *14*(1), 1–9.

Filipiak, W., Sponring, A., Baur, M. M., Filipiak, A., Ager, C., Wiesenhofer, H., Nagl, M., Troppmair, J., & Amann, A. (2012). Molecular analysis of volatile metabolites released specifically by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *BMC Microbiology, 12***,** 113.

Garcia Montero, A., Vole, C., Burda, H., Malkemper, E. P., Holtze, S., Morhart, M., Saragusty, Hildebrandt, T. B., & Begall, S. (2016). Non-breeding eusocial mole-rats produce viable sperm — spermiogram and functional testicular morphology of *Fukomys anselli*. *PLoS One*, *11*, e0150112.

Gentleman, R. C., Carey, V. J., Bates, D. M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A. J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J. Y., & Zhang, J. (2004). Bioconductor: open software development for computational biology and bioinformatics. *Genome Biology, 5***,** R80.

Gutiérrez-García, A. G., Contreras, C. M., & Saldivar-Lara, M. (2018). An alarm pheromone reduces ventral tegmental area-nucleus accumbens shell responsivity. *Neuroscience Letters, 678***,** 16–21.

Hagemeyer, P., Peuckmann, S., Papenfuhs, N., & Burda, H. (2004). Chemical communication in blind, subterranean eusocial mole-rats (genus *Coetomys*): which odour source codes information for individual recognition? *Advances in Ethology*, *38*, 56.

Hesselmann, F. (2010). Vergleich der mikroskopischen Anatomie der Haut und Hautanhangsorgane des Hausmeerschweinchens (*Cavia aperea* f. *porcellus*) und des Ansells Kleingraumulls (*Fukomys anselli*) unter Berücksichtigung ihrer Anpassung an unterschiedliche Lebensräume. Ph.D. dissertation. Tierärztliche Hochschule Hannover, Hanover, Germany.

Heth, G., Todrank, J., Begall, S., Wegner, R. E., & Burda, H. (2004). Genetic relatedness discrimination in eusocial *Cryptomys anselli* mole-rats, Bathyergidae, Rodentia. *Folia Zoologica*, *53*(3), 269–278.

Heth, G., Todrank, J., & Burda, H. (2002). Individual odor similarities within colonies and across species of *Cryptomys* mole rats. *Journal of Mammalogy*, *83*(2), 569–575.

Ingram, C. M., Burda, H., & Honeycutt, R. L. (2004). Molecular phylogenetics and taxonomy of the African mole-rats, genus *Cryptomys* and the new genus *Coetomys* Gray, 1864. *Molecular Phylogenetics and Evolution*, *31*(3), 997–1014.

Jarvis, J. U. M. (1991). Reproduction of naked mole-rats. In P. W. Sherman, J. U. M. Jarvis, & R. D. Alexander (Eds.), *The Biology of the Naked Mole-rat* (pp. 384–425). Princeton University Press.

Jastrow, H., Burda, H., & Oelschläger, H. H. (1998). Unilateral absence of the terminal nerve and distribution of gonadotropin-releasing hormone immunoreactive neurons in the brain of the common mole-rat (*Cryptomys*, Rodentia). *Brain Research, 813*(2), 229–240.

Jiao, H., Hong, W., Nevo, E., Li, K., & Zhao, H. (2019). Convergent reduction of V1R genes in subterranean rodents. *BMC Evolutionary Biology, 19*(1), 1–9.

Kawalika, M. (2004). *Rodents of Ndola (Copperbelt Province, Zambia)*. Ph.D. dissertation. Universität Duisburg-Essen, Essen, Germany.

Kean, E. F., Müller, C. T., & Chadwick, E. A. (2011). Otter scent signals age, sex, and reproductive status. *Chemical Senses*, *36*(6), 555–564.

Kimani, J. M. (2013). Comparative skin morphology and wound healing in Kenyan African mole rat (*Tachyoryctes ibeanus*) and naked mole rat (*Heterocephalus glaber*). Diploma thesis, University of Nairobi, Nairobi, Kenya.

Kuntova, B., Stopkova, R. & Stopka, P. (2018). Transcriptomic and proteomic profiling revealed high proportions of odorant binding and antimicrobial defense proteins in olfactory tissues of the house house. *Frontiers in Genetics, 9***,** 26.

Kuprina, K. V., & Smorkatcheva, A. V. (2019). Noninvasive age estimation in rodents by measuring incisors width, with the Zaisan mole vole (*Ellobius tancrei*) as an example. *Mammalia*, *83*(1), 64–69.

Leclaire, S., Nielsen, J. F., & Drea, C. M. (2014). Bacterial communities in meerkat anal scent secretions vary with host sex, age, and group membership. *Behavioral Ecology*, *25*(4), 996–1004.

Leedale, A. E., Thorley, J., & Clutton-Brock, T. (2021). Odour-based social recognition in Damaraland mole-rats, *Fukomys damarensis*. *Animal Behaviour*, *179*, 83–96.

Lövy, M., Sklíba, J. & Šumbera, R. (2013). Spatial and temporal activity patterns of the free-living giant mole-rat (*Fukomys mechowii*), the largest social Bathyergid. *PLoS ONE*, *8*, e55357

Macholán, M., Scharff, A., Burda, H., Zima, J., & Grütjen, O. (1998). The karyotype and taxonomic status of *Cryptomys amatus* (Wroughton, 1907) from Zambia (Rodentia, Bathyergidae). *Zeitschrift für Säugetierkunde*, *63*, 186–190.

Makarov, S. E., Ćurčić, B. P. M., Tešević, V. V., Jadranin, M. B., Vujisić, L. V., Ćurčić, S. B., Mandić, B. M., Sekulić, T. L., & Mitić, B. M. (2010). Defensive secretions in three species of polydesmids (Diplopoda, Polydesmida, Polydesmidae). *Journal of Chemical Ecology, 36*, 978–982.

Martan, J. (1962). Effect of castration and androgen replacement on the supracaudal gland of the male guinea pig. *Journal of Morphology*, *110*(2), 285–297.

Maswanganye, K. A., Bennett, N. C., Brinders, J., & Cooney, R. (1999). Oligospermia and azoospermia in non-reproductive male Damaraland mole-rats *Cryptomys damarensis* (Rodentia: Bathyergidae). *Journal of Zoology*, *248*(3), 411–418.

Mynhardt, S., Harris-Barnes, L., Bloomer, P., & Bennett, N. C. (2021). Spatial population genetic structure and colony dynamics in Damaraland mole-rats (*Fukomys damarensis*) from the southern Kalahari. *BMC Ecology and Evolution*, *21*(1), 1–17.

Patzenhauerová, H., Šklíba, J., Bryja, J., & Šumbera, R. (2013). Parentage analysis of Ansell's mole-rat family groups indicates a high reproductive skew despite relatively relaxed ecological constraints on dispersal. *Molecular Ecology*, *22*(19), 4988-5000.

Pavelka, N., Pelizzola, M., Vizzardelli, C., Capozzoli, M., Splendiani, A., Granucci, F. & Ricciardi-Castagnoli, P. (2004). A power law global error model for the identification of differentially expressed genes in microarray data. *BMC Bioinformatics, 5*, 203.

Peters W. (1881). Über die von Herrn Major v. Mechow von seiner letzten Expedition nach Westafrika mitgebrachten Säugetiere. *Sitzungsberichte der Gesellschaft naturforschender Freunde zu Berlin*, *1881*(1), 131–134.

Pleštilová, L., Okrouhlík, J., Burda, H., Sehadová, H., Valesky, E. M., & Šumbera, R. (2020). Functional histology of the skin in the subterranean African giant mole-rat: thermal windows are determined solely by pelage characteristics. *PeerJ*, *8*, e8883.

Pohorecky, L. A., Blakley, G. G., Ma, E. W., Soini, H. A., Wiesler, D., Bruce, K. E., & Novotny, M. V. (2008). Social housing influences the composition of volatile compounds in the preputial glands of male rats. *Hormones and Behavior*, *53*(4), 536–545.

Quay, W. B. (1965). Comparative survey of the sebaceous and sudoriferous glands of the oral lips and angle in rodents. *Journal of Mammalogy*, *46*(1), 23–37.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raina, S., & Singh, V. K. (1996). Asymmetric synthesis of 5-hexadecanolide, pheromone of the queen of the Oriental hornet, *Vespa orientalis*. *Tetrahedron, 52*, 4479–4484.

Rasmussen, L. E. L., & Perrin, T. E. (1999). Physiological correlates of musth: lipid metabolites and chemical composition of exudates. *Physiology & Behavior, 67*, 539–549.

Rohart, F., Gautier, B., Singh, A., & Lê Cao, K.-A. (2017). mixOmics: An R package for 'omics feature selection and multiple data integration. *PLoS Computational Biology, 13*, e1005752.

Scharff, A., Begall, S., Grütjen, O., & Burda, H. (1999). Reproductive characteristics and growth of Zambian giant mole-rats, *Cryptomys mechowi* (Rodentia: Bathyergidae). *Mammalia*, *63*(2), 217–230.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675.

Schulte, B. A., Müller-Schwarze, D., Tang, R., & Webster, F. X. (1994). Beaver (*Castor canadensis*) responses to major phenolic and neutral compounds in castoreum. *Journal of Chemical Ecology*, *20*(12), 3063–3081.

Scordato, E. S., Dubay, G., & Drea, C. M. (2007). Chemical composition of scent marks in the ringtailed lemur (*Lemur catta*): glandular differences, seasonal variation, and individual signatures. *Chemical Senses*, *32*(5), 493–504.

Setchell, J. M., Vaglio, S., Moggi-Cecchi, J., Boscaro, F., Calamai, L., & Knapp, L. A. (2010). Chemical composition of scent-gland secretions in an Old World monkey (*Mandrillus sphinx*): influence of sex, male status, and individual identity. *Chemical Senses*, *35*(3), 205–220.

Sokolov, V. E. (1982). *Mammal Skin*. University of California Press.

Stathopoulos, S., Bishop, J. M., & O'Ryan, C. (2014). Genetic signatures for enhanced olfaction in the African mole-rats. *PLoS One*, *9*(4), e93336.

Thody, A. J., & Shuster, S. (1989). Control and function of sebaceous glands. *Physiological Reviews*, *69*(2), 383–416.

Thorley, J., Bensch, H., Finn, K., Clutton-Brock, T., & Zöttl, M. (2021). Damaraland mole-rats are not obligate cooperative breeders. *bioRxiv*. doi: 10.1101/2021.12.08.471794.

Thoss, M., Luzynski, K. C., Enk, V. M., Razzazi-Fazeli, E., Kwak, J., Ortner, I., & Penn, D. J. (2019). Regulation of volatile and non-volatile pheromone attractants depends upon male social status. *Scientific Reports, 9*, 489.

Torrents-Ticó, M., Bennett, N. C., Jarvis, J. U., & Zöttl, M. (2018). Sex differences in timing and context of dispersal in Damaraland mole-rats (*Fukomys damarensis*). *Journal of Zoology*, *306*(4), 252–257.

Tullberg, T. (1899). Ueber das System der Nagethiere: Eine phylogenetische Studie. Akademische Buchdruckerei, Upsala.

Van Daele, P. A. A. G., Blonde, P., Stjernstedt, R., & Adriaens, D. (2013). A new species of African mole-rat (*Fukomys*, Bathyergidae, Rodentia) from the Zaire-Zambezi watershed. *Zootaxa*, *3636*(1), 171–189.

Wang, Z., Nudelman, A., & Storm, D. R. (2007). Are pheromones detected through the main olfactory epithelium? *Molecular Neurobiology*, *35*(3), 317–323.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis.* Springer.

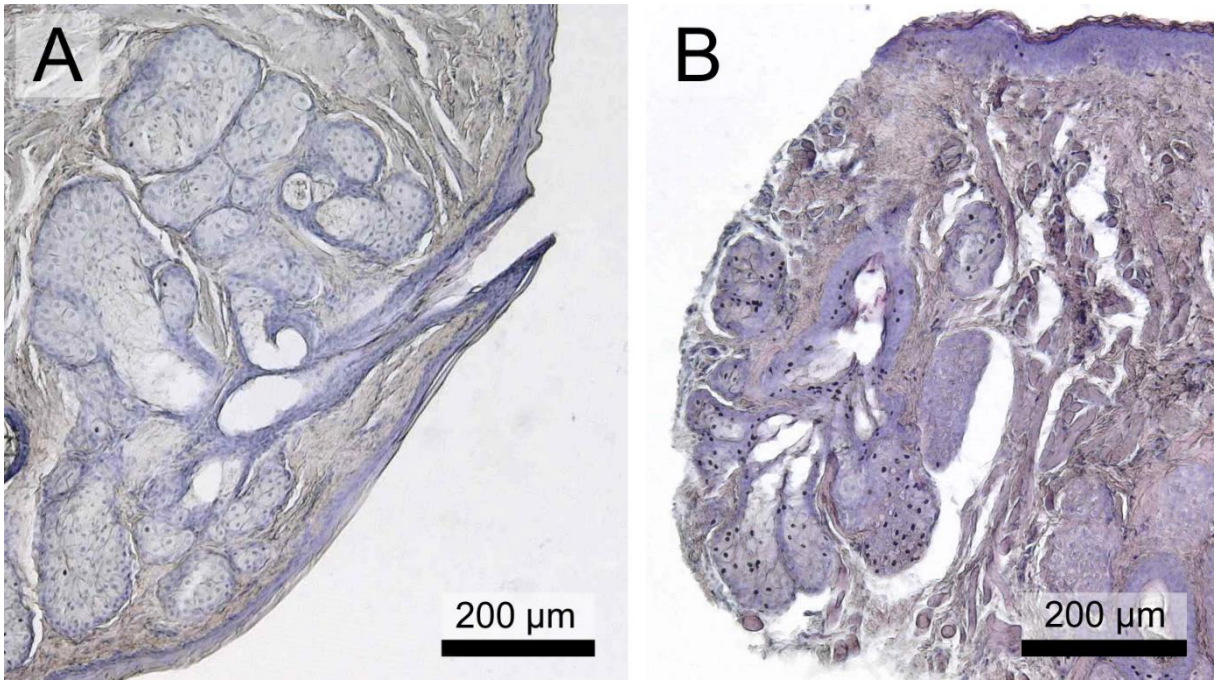
Young, A. J., & Bennett, N. C. (2013). Intra-sexual selection in cooperative mammals and birds: why are females not bigger and better armed? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1631), 20130075.

Zöttl, M., Vullioud, P., Mendonça, R., Ticó, M. T., Gaynor, D., Mitchell, A., & Clutton-Brock, T. (2016). Differences in cooperative behavior among Damaraland mole rats are consequences of an age-related polyethism. *Proceedings of the National Academy of Sciences*, *113*(37), 10382–10387.

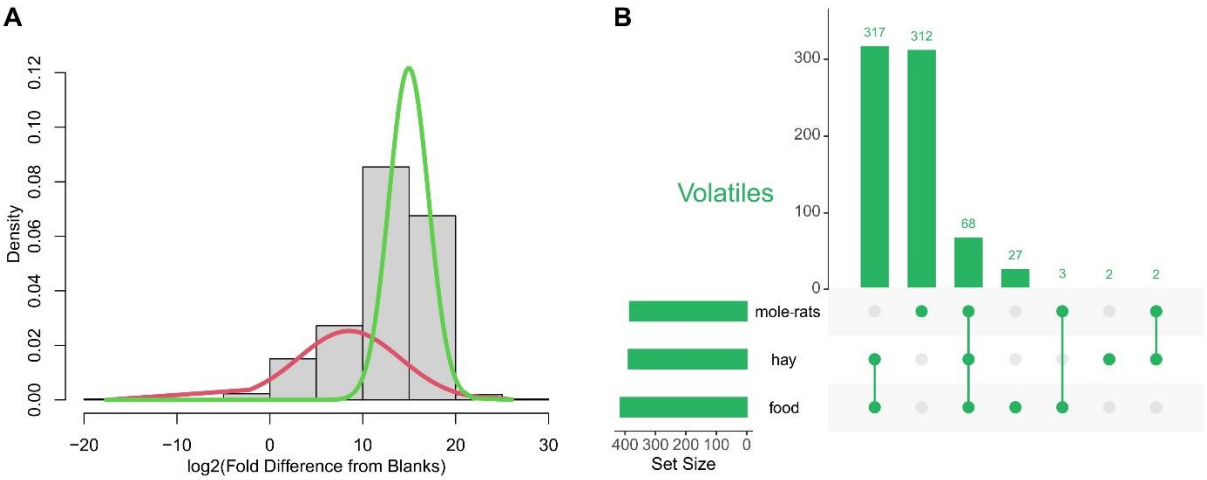
Supplementary Material

Supplementary Tables 4 & 5 contain large bioinformatic datasets and are thus only included in the electronic appendix of this thesis.

Supplementary Figure 1: Histology of perioral glands in the naked mole-rat (*Heterocephalus glaber*). A: male. B: female.



Supplementary Figure 2: A: Gaussian modeling of cereal (here denoted as "food") and hay volatiles derived from the mole-rats' regular food, only true positives under the green line were selected. B: Intersection plot showing that similar numbers of compounds are found in hay plus food and in mole-rats while only ~10 % (n = 68) are shared between all the three sets.



Supplementary Table 1: Information on histologically examined mole-rats.

Species	ID	Sex	Reproductive status	Perioral gland tissue found?
Fukomys anselli	FA12 5426	male	non-breeder	yes
Fukomys anselli	FA89 2922	male	non-breeder	no
Fukomys anselli	FA55 5427	male	non-breeder	no
Fukomys anselli	FA55 5439	male	non-breeder	no
Fukomys anselli	FA41 5429	male	non-breeder	yes
Fukomys anselli	FA89 4706	female	non-breeder	yes
Fukomys mechowii	FM26 2910	male	non-breeder	yes
Fukomys mechowii	FM38 8192	female	breeder	yes
Fukomys mechowii	FM46 0264	female	breeder	yes
Fukomys mechowii	FMC-sep	female	non-breeder	yes
Fukomys micklemi	Fmi-m 6979	male	breeder	no
Fukomys micklemi	Fmi16 6631	male	breeder	no
Fukomys micklemi	Fmi13 16C1	male	wild-caught, presumably non-breeder	no
Heterocephalus glaber	HG4 5408	female	breeder	yes
Heterocephalus glaber	HG2 7311	female	non-breeder	yes
Heterocephalus glaber	HG5 5374	female	non-breeder	yes
Heterocephalus glaber	HG3 5400	male	non-breeder	yes

Supplementary Table 2: Information on mole-rats examined to score perioral stain patterns.

Species	Status	Sex	Family	ID	Age (months)	Mass (g)	Stain score	Location
Fukomys mechowii	non	f	FM38	Fm38_2751	40	382.2	1	Essen
Fukomys mechowii	non	f	FM38	Fm38_5339	17	238.8	1	Essen
Fukomys mechowii	non	f	FM38	Fm38_5338	17	196.4	2	Essen
Fukomys mechowii	non	f	FM38	Fm38_5340	17	184	1	Essen
Fukomys mechowii	non	f	FM-I	Fm-I_0775	60	199	1	Essen
Fukomys mechowii	non	f	FM-I	Fm-I_2438	60	229.1	2	Essen
Fukomys mechowii	non	f	FM-C	Fm-C_4812	80	321.5	2	Essen
Fukomys mechowii	non	f	FM-C	Fm-C_6207	74	255.2	2	Essen
Fukomys mechowii	non	f	FM-C	Fm-C_5642	74	339.2	1	Essen
Fukomys mechowii	non	f	FM-C	Fm-C_6104	74	369.6	1	Essen
Fukomys mechowii	non	f	FM26	Fm26_8395	84	209.1	1	Essen
Fukomys mechowii	non	f	FM26	Fm26_8245	73	423	1	Essen
Fukomys mechowii	non	f	FM43sep.	Fm43sep_0752	43	240.4	2	Essen
Fukomys mechowii	non	f	FM43sep.	Fm43sep_2761	33	201.2	1	Essen
Fukomys mechowii	non	f	FM-L	Fm-L_1387	81	242.2	1	Essen
Fukomys mechowii	non	f	FM-L	Fm-L_5575	77	200.4	1	Essen
Fukomys mechowii	non	f	FM-I neu	Fm-I neu_6838	73	311.5	1	Essen
Fukomys mechowii	non	f	FM-I neu	Fm-I neu_7456	69	336.4	1	Essen
Fukomys mechowii	non	f	FM43	Fm43 _2753	36	164.8	2	Essen
Fukomys mechowii	non	f	FM43	Fm43 _2739	36	224.6	2	Essen
Fukomys mechowii	non	f	FM43	Fm43 _2743	33	301.3	2	Essen
Fukomys mechowii	non	f	FM43	Fm43 _2779	29	116.6	1	Essen
Fukomys mechowii	non	f	FM43	Fm43 _5413_IV	25	236.4	2	Essen
Fukomys mechowii	non	f	FM43	Fm43 _5415	22	191.3	1	Essen
Fukomys mechowii	non	f	FM43	Fm43 _5414	22	184.8	2	Essen
Fukomys mechowii	non	f	Fm38-neu	Fm38-neu_0766	55	425.8	2	Essen
Fukomys mechowii	non	f	Fm38-neu	Fm38-neu_0758	44	297.7	1	Essen
Fukomys mechowii	non	f	Fm41	Fm41_2748	23	200	1	Essen
Fukomys mechowii	non	f	Fm5	Fm5_8714	217	185	1	Essen
Fukomys mechowii	non	f	Fm41	Fm41_0724	25	227	1	Essen
Fukomys mechowii	non	f	Fm4	Fm4_1185	261	260	1	Essen
Fukomys mechowii	non	f	FmH	FmH_5250	49	303	1	Essen
Fukomys mechowii	non	f	FmH	FmH_6216	53	234	1	Essen
Fukomys mechowii	non	f	FmH	FmH_5634	56	220	1	Essen
Fukomys mechowii	non	f	Fm41	Fm41_0733	25	266	2	Essen
Fukomys mechowii	non	f	Fm42	Fm42_2941	120	288	2	Essen
Fukomys mechowii	non	f	FmH	FmH_8749	56	336	2	Essen
Fukomys mechowii	non	f	FmH	FmH_7589	60	286	2	Essen
Fukomys mechowii	non	f	FmH	FmH_4930	53	357	3	Essen
Fukomys mechowii	non	f	FMK	FMK_0496	13	193	1	Essen
Fukomys mechowii	non	f	FM43	FM43_0485	20	146	2	Essen
Fukomys mechowii	non	f	FM43	FM43_0487	20	152	1	Essen
Fukomys mechowii	non	f	FMD	FMD_0817	62	165	1	České Budějovice
Fukomys mechowii	non	f	FMD	FMD_0739	75	213	1	České Budějovice
Fukomys mechowii	non	f	FMD	FMD_9651	81	216	2	České Budějovice
Fukomys mechowii	non	f	FMD	FMD_2778	24	345	2	České Budějovice
Fukomys mechowii	non	f	FMD	FMD_3550	17	155	1	České Budějovice
Fukomys mechowii	non	f	FMD	FMD_8364	81	236	2	České Budějovice
Fukomys mechowii	non	f	FM32	FM32_2275	59	377	1	České Budějovice

Supplementary Table 2 (continued)

Species	Status	Sex	Family	ID	Age (months)	Mass (g)	Stain score	Location
Fukomys mechowii	non	f	FM32	FM32_8145	59	286	1	České Budějovice
Fukomys mechowii	non	f	FM32	FM32_6765	99	297	1	České Budějovice
Fukomys mechowii	non	f	FM32	FM32_1450	59	243	1	České Budějovice
Fukomys mechowii	non	f	FM44	FM44_5384	15	162	1	České Budějovice
Fukomys mechowii	non	f	FM44	FM44_2777	15	196	1	České Budějovice
Fukomys mechowii	repro	f	FM47	Fm47_4092	120	272.2	1	Essen
Fukomys mechowii	repro	f	FM38	Fm38_8192	105	440.5	1	Essen
Fukomys mechowii	repro	f	FM41	Fm41_6453	80	310.5	1	Essen
Fukomys mechowii	repro	f	FM-I	Fm-I_4122	105	280	1	Essen
Fukomys mechowii	repro	f	FM45	Fm45_7146	194	163	1	Essen
Fukomys mechowii	repro	f	FM46	Fm46_0264	134	238.7	2	Essen
Fukomys mechowii	repro	f	FM-K	Fm-K_6663	118	305	1	Essen
Fukomys mechowii	repro	f	FM43	Fm43 _9794	102	223.2	2	Essen
Fukomys mechowii	repro	f	Fm41sep	Fm41sep_4855	80	257.8	1	Essen
Fukomys mechowii	repro	f	Fm4	Fm4_6321	301	225	2	Essen
Fukomys mechowii	repro	f	Fm41	Fm41_8248	61	455	1	Essen
Fukomys mechowii	repro	f	Fm44	Fm44_7246	67	220	1	Essen
Fukomys mechowii	repro	f	FM22	FM22_2646	163	266	1	České Budějovice
Fukomys mechowii	repro	f	FMD	FMD_1699	111	281	1	České Budějovice
Fukomys mechowii	non	m	FM46	Fm46_0818	53	177.4	4	Essen
Fukomys mechowii	non	m	FM43	Fm43 _0723	48	452.2	2	Essen
Fukomys mechowii	non	m	FM43	Fm43 _2795	43	336.2	3	Essen
Fukomys mechowii	non	m	Fm37	Fm37_8562	118	353	3	Essen
Fukomys mechowii	non	m	Fm30	Fm30_7962	61	565	2	Essen
Fukomys mechowii	non	m	Fm32	Fm32_6870	68	450	2	Essen
Fukomys mechowii	non	m	Fm32	Fm32_1331	68	640	3	Essen
Fukomys mechowii	non	m	Fm30	Fm30_5565	48	690	3	Essen
Fukomys mechowii	non	m	Fm30	Fm30_7401	48	474	3	Essen
Fukomys mechowii	non	m	Fm30	Fm30_4911	48	454	3	Essen
Fukomys mechowii	non	m	Fm26	Fm26_2910	131	280	3	Essen
Fukomys mechowii	non	m	Fml	Fml_5104	37	160	3	Essen
Fukomys mechowii	non	m	Fm5	Fm5_8322	217	353	4	Essen
Fukomys mechowii	non	m	FM_Prag	FM_Prag	84	399	4	Essen
Fukomys mechowii	non	m	FMK	FMK_0495	13	239	2	Essen
Fukomys mechowii	non	m	FMD	FMD_3559	17	162	3	České Budějovice
Fukomys mechowii	repro	m	FM47	Fm47_0761	55	691	3	Essen
Fukomys mechowii	repro	m	FM41	Fm41_0755	48	468.5	3	Essen
Fukomys mechowii	repro	m	FM45	Fm45_6694	63	603	4	Essen
Fukomys mechowii	repro	m	FM-K	Fm-K_0721	44	482.9	3	Essen
Fukomys mechowii	repro	m	FM43	Fm43 _5247	80	465	3	Essen
Fukomys mechowii	repro	m	Fm41sep	Fm41sep_5877	73	232.5	3	Essen
Fukomys mechowii	repro	m	Fm41	Fm41_2246	60	552	3	Essen
Fukomys mechowii	repro	m	Fm44	Fm44_6211	40	317	3	Essen
Fukomys mechowii	repro	m	Fm38	Fm38_4808	137	428	3	Essen
Fukomys mechowii	repro	m	Fml	Fml_8780	82	504	3	Essen
Fukomys mechowii	repro	m	Fm30	Fm30_4120	144	707	3	Essen
Fukomys mechowii	repro	m	Fm32	Fm32_0625	144	535	3	Essen
Fukomys mechowii	repro	m	FMD	FmD_9510	99	521	4	Essen
Fukomys mechowii	repro	m	FM22	FM22_5565	80	511	3	České Budějovice

Supplementary Table 2 (continued)

Species	Status	Sex	Family	ID	Age (months)	Mass (g)	Stain score	Location
Fukomys micklemi	non	f	FmiA	FmiA_5294	52	81	1	Essen
Fukomys micklemi	non	f	FmiB	FmiB_2773	14	50	1	Essen
Fukomys micklemi	non	f	Fmi4	Fmi4_7448	48	84	1	Essen
Fukomys micklemi	non	f	Fmi4	Fmi4_5240	56	102	1	Essen
Fukomys micklemi	non	f	Fmi14	Fmi14_0482	14	59	1	Essen
Fukomys micklemi	non	f	Fmi18	Fmi18_5337	15	54	1	Essen
Fukomys micklemi	non	f	Fmi-g neu	Fmi-g_0426	12	67	1	Essen
Fukomys micklemi	non	f	Fmi17	Fmi17_0493	12	61	1	Essen
Fukomys micklemi	non	f	Fmi7	Fmi7_7F7BF	101	96	1	České Budějovice
Fukomys micklemi	non	f	Fmi7	Fmi7_0355	101	68	2	České Budějovice
Fukomys micklemi	non	f	Fmi4	Fmi4_2738	52	91	1	České Budějovice
Fukomys micklemi	non	f	Fmi6	Fmi6_5986	87	94	1	České Budějovice
Fukomys micklemi	repro	f	Fmi18	Fmi18_7118	123	108.1	2	Essen
Fukomys micklemi	repro	f	Fmi19	Fmi19_8436	69	80.6	1	Essen
Fukomys micklemi	repro	f	Fmi17	Fmi17_6509	108	114.6	1	Essen
Fukomys micklemi	repro	f	Fmi22	Fmi22_0714	51	81.2	1	Essen
Fukomys micklemi	repro	f	Fmi24	Fmi24_5192	68	105	1	Essen
Fukomys micklemi	repro	f	Fmi25	Fmi25_5760	76	85.4	1	Essen
Fukomys micklemi	repro	f	Fmi-g neu	Fmi-g-neu_0715	51	81.5	2	Essen
Fukomys micklemi	repro	f	Fmi27	Fmi27_6700	27	81.3	2	Essen
Fukomys micklemi	repro	f	Fmi14	Fmi14_8104	166	96.6	1	Essen
Fukomys micklemi	repro	f	Fmi7	Fmi7_8350	72	101.4	1	Essen
Fukomys micklemi	repro	f	Fmi3	Fmi3_8607	118	55	2	Essen
Fukomys micklemi	repro	f	FmiB	FmiB_9656	101	89	1	Essen
Fukomys micklemi	repro	f	FmiM	FmiM_2281	64	69	3	Essen
Fukomys micklemi	repro	f	Fmi9	Fmi9_8609	80	96	1	České Budějovice
Fukomys micklemi	repro	f	Fmi-e	Fmi-e_6241	80	111	1	České Budějovice
Fukomys micklemi	repro	f	Fmi3	Fmi3_2734	51	117	1	České Budějovice
Fukomys micklemi	repro	f	Fmi10	Fmi10_0778	68	85	1	České Budějovice
Fukomys micklemi	repro	f	Fmi6	Fmi6_6713	87	68	1	České Budějovice
Fukomys micklemi	repro	f	Fmi-h	Fmi-h_0778	70	85	1	České Budějovice
Fukomys micklemi	repro	f	Fmi-f	Fmi_f_0751	56	113	1	České Budějovice
Fukomys micklemi	non	m	FmiA	FmiA_5028	45	118	1	Essen
Fukomys micklemi	non	m	FmiG	FmiG_6809	46	108	1	Essen
Fukomys micklemi	non	m	FmiB	FmiB_0725	27	101	1	Essen
Fukomys micklemi	non	m	Fmi14	Fmi14_0764	33	111	1	Essen
Fukomys micklemi	non	m	Fmi15	Fmi15_2291	75	76	1	Essen
Fukomys micklemi	non	m	Fmi9	Fmi9_7FB0F	104	127	1	Essen
Fukomys micklemi	non	m	Fmi9	Fmi9_7571	85	119	1	Essen
Fukomys micklemi	non	m	FmiA	FmiA_6606	58	102	2	Essen
Fukomys micklemi	non	m	Fmi9	Fmi9_6608	81	120	2	Essen
Fukomys micklemi	non	m	FmiG	FmiG_8372	52	130	3	Essen
Fukomys micklemi	non	m	Fmi7	Fmi7_5765	56	120	3	Essen
Fukomys micklemi	non	m	Fmi14	Fmi14_8108	56	125	3	Essen
Fukomys micklemi	non	m	Fmi4	Fmi4_0000	48	82	3	Essen
Fukomys micklemi	non	m	Fmi9	Fmi9_6932	81	167	3	Essen
Fukomys micklemi	non	m	FmiG	FmiG_4844	58	133	4	Essen
Fukomys micklemi	non	m	Fmi9	Fmi9_7451	81	172	4	Essen
Fukomys micklemi	non	m	Fmi18	Fmi18_5336	15	63	1	Essen

Supplementary Table 2 (continued)

Species	Status	Sex	Family	ID	Age (months)	Mass (g)	Stain score	Location
Fukomys micklemi	non	m	Fmi-g neu	Fmi-g_0428	12	62	1	Essen
Fukomys micklemi	non	m	Fmi27	Fmi27_0425	12	85	2	Essen
Fukomys micklemi	non	m	Fmi24	Fmi24_0483	14	103	2	Essen
Fukomys micklemi	non	m	Fmi24	Fmi24_0500	14	95	3	Essen
Fukomys micklemi	repro	m	Fmi18	Fmi18_6288	106	95.5	1	Essen
Fukomys micklemi	repro	m	Fmi19	Fmi19_7088	81	107.3	3	Essen
Fukomys micklemi	repro	m	Fmi17	Fmi17_1587	143	118.6	4	Essen
Fukomys micklemi	repro	m	Fmi22	Fmi22_1496	84	90.5	4	Essen
Fukomys micklemi	repro	m	Fmi24	Fmi24_7527	95	121.7	2	Essen
Fukomys micklemi	repro	m	Fmi25	Fmi25_2452	89	106.8	2	Essen
Fukomys micklemi	repro	m	Fmi-g neu	Fmi-g-neu_6699	20	92.8	1	Essen
Fukomys micklemi	repro	m	Fmi27	Fmi27_0797	60	127.7	3	Essen
Fukomys micklemi	repro	m	Fmi14	Fmi14_4215	179	86.1	1	Essen
Fukomys micklemi	repro	m	Fmi7	Fmi7_7715	123	104.2	1	Essen
Fukomys micklemi	repro	m	FmiM	FmiM_6979	101	131	2	Essen
Fukomys micklemi	repro	m	FmiB	FmiB_6239	98	100	4	Essen
Fukomys micklemi	repro	m	Fmi15	Fmi15_6631	120	103	4	Essen
Fukomys micklemi	repro	m	Fmi3	Fmi3_8116	130	94	4	Essen
Fukomys micklemi	repro	m	Fmi-e	Fmi_e2736	54	125	1	České Budějovice
Fukomys micklemi	repro	m	Fmi3	Fmi3_6581	116	123	1	České Budějovice
Fukomys micklemi	repro	m	Fmi10	Fmi10_5385	87	112	2	České Budějovice
Fukomys micklemi	repro	m	Fmi-h	Fmi-h_5385	87	112	3	České Budějovice
Fukomys micklemi	repro	m	Fmi-f	Fmi_f_6297	80	132	2	České Budějovice

Supplementary Table 3: Results from olfactory preference tests in Micklems mole-rats (*Fukomys micklemi*).

Trial	Sex	Donor / Situation	Presented odor type / sniffing time		
			perioral	back	anogenital
1	f	male_back	15.2	8.3	NA
2	f	male_back	66.6	53.9	NA
3	f	male_back	43.5	12.5	NA
4	f	male_back	43.7	8.5	NA
5	f	male_back	22.2	14.4	NA
6	f	male_back	5.0	2.0	NA
7	f	male_back	6.8	0.4	NA
8	f	male_back	51.7	20.2	NA
9	f	male_back	64.5	19.4	NA
10	f	male_back	68.4	17.7	NA
11	f	male_back	46.9	34.7	NA
12	f	male_back	40.9	16.2	NA
13	f	male_back	8.9	1.6	NA
14	f	male_back	86.9	28.6	NA
15	m	male_back	14.6	9.8	NA
16	m	male_back	23.2	11.6	NA
17	m	male_back	11.7	14.8	NA
18	m	male_back	7.6	3.8	NA
19	m	male_back	11.5	6.2	NA
20	m	male_back	14.1	6.7	NA
21	m	male_back	12.4	12.5	NA
22	m	male_back	8.3	7.2	NA
23	m	male_back	23.2	5.8	NA
24	m	male_back	10.9	38.6	NA
25	m	male_back	23.4	10.9	NA
26	m	male_back	82.4	59.3	NA
27	m	male_back	77.1	22.6	NA
28	f	female_back	10.3	8.9	NA
29	f	female_back	21.5	26.5	NA
30	f	female_back	13.7	17.4	NA
31	f	female_back	4.3	4.8	NA
32	f	female_back	7.9	2.5	NA
33	f	female_back	2.5	2.5	NA
34	f	female_back	12.9	28.2	NA
35	f	female_back	37.1	41.5	NA
36	f	female_back	6.9	11.5	NA
37	f	female_back	3.6	7.7	NA
38	f	female_back	7.1	15.7	NA
39	m	female_back	10.2	6.7	NA
40	m	female_back	9.7	29.3	NA
41	m	female_back	53.3	44.0	NA
42	m	female_back	28.3	12.1	NA
43	m	female_back	2.6	2.9	NA
44	m	female_back	23.7	7.8	NA
45	m	female_back	15.9	48.2	NA
46	f	male_anogenital	7.0	NA	4.1
47	f	male_anogenital	27.1	NA	18.1
48	f	male_anogenital	22.8	NA	11.9
49	f	male_anogenital	64.1	NA	42.0
50	f	male_anogenital	29.5	NA	15.1
51	f	male_anogenital	24.2	NA	9.5
52	f	male_anogenital	57.2	NA	29.3
53	f	male_anogenital	17.9	NA	7.8
54	f	male_anogenital	32.6	NA	51.3
55	f	male_anogenital	7.3	NA	3.2
56	f	male_anogenital	15.4	NA	31.5
57	m	male_anogenital	12.8	NA	11.8
58	m	male_anogenital	34.5	NA	44.8
59	m	male_anogenital	28.6	NA	24.2
60	m	male_anogenital	17.5	NA	25.2
61	m	male_anogenital	24.1	NA	22.1
62	m	male_anogenital	16.5	NA	13.7
63	m	male_anogenital	2.4	NA	11.4
64	m	male_anogenital	44.8	NA	56.3
65	m	male_anogenital	21.0	NA	6.9
66	m	male_anogenital	37.6	NA	26.3

Supplementary Table 4: Information on giant mole-rats (*Fukomys mechowii*) sampled for GC-MS.

Species	Sex	Status	Specimen	GC-MS ID	Locality	Age (months)	Mass (g)	Notes
Fukomys mechowii	female	non-reproductive	FM D 9651		České Budějovice	81	216	Used to train the mass spectrometer
Fukomys mechowii	female	non-reproductive	FM 32 2275		České Budějovice	59	377	Used to train the mass spectrometer
Fukomys mechowii	female	non-reproductive	FM 44 2777		České Budějovice	15	196	Used to train the mass spectrometer
Fukomys mechowii	female	non-reproductive	FM 43 2739	FN2739	Essen	45	270.1	
Fukomys mechowii	female	non-reproductive	FM 43 0232	FN0232	Essen	38	399.9	
Fukomys mechowii	female	non-reproductive	FM 43 0239	FN0239	Essen	34	230.7	
Fukomys mechowii	female	non-reproductive	FM 43 2743	FN2743	Essen	42	345.8	
Fukomys mechowii	female	non-reproductive	FM K 0455	FN0455	Essen	7	173	juvenile
Fukomys mechowii	female	non-reproductive	FM L 5575	FN5575	Essen	86	199.9	
Fukomys mechowii	female	non-reproductive	FM L 1387	FN1387	Essen	90	253.5	
Fukomys mechowii	female	reproductive	FM22 2646	FR2646	České Budějovice	163	266	mated but no offspring
Fukomys mechowii	female	non-reproductive	FM D 8364		České Budějovice	81	236	Used to train the mass spectrometer
Fukomys mechowii	female	reproductive	FM 48 4855	FR4855	Essen	88	272	
Fukomys mechowii	female	reproductive	FM 45 8245	FR8245	Essen	80	408.3	
Fukomys mechowii	female	reproductive	FM 49 0766	FR0766	Essen	63	364.8	mated but no offspring
Fukomys mechowii	female	non-reproductive	FM D 2778	FN2778	České Budějovice	24	345	
Fukomys mechowii	female	non-reproductive	FM C 5642	FN5642	Essen	83	342	
Fukomys mechowii	female	non-reproductive	FM C 6104	FN6104	Essen	83	375	
Fukomys mechowii	male	reproductive	FM 46 0818	MR0818	Essen	53	177.4	mated but no offspring
Fukomys mechowii	male	non-reproductive	FM 43 0732	MN0732	Essen	48	452.2	
Fukomys mechowii	male	reproductive	FM 48 5877	MR5877	Essen	80	203.1	
Fukomys mechowii	male	non-reproductive	FM K 0495	MN0495	Essen	12	255.4	juvenile
Fukomys mechowii	male	reproductive	FM 22 5565	MR5565	České Budějovice	80	511	mated but no offspring
Fukomys mechowii	male	reproductive	FM 41 0755	MR0755	Essen	48	468.5	mated but no offspring
Fukomys mechowii	male	reproductive	FM 43 5247	MR5247	Essen	89	490.3	
Fukomys mechowii	male	reproductive	FM K 0721	MR0721	Essen	52	501.8	
Fukomys mechowii	male	reproductive	FM 47 0761	MR0761	Essen	64	655	
Fukomys mechowii	male	reproductive	FM 49 2795	MR2795	Essen	51	308.7	mated but no offspring
Fukomys mechowii	male	non-reproductive	FM 50 0257	MN0257	Essen	84	399	

2.5 – Sexual dimorphism

Effects of sex and breeding status on skull morphology in cooperatively breeding Ansell's mole-rats and an appraisal of sexual dimorphism in the Bathyergidae

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Contributions:

- **Conception** – 90 %: I conceived the study, including its objectives and methodology, with input from SB.

- **Data collection** – 70%: I researched body weights of bathyergids, collected linear measurements from skulls and feet, and collected landmarks from mandibles. Remaining data were collected by JM.

- **Data analyses** – 25 %: Most parts of data analysis were carried out by JM and SB with methodological input from my side.

- **Writing the manuscript** – 85 %: I wrote the initial draft of the manuscript and revised it with input from JM & SB and created figures together with SB.

- **Revising the manuscript** – 90 %: I revised the manuscripts following the reviewer's comments together with input from SB and JM.

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Signature of Ph.D. student Signature of supervisor

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Effects of Sex and Breeding Status on Skull Morphology in Cooperatively Breeding Ansell's Mole-Rats and an Appraisal of Sexual Dimorphism in the Bathyergidae

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African mole-rats of the genus *Fukomys* (Northern common mole-rats) combine a monogamous mating system and pronounced sexual size dimorphism; a pattern highly untypical for mammals. At the same time, they live in cooperatively breeding groups composed of reproductive and non-reproductive members of both sexes. How and to which degree sex and breeding status influence morphofunctional characters in eusocial mole-rats is not well characterized but essential to come to a comprehensive understanding of their peculiar social system. Here, we explore patterns of morphological differentiation in skulls of Ansell's mole-rats (*Fukomys anselli*) by means of multivariate analysis of linear skull measurements combined with a 2D shape analysis of cranium and mandible. Compared to females, males display larger skulls relative to body size and show an expansion of the facial portion of the cranium, while reproductive status did not have an effect on any of the traits studied. We also show that species of *Fukomys* mole-rats display a scaling of relative sexual body size dimorphism in compliance to Rensch's rule, which is deemed indicative of intense male intrasexual competition. For the bathyergid family as a whole, results of scaling analyses were more ambiguous, but also indicative of Rensch's rule conformity. In line with genetic field data, our results point to a greater role of male-male conflicts in *Fukomys* than is traditionally assumed and support the notion that reproductive status does not correlate with morphofunctional segregation in these unusual rodents.

Keywords: Bathyergidae, Rensch's rule, shape analysis, osteology, geometric morphometrics

INTRODUCTION

African mole-rats (Bathyergidae) are a speciose group of sub-Saharan rodents which are renowned for their superb adaptation to life underground (Gomes Rodrigues et al., 2016). Despite their ecological uniformity and specialization, bathyergids encompass species with strongly contrasting social systems (**Figure 1**). The genera *Bathyergus* (dune mole-rats), *Georychus* (Cape mole-rat), and *Heliophobius* (silvery mole-rat) lead strictly solitary lives, while the sister genera *Cryptomys* (Southern common mole-rats) and *Fukomys* (Northern common mole-rats) as well as the basalmost branching

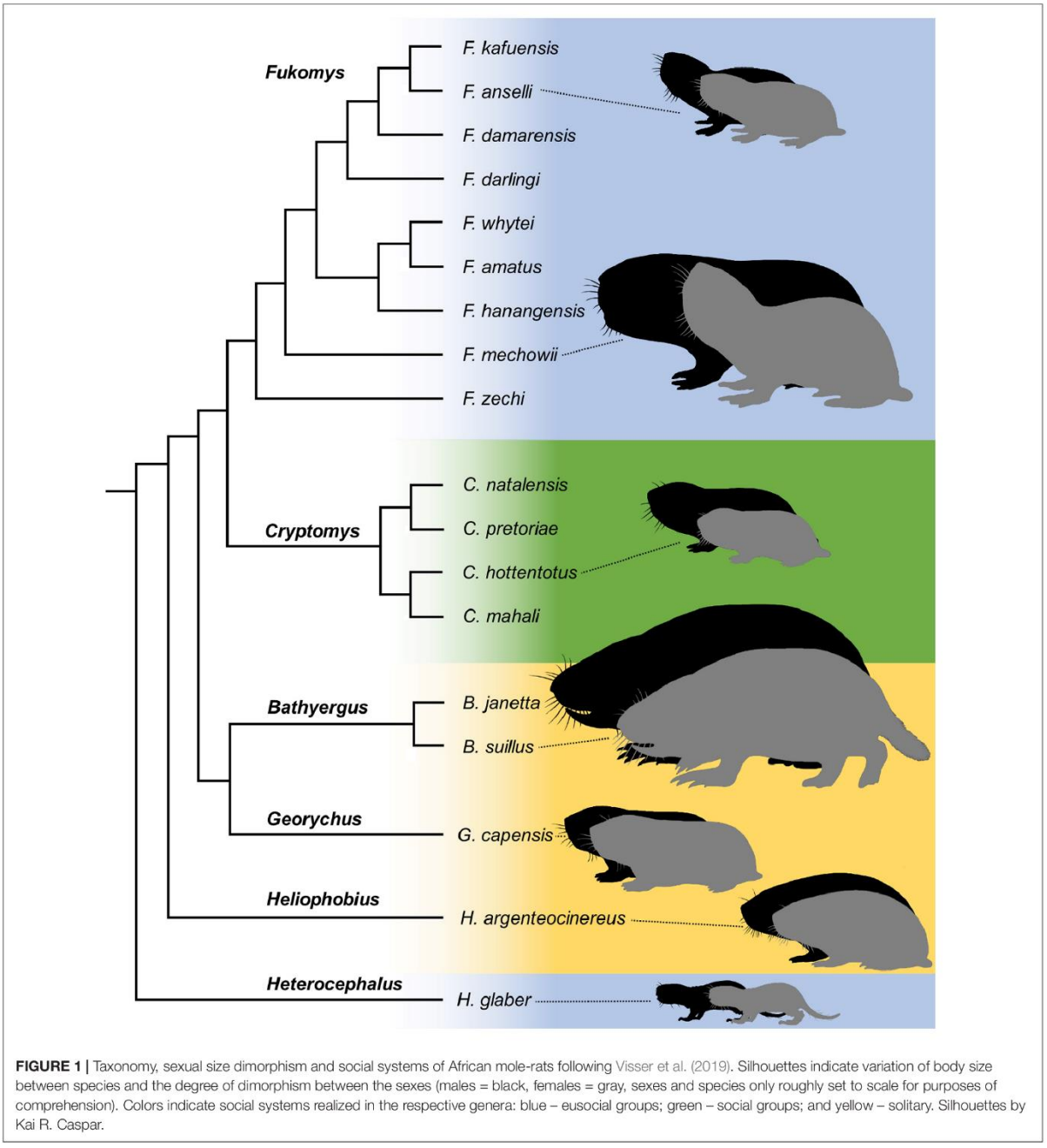

FIGURE 1 | Taxonomy, sexual size dimorphism and social systems of African mole-rats following Visser et al. (2019). Silhouettes indicate variation of body size between species and the degree of dimorphism between the sexes (males = black, females = gray, sexes and species only roughly set to scale for purposes of comprehension). Colors indicate social systems realized in the respective genera: blue – eusocial groups; green – social groups; and yellow – solitary. Silhouettes by Kai R. Caspar.

bathyergid genus *Heterocephalus* (naked mole-rat), live in cooperatively breeding groups. These families typically comprise only a single breeding female in all social genera, while the number of simultaneously active reproductive males varies.

In *Fukomys*, the reproductive female is typically monogamous (Burland et al., 2002; Šumbera et al., 2012; Patzenhauerová et al., 2013). When applying the term monogamy, it is important to differentiate between social monogamy (living and raising offspring with a single partner) and genetic monogamy (exclusively mating and producing offspring with such partner). Many socially monogamous mammals are not genetically monogamous (Cohas and Allainé, 2009). However,

current evidence suggests that *Fukomys* indeed exhibits strict social and predominant sexual monogamy, the latter fluctuating in frequency between populations (Burland et al., 2002; Patzenhauerová et al., 2013). While staying faithful to their current mate for prolonged periods (Bappert et al., 2012; Begall et al., 2021), *Fukomys* may experience serial changes in partners over their lifetime (Burland et al., 2002; Šumbera et al., 2012; Patzenhauerová et al., 2013). Different mating systems are expressed in the other two social bathyergid genera. The breeding female in *Heterocephalus* families may mate with two (seldom more) males at a time (Braude et al., 2020) and in *Cryptomys* it often has multiple partners (Bishop et al., 2004). In *Heterocephalus* as well as in *Fukomys*, more than six generations of pups overlap in average family groups and the majority of offspring remains with their parents and assist in provisioning their siblings instead of reproducing themselves (Burda et al., 2000; Torrents-Ticó et al., 2018). Because of this high degree of philopatry in combination with partitioning of reproductive labor and cooperative breeding, these genera have at times been characterized as eusocial mammals (Burda et al., 2000). In *Cryptomys*, levels of philopatry and male reproductive skew are notably lower than in the other social genera (Bishop et al., 2004; Ingram et al., 2004) so that they are generally not considered to be eusocial. Less is known about the mating systems of solitary genera, but morphological, genetic and behavioral evidence suggests that they are either polygynous or promiscuous (Patzenhauerová et al., 2010; Bray et al., 2012; Visser et al., 2017).

In social mole-rats such as *Fukomys*, non-reproductive individuals are believed to reduce the workload of the breeding female, granting a fitness benefit (Burda et al., 2000). Empirical evidence in support of this assumption has been provided for *Fukomys damarensis*, the Damaraland mole-rat. In captivity, the fecundity of *F. damarensis* breeding females as well as the time they spend resting and feeding correlates positively with the number of non-reproductive helpers (Houslay et al., 2020) and in the wild, breeders spend significantly less time foraging than helpers (Francioli et al., 2020). Although at times stated differently, mole-rat helpers do not show developmentally fixed patterns of task specialization (Lacey and Sherman, 1991; Zöttl et al., 2016; Thorley et al., 2018b; Van Daele et al., 2019), meaning that no helper casts dedicated to specific tasks (e.g., foraging, pup raising, and nest defense) exist, as it is the case in many social insects. Rather than that, an individual's age influences the frequency in which it engages in specific helping behaviors (Zöttl et al., 2016), with differences in the contributions of male and female helpers being negligible (Thorley et al., 2018b).

Due to the differing social and mating systems among bathyergids, it would be predicted that mole-rat genera display varying patterns of sexual size dimorphism (SSD) and sexually selected weaponry directly linked to their mating system (Heske and Ostfeld, 1990; Schulte-Hostedde, 2007). In the solitary genera, one would predict pronounced SSD since access to partners is contested and individual males can gain a reproductive advantage by mating with multiple females via the monopolization of defendable resources. Fitting this assumption, pronounced SSD is found in many solitary subterranean rodents that belong to diverse evolutionary lineages (Daly and Patton, 1986; Mauk et al., 1999; Su et al., 2018). A conjoint prediction would be that males in these species evolve more formidable weapons, in case of mole-rats more powerful and robust jaws and incisors, to solve this task. More subtle or absent sexual dimorphism would be expected in the social genera, particularly so in monogamous *Fukomys*, where physical breeding competition is low for prolonged periods once a pair-bond is established (Patzenhauerová et al., 2013). Among the few non-bathyergid social subterranean rodents, monogamy and a lack of SSD is for instance evident in the Northern mole vole (*Ellobius talpinus*; Moshkin et al., 2001). On the other hand, it might be expected that these social genera display differences in functional morphology that relate to reproductive status, for example more strongly developed weaponry in breeders of both sexes to defend their status against challengers (Young and Bennett, 2013). Such hypotheses appear reasonable, since at least female breeders in *Fukomys* and *Heterocephalus* show marked changes in their postcranial skeletal anatomy when attaining breeding status (Dengler-Crish and Catania, 2007; Thorley et al., 2018a), indicating a notable degree of developmental plasticity. So far, such differences are not evident in the skulls of female mole-rats (Thorley et al., 2018a) but precise methods such as geometric morphometrics have not yet been employed to differentiate between reproductive status groups and male mole-rats were never studied at all in this respect.

Surprisingly, SSD expression does not appear to correlate with social systems or phylogeny among bathyergids (Burda, 1990, **Figure 1**). Although these patterns are inconsistent and counterintuitive, the phenomenon received only little scientific attention and is seldom comparatively assessed: The solitary *Bathyergus* and at least most species of eusocial *Fukomys* are highly sexually dimorphic in body size with adult males being the larger sex with more massive skulls (Hart et al., 2007; Chimimba et al., 2010; Young and Bennett, 2013). Nevertheless, potential dimorphism in skull shape unrelated to size remains essentially unaddressed in these genera (but see Faulkes et al., 2017 for *Fukomys*). Within the remaining groups of African mole-rats, available studies suggest that skulls are not sexually dimorphic (Taylor et al., 1985; van Rensburg et al., 2004; Barčíová et al., 2009) and that differences in body size are variably expressed. Solitary *Heliophobius* are notably dimorphic in mass (Šumbera et al., 2003). For male-biased SSD in social *Cryptomys*, contradictory results have been published, but studies relying on data from multiple family groups agree that it is indeed present to varying degrees (Spinks et al., 2000; van Rensburg et al., 2004). Finally, eusocial *Heterocephalus* as well as solitary *Georychus* are assumed to lack SSD (Brett, 1991; Jarvis and Bennett, 1991; Bennett et al., 2006, see also Thomas et al., 2012).

If no clear relationship with sociality is evident, what factors underly the expression of SSD in African mole-rats? In many animal groups, SSD scales with body mass, a phenomenon most prominently described by Rensch's rule (Abouheif and Fairbairn, 1997). Rensch's rule posits that among closely related species, SSD grows with increasing general body size when males are the larger sex (Rensch, 1950) and *vice versa* when the opposite is the case (Rensch, 1960, but see Webb and Freckleton, 2007). However, it is commonly assumed that Rensch's rule can only be observed

among socially polygynous or polygamous species (Dale et al., 2007; Bidau and Martinez, 2016) so that bathyergids and even more so *Fukomys* species would not be expected to comply to it. In other subterranean rodents studied so far, the polygynous South American tuco-tucos (*Ctenomys*) and Central Asian zokors (*Eospalax* and *Myospalax*), Rensch's rule is not in effect (Martínez and Bidau, 2016; Su et al., 2018), and notable SSD appears to be rare among small-bodied mammals in general (Lu et al., 2014). Finding Rensch's rule among bathyergids would be unexpected and could indicate so far unappreciated social dynamics acting across the boundaries of social systems in this group.

In this exploratory study, we focus on patterns of sexual dimorphism and correlates of breeding status in *Fukomys anselli*, the Ansell's mole-rat. *F. anselli* is a typical representative of its genus in displaying the puzzling combination of pronounced SSD in conjunction with prolonged sexual monogamy and cooperative breeding (Burda and Begall, 1998; Patzenhauerová et al., 2013). Besides employing craniometric methods to assess morphological differentiation, we also quantify differences in relative skull size between the sexes. This phenomenon received little study in mammals (but see Young and Bennett, 2013) but is well investigated in squamate reptiles, where it is prominently discussed as an indicator for intrasexual competition (e.g., Baird, 2013). To place sexual dimorphism in the Ansell's mole-rat into its phylogenetic context, we further compile a dataset on body mass in the sexes of various bathyergid species and test for SSD scaling conforming to Rensch's rule. By combining these different approaches, we aim to arrive at a comprehensive characterization of sexual dimorphism in cooperatively breeding *Fukomys* mole-rats, which is a crucial step to understand the interplay between monogamous mating systems and pronounced SSD found in these animals.

MATERIALS AND METHODS

SSD and the Validity of Rensch's Rule in the Bathyergidae

We conducted a literature search for information on sex-specific body mass in the Bathyergidae and concluded with a dataset spanning 18 species from all six extant genera, which cover almost the complete spectrum of body sizes found in the family, including size extremes (**Table 1**). The genus *Fukomys* is represented by nine species. Species were included when data for at least five specimens per sex and species corresponding to wild adult animals were available. Data from pregnant females were excluded, whenever provided by the respective sources. Following Ingram et al. (2004), we refer to populations of *Cryptomys* (*C. hottentotus*, *C. mahali*, *C. natalensis*, and *C. pretoriae*) as full species, since the age of these lineages as well as their genetic divergence exceeds that of many *Fukomys* populations which are differentiated at the species level.

We tested for the validity of Rensch's rule in both bathyergids in general and in *Fukomys* by employing two established methods to investigate SSD scaling. If both were to yield comparable outcomes, the respective results could be assumed to be robust. First, we employed phylogenetic reduced major axis (pRMA) regression, a widely accepted method to study scaling in SSD (Abouheif and Fairbairn, 1997; Fairbairn, 1997; Bidau and Martinez, 2016; Martínez and Bidau, 2016), by utilizing the *phytools* package in R (Revell, 2012). For such interspecific comparisons, phylogenetic relationship must be accounted for to address the non-independence of species data points due to shared ancestry. Based on the bathyergid phylogeny of Visser et al. (2019; see **Figure 1**), we calculated pRMA regressions of $\log_{10}$(male body mass) on $\log_{10}$(female body mass) to estimate whether SSD in bathyergids scales with body mass in compliance with Rensch's rule. The rule is in effect when the coefficient β of said regressions is significantly greater than the expected value 1 (Abouheif and Fairbairn, 1997; Martínez and Bidau, 2016). Clarke's T statistic with adjusted degrees of freedom was employed to assess the deviation of the slope from the expectation (Bidau and Martinez, 2016).

Second, we calculated phylogenetic linear regression models based on modified Lovich-Gibbons' ratios (two-step LG ratio) on $\log_{10}$(female body mass) as recommended by Smith (1999). The two-step LG ratio is calculated as follows: If the mean body mass of males is higher than that of females: M/F; if the mean body mass of females is higher than that of males: 2-F/M. Smith (1999) recommends the application of the two-step LG ratio for typical mammalian data sets where male-biased SSD is prevalent. Linear regression models were corrected for phylogeny by using phylogenetic independent contrasts. According to this method, Rensch's rule is in effect when the coefficient β of the regression line is significantly greater than 0.

Skull Morphometrics of *Fukomys anselli*

All morphometric data was collected blindly with the researchers taking measurements being unaware of sex and reproductive status of the specimen concerned. All analyses, if not otherwise indicated, were carried out in R Studio for Mac Version 1.3.1093 (RStudio Team, 2020).

Material

Skulls of 40 adult *Fukomys anselli*, representing ten non-breeders and ten breeders of either sex were extracted from ethanol-fixed or frozen animals from the research collection of the Department of General Zoology, University of Duisburg-Essen (**Supplementary Table 1**). Respective individuals were kept and often bred in the laboratories of the University but genealogically derive from animals captured close to the type locality of the species in the Lusaka area of Central Zambia. Relying on captive subjects allowed for an unequivocal determination of an individual's reproductive status, as breeding was closely monitored in the laboratory. Only captive-born individuals that never reproduced where classified as non-breeders, while breeders where required to have produced at least one offspring. All skulls derived from animals that were at least 30 months old at the time of death and therefore fully mature. *F. anselli* is full grown at an age of approximately 12 months (Burda and Begall, 1998). Mean age for breeders was 111.2 months (=9.3 years; SD: 41.4 months), while mean age for helpers was 57.9 months (=4.8 years; SD: 36.3 months). This stark discrepancy is largely explained by the bimodal aging pattern in the genus *Fukomys*,

TABLE 1 | Mean body mass (BM, *g*) and sexual size dimorphism (SSD, male:female) based on BM in African mole-rats.

Species	n_{males}	$n_{females}$	BM_{males}	$BM_{females}$	SSD	References
Bathyergus janetta	100	106	429.4	330.4	1.30	Herbst et al., 2004
Bathyergus suillus	87	100	955.2	778.5	1.23	Bennett et al., 2009
Cryptomys hottentotus	31	19	77	57	1.35	Jarvis and Bennett, 1991
Cryptomys mahali	8	8	131	92.1	1.42	van Jaarsveld et al., 2019
Cryptomys natalensis	106	95	108.8	77.3	1.41	Oosthuizen, 2008
Cryptomys pretoriae	96	184	100.5	90.7	1.11	van Rensburg et al., 2004
Fukomys amatus	9	5	71.6	65.2	1.10	Scharff, 1998
Fukomys anselli	87	86	63	52.9	1.19	Sichilima et al., 2011
Fukomys damarensis	290	281	165	141.5	1.17	Bennett and Jarvis, 2004
Fukomys darlingi	23	20	69.5	63.5	1.10	Bennett et al., 1994; Gabathuler et al., 1996
*Fukomys hanangensis**	12	5	89.7	78.2	1.15	Faulkes et al., 2017
Fukomys kafuensis sensu lato*	5	12	89.8	75.6	1.19	Van Daele et al., 2019, reclassified according to Visser et al., 2019
Fukomys mechowii	79	76	570.7	391.8	1.46	Sichilima et al., 2008
*Fukomys whytei**	11	11	131.1	107.3	1.22	Burda et al., 2005; Faulkes et al., 2017
Fukomys zechi	28	29	234.1	202.2	1.16	Yeboah and Dakwa, 2002
Georychus capensis	189	277	193.1	195.8	0.99	Bennett et al., 2006; Thomas et al., 2012; Visser et al., 2017
Heliophobius argenteocinereus	70	74	190.1	162.1	1.17	Šumbera et al., 2003
Heterocephalus glaber	n_{total} = 651		34.1	36.5	0.93	Brett, 1991

*In case that data were pooled from different studies, weighted means have been calculated. Data presented refer to adult animals measured in the wild (age class assignment followed respective references). *only unequivocally sexed specimens with body masses equal to or above 50 g were considered and, if applicable, those in adult dental age categories as assigned by the respective authors. The two-step Lovich-Gibbons' ratios (Smith, 1999) were identical with SSD values to the second decimal place.*

with breeders reaching on average two times the age of helpers in captivity (Dammann and Burda, 2006). However, mean ages of males (7.8 years; SD: 49.2 months) and females (6.3 years; SD: 44 months) did not differ significantly (Student's *t*-Test, $p > 0.2$). Each specimen derived from a different litter and for the most part from different families. Only two pairs of different aged siblings were sampled, all of the respective individuals being non-breeders.

Multivariate Skull Morphometrics

A set of 24 linear measurements from cranium and mandible (**Table 2**) were collected with digital calipers (fixpoint® model 77001). Measurements were taken by a single observer (KRC) to 0.1 mm from either the sagittal plane or the right side of the skull (see **Table 2** and **Supplementary Table 1**) and are visualized in **Figure 2**. In case relevant structures of the right side of the skull were damaged, measurements were taken from the left one (11 measurements = 1.1% of total measurements). Missing measurements [10 measurements = 1% of total measurements (n = 950), see **Supplementary Table 1**] were estimated using the *LOST* package (Arbour and Brown, 2020). Hindfoot length was chosen as a proxy for body size to assess its influence on cranial measurements. Foot length is a common metric in small mammal research that is insensitive to changes in body condition and was measured to the nearest 0.1 mm excluding claws, following Ansell (1965).

Measurements were log-transformed and analyzed using principal component analysis (PCA). Subsequently, leave-one-out cross-validated linear discriminant function analysis (LDA) was performed on the principal components (PC) generated by the PCA to estimate how well sexes and breeding status groups could be differentiated based on cranial measurements. One-tailed exact binomial tests were used to check whether correct assignment rates differed significantly from chance level. Suitability of PC covariance for LDA was tested with Box's *M* test. The assumption of multivariate normality was tested using the Shapiro–Wilk multivariate normality test. A MANOVA was used to test for the effects of body size, sex, and reproductive status as well as their interaction on skull morphology. Comparisons of each linear measurement between the respective groups of specimens were performed by employing Welch's two sample *t*-test for normally distributed measurements and Wilcoxon rank sum test for non-normally distributed ones, respectively, controlling for alpha error accumulation via Bonferroni correction. This resulted in an adjusted significance level of $\alpha_{adjusted} = 0.002$.

2D Geometric Morphometrics of Cranium and Mandible

Size-independent differences in skull shape were analyzed by employing a 2D landmark approach. Crania were photographed with a Canon EOS 200D reflex digital camera in a standardized fashion from dorsal and ventral perspectives. Mandibles were photographed from a lateral perspective. Specimens were placed on a checkered mat with squares of 1 cm edge length that was used to provide spatial orientation and acted as a size reference. The inclination of the crania was adjusted with a piece of plasticine. The camera focus was adjusted on the molars

TABLE 2 | Summary of linear cranial measurements collected for multivariate morphometric analysis.

Measurement	Abbreviation	Description
Length of diastema	DIAS	Distance between alveoli of the upper incisor and premolar.
Palatilar length	PALA	Distance between staphylion and the alveoli of the upper incisors
Length of upper alveolar row	UPAL	Length of upper tooth row, measured at the alveolar margins.
Width of premolar	PREW	Breadth of upper premolar, measured at the alveolar margin
Length of bulla	BULL	Length of bulla measured from styliform process to the external auditory meatus
Width of bulla	BULW	Width of bulla measured from the jugular foramen to the external auditory meatus
Basilar length	BASI	Distance between the anterior margin of the foramen magnum and the anterior margin of the incisor alveoli
Greatest length of skull	SKUL	Distance between the premaxillary tip and the posteriormost extension of the occipital in the sagittal plane
Interorbital width	IORW	Smallest distance between the outer margins of the frontals at the reduced bony orbits
Width of posterior portion of zygomatic arch	ZYPW	Width of squamosal portion of zygomatic arch measured in parallel to the sagittal plane
Width of zygomatic arches	ZYAW	Maximum width of zygomatic arches measured at the anterior tips of the squamosals
Width of skull base	BASW	Minimal distance between squamosals measured ventrally
Width of skull at paroccipital processes	PAPW	Maximum width of cranium measured at paroccipital processes
Size of infraorbital foramen	FIOL	Maximum extension of the infraorbital foramen
Width of processus zygomaticus maxillaris	PZMW	Width of the processus zygomaticus maxillaris at the infraorbital foramen
Width of incisors	INCW	Width of upper incisors measured at the alveolar margins
Length of rostrum	ROSL	Distance between alveolar margins of incisors and the nasofrontal suture.
Height of cranium	SKUH	Distance between anterior alveolar margin of the premolar and the nasofrontal suture.
Width of condyle	CONW	Greatest width of condyle
Height of occiput	SOCC	Greatest length of the supraoccipital bone (including skull crests, if present)
Mandible length	MANL	Length of the ramus mandibulae measured from condyle to the incisors' alveolar margin
Total height of mandible	MANH	Distance between tip of coronoid process and the ventral margin of the angular process
Height of coronoid process	CONH	Distance between tip of coronoid and ventral margin of the mandibular ramus
Length of angular process	ANGL	Greatest length of angular process

Measurements were adopted and modified from Van Daele et al. (2013).

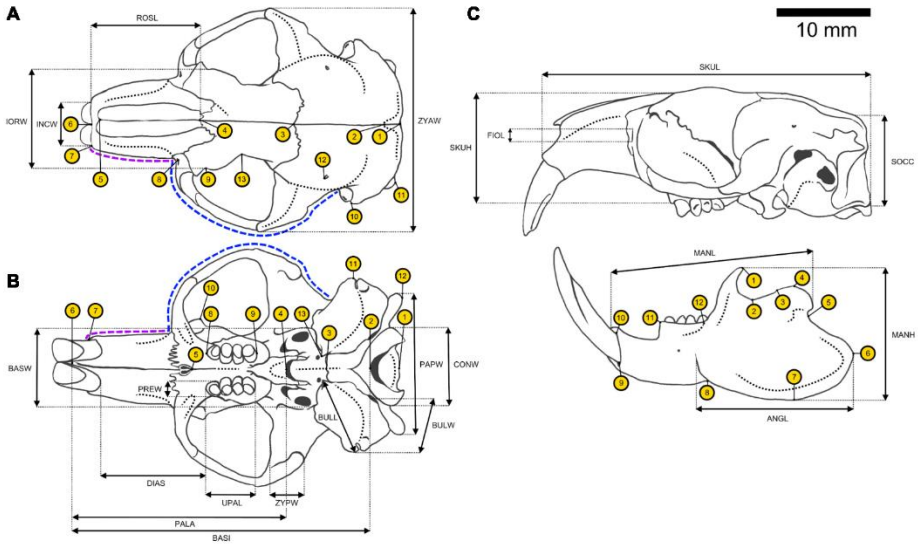

FIGURE 2 | Schematic drawing of a male Ansell's mole-rat skull with annotated linear measurements and landmarks used in this study. Definitions of landmarks and measurements are provided in **Table 2** and **Supplementary Table 2**. Semilandmarks are indicated as dashed lines. Rostral semilandmarks (purple) encompassed 8 landmark points, while zygomatic semilandmarks (blue) comprised 24. **(A)** Dorsal perspective of cranium with dorsal landmark set. **(B)** Ventral perspective of cranium with ventral landmark set. **(C)** Lateral perspective of cranium and mandible with mandibular landmark set.

for both ventral cranium and mandible photos and on the interfrontal suture of the skull roof for pictures taken from the dorsal perspective. Unilateral sets of 13 landmarks for the dorsal and ventral cranium and 12 landmarks for the mandible were selected and analyzed independently from each other (**Figure 2**; definitions are listed in **Supplementary Table 2**). Dorsal and ventral sets were amended by two lateral semilandmarks. The first one consisted of 8 semilandmark points and was set

along the rostrum from the incisor to the zygomatic arch. The second one comprised 24 landmark points and demarcated the zygomatic arch (**Figure 2**). Bending energy was used as criterion to optimize landmark positioning during sliding. Landmarks' digitization and scaling of photographs was achieved in TPSDig2 version 2.31 (Rohlf, 2018) and done by a single researcher for each landmark set (JM: dorsal/ventral; KRC: mandible). The geomorph package (Adams et al., 2020) was employed to analyze shape data. Occasional damage to the coronoid process led to missing landmarks (6 landmark points = 1.25%) in the mandible dataset, which were estimated and replaced by aid of the *LOST* package (Arbour and Brown, 2020). Landmarks were Procrustes superimposed to allow for subsequent analysis. Allometry was assessed by an ANOVA, regressing shape variables against centroid size, which is a measure of overall size in superimposed landmark datasets. PCA and LDA were employed with procedures analogous to those used in the analysis of linear cranial measurements and served the same purpose. However, the mandible dataset was not normally distributed. Therefore, statistical differences in measurements for sexes and reproductive status groups as well as interaction effects were assessed via MANOVA for dorsal and ventral cranial datasets and via a PERMANOVA for the mandibular one, respectively. Since LDA requires normal distribution of data as well, the randomForest method was used as an alternative classification procedure for mandibular landmark data (Liaw and Wiener, 2002).

Sex Differences in Relative Skull Dimensions

The greatest lengths of the skull (SKUL) and zygomatic arch width (ZYAW) were chosen to represent skull length and width, respectively, while hindfoot length was again used as a proxy for body size. Differences in hindfoot length between sexes and reproductive status groups were assessed in a linear regression model with hindfoot length as the dependent variable and sex and breeding status as predictors. Subsequently, the influence of body size and sex on skull size was tested the same way with either skull length or width as the dependent and hindfoot length as well as sex as predictor variables. Based on results from our previous morphometric analyses, the influence of breeding status on these parameters was not tested. Data were inspected visually for biasing outliers, linearity, normality, and homogeneity of variances in diagnostic plots. The latter two aspects were also assessed through Shapiro–Wilk and Levene tests, respectively, and found to be suitable for linear regression. Values for Cohen's d were derived from models' t statistics to provide a measure of effect size.

RESULTS

Occurrence and Scaling of SSD in the Bathyergidae

We found large differences in SSD among bathyergids and within the most speciose genus, *Fukomys* (**Table 1**). Lowest SSD values indicating sexual monomorphism were recovered for *Georychus* and *Heterocephalus*. All other bathyergids show a male-biased SSD which is pronounced in *Fukomys mechowii*, the giant mole-rat, and in the small-bodied genus *Cryptomys*. There was only a modest correlation between $\log_{10}$ female body mass and SSD in the Bathyergidae in general (Pearson correlation: $r = 0.3$; $t = 1.26$; and $p = 0.23$) but a strong one among *Fukomys* species (Pearson correlation: $r = 0.76$; $t = 3.1$; and $p = 0.017$).

Analyses of body mass data via pRMA models (**Table 3** and **Figure 3**) recovered that bathyergids as a whole show a trend to follow Rensch's rule of SSD scaling ($\beta = 1.072$, $p = 0.079$) while *Fukomys* species clearly comply to it ($\beta = 1.102$; $p = 0.021$). For both groups a strong phylogenetic signal associated with SSD was found ($\lambda = 0.999$), indicating a tight correlation between the expression of SSD and phylogenetic affiliation in bathyergids. Results of the regression models on two-step LG ratios did only partially align with the ones of the pRMA models. The former found bathyergids to comply to Rensch's rule ($\beta = 0.2214$; $p = 0.01$) while the data for *Fukomys* is approaching significance ($\beta = 0.2611$; $p = 0.05$).

Multivariate Skull Morphometrics

Male and female *F. anselli* were recovered as almost completely separated in the PCA morphospace based on linear cranial measurements, while reproductive groups were not discernible from each other (**Figure 4** and **Table 4**). Further analyses provided results consistent with this finding. MANOVA indicated a strong influence of sex on cranial morphometrics ($F = 12.13$, $p \ll 0.001$) but none of reproductive status ($F = 0.63$, $p = 0.74$) or the interaction between both ($F = 0.34$, $p = 0.84$). Hindfoot length was a strong general predictor of cranial morphology ($F = 7.33$, $p < 0.001$), but not among individuals of the same sex ($F = 0.40$, $p = 0.8$), and irrespective of their reproductive status ($F = 0.1$, $p = 0.98$). While males (90%) and females (80%) could be reliably classified in the linear measurement LDA ($p < 0.01$), individuals of the same sex were randomly assigned to reproductive status groups ($p > 0.1$, **Table 5**).

For the linear measurements, PCA computed 24 principal components, the first four of which had eigenvalues > 1 and explained 79.9% of total variance in the sample (**Table 4**). The most important measurements for group separation in PC1 were indicators of overall skull size, but particularly of its facial portion: mandibular length (MANL: 6.35%), basilar

TABLE 3 | Results from phylogenetic major reduced axis models investigating the relationship between male and female body mass in African mole-rats.

Group	N	λ	r^2	a	β	p
Bathyergidae	18	0.999	0.979	–0.093	1.072	0.079
Fukomys	9	0.999	0.993	–0.136	1.102	0.021

N gives the number of analyzed species in the respective group. Northern common mole-rats (Fukomys sp.) obey Rensch's rule, while the family in total does not, according to this method. Different from that, the regression models on two-step LG ratios suggest that bathyergids follow Rensch's rule as well (β = 0.2214; p = 0.01).

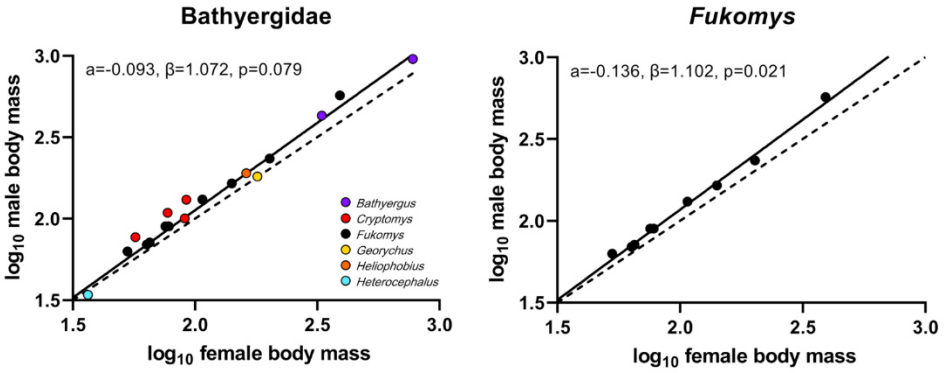

FIGURE 3 | Scaling of sexual size dimorphism in the family Bathyergidae and *Fukomys* species. The broken line indicates a size ratio of 1.0, equating to isometric scaling. *Fukomys* follows Rensch's rule as indicated by a significant deviation of $\beta > 1$. At family level, a similar trend could be recovered using phylogenetic major reduced axis models. However, the regression models on two-step LG ratios found that also bathyergids follow Rensch's rule revealed by a significant deviation of $\beta > 0$.

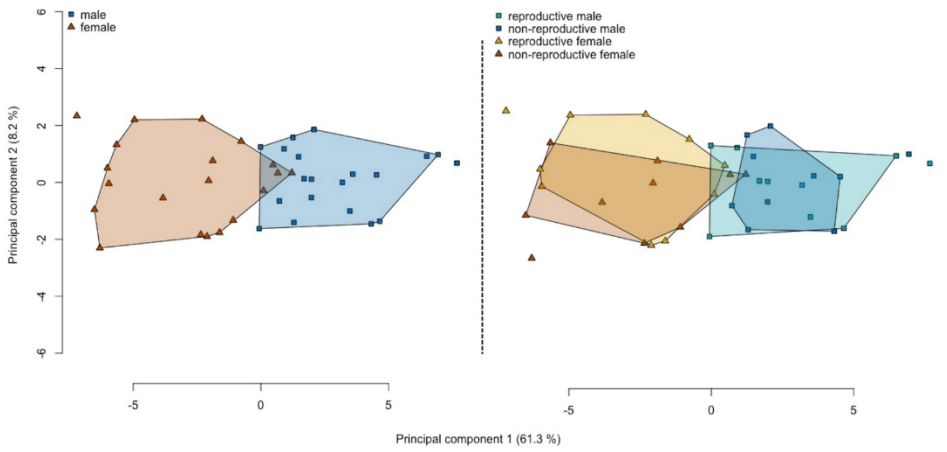

FIGURE 4 | Visualization of PCA results based on linear cranial measurements of *F. anselli*. Specimens are well separated by sex but not reproductive status.

length (BASI: 6.35%), and zygomatic arch width (ZYAW: 6.29%). However, PC1 factor loadings were to a large extent evenly distributed over the measurements, excluding several measurements concerned with dental characters and the skull base, which were of specific relevance to PC2. Width of skull base (BASW: 22.04%), width of premolar (PREW: 21.43%), and upper tooth row length (UPAL: 15.9%) contributed most to PC2 (**Table 4**).

The comparison of individual measurements between sexes and reproductive groups supported results of other analyses in showing distinct sexual dimorphism and the absence of cranial differences between breeders and non-breeders (**Table 5**). All but six variables differed significantly between the sexes ($p_{adjusted} < 0.002$). Non-significant variables primarily encompassed measurements relevant to the dentition and skull base. They related to the upper cheek dentition (PREW, UPAL), condyle (CONW) and paroccipital processes (PAPW), infraorbital foramen size (FIOL) and the width of the tympanic bulla (BULW). Differences between breeders and non-breeders of the same sex were consistently non-significant.

Sex Differences in Relative Skull Dimensions

Hindfoot length, approximating body size, was not found to differ intrasexually within reproductive status groups (t = -0.355, $p > 0.7$, and $d = 0.12$), but between the sexes ($t = 2.626$, $p = 0.012$, and $d = 0.88$). Skull length as well as width was found to increase proportionally with body size without significant differences among regression slopes between the sexes (skull length: $t = 1.543$, $p = 0.131$, and $d = 0.51$; skull width: $t = 1.773$, $p = 0.085$, and $d = 0.59$; **Figure 5**). However, relative skull length and width was greater in males compared to females as

TABLE 4 | Results of PCA based on linear cranial measurements in *F. anselli*.

Variable	PC1		PC2		PC3		PC4	
	EV: 14.70	Var. expl. 61.3%	EV: 1.95	Var. expl. 8.2%	EV: 1.44	Var. expl. 6.0%	EV: 1.09%	Var. expl. 4.55%
DIAS*	5.44%	0.23	2.82%	0.17	0.12%	–0.04	0.27%	0.05
PALA*	6.26%	0.25	0.16%	0.04	0.12%	0.04	0.11%	–0.03
UPAL	0.54%	0.07	15.90%	–0.40	5.65%	0.24	1.36%	–0.12
PREW	0.10%	0.03	21.53%	–0.46	2.78%	0.17	27.81%	–0.53
BULL*	4.68%	0.22	1.53%	0.12	9.76%	–0.31	0.58%	–0.08
BULW	3.49%	0.19	3.75%	0.19	10.98%	–0.33	0.08%	–0.03
BASI*	6.35%	0.25	0.56%	0.07	0.25%	0.05	0.02%	0.02
SKUL*	6.11%	0.25	0.16%	0.04	0.20%	0.04	0.32%	0.06
IORW*	5.56%	0.24	0.77%	0.09	0.94%	0.10	1.86%	–0.14
ZYPW*	3.86%	0.20	0.92%	–0.10	5.02%	–0.22	3.08%	–0.18
ZYAW*	6.29%	0.25	0.43%	0.07	0.14%	0.04	0.30%	0.05
BASW*	0.12%	0.04	22.04%	–0.47	0.35%	–0.06	37.99%	0.62
PAPW	3.34%	0.18	5.77%	–0.24	0.60%	–0.08	3.05%	0.17
FIOL	0.92%	0.10	9.37%	–0.31	22.05%	–0.47	8.46%	–0.29
PZMW*	3.78%	0.19	2.11%	–0.15	11.14%	0.33	0.15%	0.04
INCW*	5.55%	0.24	0.96%	0.10	2.55%	0.16	1.53%	0.12
ROSL*	5.19%	0.23	0.73%	0.09	0.32%	0.06	5.48%	–0.23
SKUH*	5.44%	0.23	1.21%	0.11	0.02%	0.01	0.05%	0.02
CONW	1.97%	0.14	5.09%	–0.23	11.35%	–0.34	0.05%	0.02
SOCC*	3.00%	0.17	0.41%	0.06	14.21%	0.38	2.59%	–0.16
MANL*	6.35%	0.25	0.36%	0.06	0.32%	–0.06	0.02%	0.01
MANH*	5.42%	0.23	0.37%	–0.06	0.01%	0.01	1.32%	0.11
CONH*	4.69%	0.22	2.91%	–0.17	0.98%	0.10	0.83%	0.09
ANGL*	5.54%	0.24	0.13%	–0.04	0.15%	0.04	2.72%	0.16

indicates measurements that differed significantly ($p_{adjusted} < 0.002$) between the sexes. See **Table 2 for abbreviations of linear measurements; EV, eigenvalue; Var. expl., percentage of explained variance.*

TABLE 5 | Correct assignment rates (%) of *F. anselli* skulls based on sex and reproductive status according to discriminant function analyses (based on LDA or in the case of mandibular landmarks on randomForest).

	Linear measurements				Dorsal landmarks				Ventral landmarks				Mandibular landmarks			
Sex (n = 20)	♂♂		♀♀		♂♂		♀♀		♂♂		♀♀		♂♂		♀♀	
	90		**80**		**90**		**85**		**95**		**85**		70		65	
Breeding status (n = 10)	*R*	NR	*R*	NR	*R*	NR	*R*	NR	*R*	NR	*R*	NR	*R*	NR	*R*	NR
	30	20	60	50	40	50	50	50	40	60	50	50	60	70	50	60

Bold numbers indicate correct assignments significantly different from chance ($p < 0.05$, one-tailed exact binomial test). NR, non-reproductive, R, reproductive.

indicated by significantly different regression constants (skull length: $t = 3.849$, $p < 0.001$, and $d = 1.27$; skull width: $t = 6.665$, $p < 0.001$, and $d = 2.19$). There was a mean sex difference of 1.5 mm (SD: 0.39) in skull length and 2.4 mm (SD: 0.36) in width at equal body size, conforming to 4.4 and 10.1% of mean female skull length and width, respectively.

2D Geometric Morphometrics of Cranium and Mandible

Analyses on the two landmark sets corresponding to dorsal and ventral cranium yielded similar results, while the mandibular dataset deviated from the latter ones in some respects, being less sensitive to influences of sex and skull size. In the dorsal and ventral cranial datasets, the PCA was able to separate specimens well regarding sex but not breeding status. However, the first two principal components encompassed only a fraction of total variance (38.1 and 44.8%, respectively; **Figure 6**), indicating high overall morphological variability. A similar pattern was recovered for the mandibular dataset, although morphospace overlap between the sexes as well as the variance explained by the first two PCs (49.3%) was greater compared to the other ones (**Figure 6**, see below). Accordingly, only sex was recovered to significantly influence the shape of the dorsal ($F = 6.36$, $p = 0.001$) and ventral cranium ($F = 5.32$, $p = 0.001$) as well as mandibular shape ($F = 4.82$, $p = 0.001$). Reproductive status or the combined

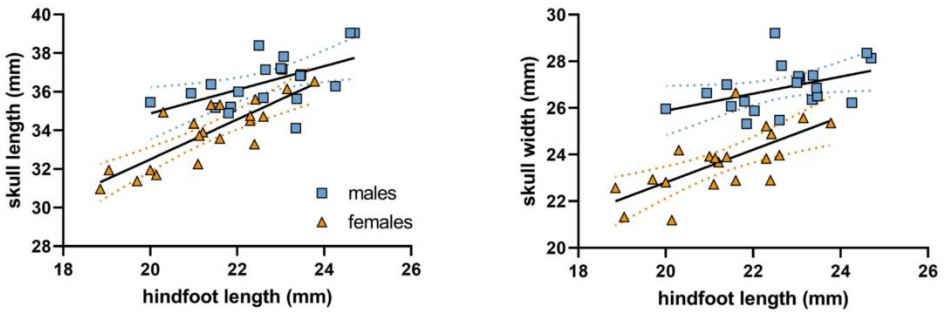

FIGURE 5 | Cranial length and width in Ansell's mole-rat in relation to body size (approximated by hindfoot length). Dotted lines indicate 95% confidence intervals of sex-specific regression lines.

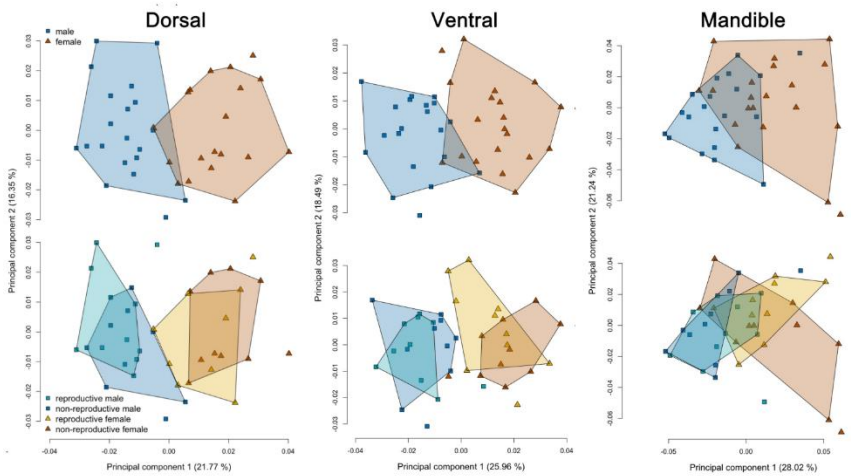

FIGURE 6 | Visualization of PCA-results based on geometric morphometrics of the cranium and mandible in *F. anselli*. Specimens are well separated by sex in plots based of dorsal and ventral landmarks, while overlap between the sexes is large for the mandibular dataset. No separation of reproductive groups was achieved. Polygons indicate the 95% convex hulls.

effect of sex and breeding status had no significant effect on these traits ($p > 0.1$). Shape allometry was detected for the dorsal ($F = 3.80$, $p = 0.001$) and ventral ($F = 3.17$, $p = 0.001$) cranium, but not for the mandible ($F = 1.97$, $p = 0.06$). However, since males had almost consistently larger skulls than females (**Figure 6**), this result is trivial. No pattern of allometry was found among specimens of the same sex in either landmark dataset (dorsal: $F = 0.69$, $p = 0.25$; ventral: $F = -0.44$, $p = 0.66$; and mandible: $F = 1.1$, $p = 0.40$).

Compared to females, the facial portion of the skull in males is enlarged, with longer rostra and widened zygomatic arches, which flare out more anteriorly (**Figure 7**). When scaled to the same size, the braincase and occipital region of male skulls therefore appear notably compressed compared to those of females. **Figure 7** visualizes shape differences between male and female *F. anselli* in a vector plot, highlighting relevant shape differences. Shape variable contributions and eigenvalues of PCs can be derived from **Supplementary Table 3**. Male mandibles were found to display a deeper and slightly more elongated processus angularis (landmark 6: 7.8% of PC1 variance, 16.9% of PC2 variance; landmark 7: 41.2% of PC1 variance, 22.7% of PC 2 variance) thicker lower incisors (landmark 9: 23.2% of PC1 variance, 14% of PC2 variance) and a longer, more sickle shaped coronoid process (landmark 1: 14.4% of PC1 variance, 12% of PC2 variance), when compared to females (**Figure 7**). However, sex differences in the shape of the lower jaw were altogether less pronounced than the ones in cranial shape. This was indicated by a more pronounced morphospace overlap and markedly higher error rates for assignments of individuals to one sex based on mandibular compared to cranial shape (**Table 5**, see below).

Cranial shape was found to be highly diagnostic for the sexes in *F. anselli*, but more so for males than for females mirroring the results from linear measurements (**Table 5**). LDA assigned 90% of males and 85% of females correctly based on dorsal cranial shape, while the ventral dataset allowed to classify 90% of males and 85% of females correctly. In both cases, these proportions

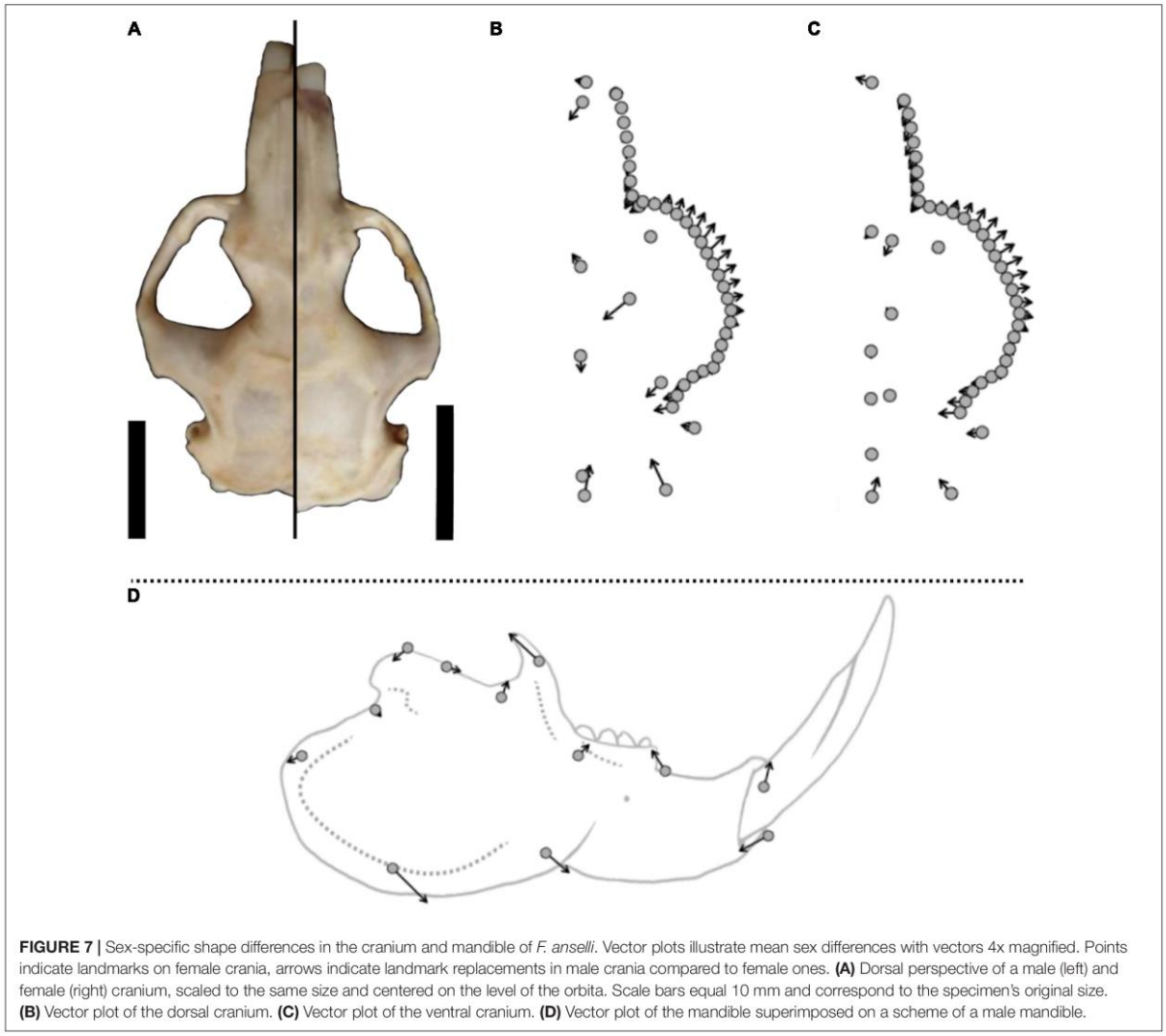

FIGURE 7 | Sex-specific shape differences in the cranium and mandible of *F. anselli*. Vector plots illustrate mean sex differences with vectors 4x magnified. Points indicate landmarks on female crania, arrows indicate landmark replacements in male crania compared to female ones. **(A)** Dorsal perspective of a male (left) and female (right) cranium, scaled to the same size and centered on the level of the orbita. Scale bars equal 10 mm and correspond to the specimen's original size. **(B)** Vector plot of the dorsal cranium. **(C)** Vector plot of the ventral cranium. **(D)** Vector plot of the mandible superimposed on a scheme of a male mandible.

differed significantly from random assignments ($p \leq 0.001$). The mandibular shape yielded less definite results: 70% of males and 65% of females were assigned to the correct categories, rates that did not significantly deviate from chance levels ($p > 0.05$). All intrasexual assignments based on reproductive status were randomized ($p > 0.1$).

DISCUSSION

Occurrence and Scaling of SSD in the Bathyergidae

Regression analyses based on both pRMA and two step LG ratios found Rensch's rule expressed in the monogamous mole-rat genus *Fukomys*, while only the latter did so for the bathyergid clade as a whole. The evidence for *Fukomys* following Rensch's rule is therefore robust, while its validity for bathyergids might still be questioned. However, although the pRMA regression did not reveal bathyergid SSD scaling significantly deviating from an isometric trajectory, a statistical trend ($p < 0.1$) emerged. If this is considered together with the positive results from the LG ratio based regression, one could therefore tentatively assume that Rensch's rule could apply to bathyergids on family level as well. Differences in the clarity of analytical outcomes between the two studied groups (*Fukomys* versus all bathyergids) might result from more uniform intrasexual selection pressures in *Fukomys*, when compared to other genera, which encompass a greater diversity of mating systems (compare Bray et al., 2012; Visser et al., 2017; Braude et al., 2020). Still, with the exception of *Heterocephalus*, aggressive competition between males, deduced either from direct observations (see below for *Fukomys* and, e.g., Jarvis and Bennett, 1991 for *Bathyergus* and Oosthuizen, 2008

for *Georychus*) or from morphological correlates such as sexual dimorphism, is evident throughout the bathyergid radiation. As such, it could underly the prevalence and scaling of SSD in these different taxa.

It is, however, important to point at the limitations of our dataset: Various studies have shown that intraspecific variation in bathyergid SSD (as approximated by body mass) can correlate with geographic provenance (*Cryptomys hottentotus* – Spinks et al., 2000; *Fukomys mechowii* — Scharff, 1998) and season of the year (*Georychus capensis* – Oosthuizen, 2008), factors for which we did not and could not control. Additionally, as we only included adult animals as assigned by the respective studies, we have to expect inconsistencies in the assignment of somatic maturity by the various authors we drew data from (see below). We must therefore consider a certain bias to our dataset. However, since most recovered rates of SSD correspond well to general reporting in the literature, we do not see the validity of our results compromised in that regard. Still, a more comprehensive dataset would have been desirable, especially regarding *Fukomys* species occurring North to the equator and for unambiguously localized populations of the solitary bathyergid genera, which might encompass significantly more species than currently recognized (Visser et al., 2019). However, biometric information on these mole-rats is currently unavailable.

Smaller-bodied species in our dataset (<150 *g*) are more variable regarding the expression of dimorphism than larger ones, where marked male-biased SSD is prevalent (**Table 1**). Among the small species, SSD is particularly pronounced in *Cryptomys*, in which male reproductive competition can be intense since multiple breeding males are regularly cohabiting a burrow and high incidences of extra-group paternity are common (Bishop et al., 2004). Bathyergids with strongly expressed SSD such as *Cryptomys* ssp. and *F. mechowii* are even among the most dimorphic of all rodents (Schulte-Hostedde, 2007), pointing to a crucial biological relevance of SSD in these animals. The latter must be especially emphasized for the genus *Fukomys*, where SSD scaling unambiguously follows Rensch's rule.

It appears that if at all, only modest SSD should be considered the ancestral condition in the Bathyergidae. Notable SSD is predominately found in derived bathyergids and is missing in the most basal genus *Heterocephalus* as well as among the closely allied rodent taxa Thryonomyidae and Petromuridae (Adu et al., 2002; Rathbun and Rathbun, 2006). Still, future studies might try to retrace the evolution of SSD in African hystricognaths in detail to eventually solve this question. Clarifying the matter might be specifically relevant to add to our understanding of the independent evolution of eusociality in *Fukomys* and *Heterocephalus*, which apparently derived from ancestors that strongly differed in SSD. In any case, some bathyergid SSD patterns will remain challenging to explain (compare **Figure 1**). The lack of SSD in *Georychus* appears to be secondarily acquired and is unexpected in an aggressively territorial solitary species. Not only are other non-social bathyergids, including its sister genus *Bathyergus*, dimorphic, but well-developed SSD is found in the majority of solitary subterranean rodents (Ctenomyidae – Martínez and Bidau, 2016; Geomyidae – Daly and Patton, 1986; Mauk et al., 1999; and Spalacidae – Su et al., 2018). On the other hand, the presence of pronounced SSD in at least several of the monogamous *Fukomys* species is surprising: their social system is commonly assumed to result in monomorphic sexes, and compliance to Rensch's rule could not be expected *a priori*. Possible explanations for this trait combination based on male-biased dynamic replacement of breeders will be discussed below.

Effects of Breeding Status on Skull Morphology in *F. anselli*

None of our analyses recovered significant differences between breeding and non-breeding mole-rats of the same sex, while sexual dimorphism was found to be pronounced within both groups. An important caveat to our analysis is the limited sample size of only ten individuals per status group and sex, especially in light of the high morphological variability we recover. However, since none of the different datasets we compared indicated even a trend of morphological segregation, we are reasonably confident that our findings reflect the actual conditions. Additionally, although our study is the first to explicitly address potential differences in skull morphology between mole-rats of different reproductive status, previous publications have already alluded to the lack of status-dependent cranial differentiation in *Fukomys* (Thorley et al., 2018a). Still, there surely is the possibility that future studies relying on more refined methods, such as 3D geometric morphometrics, may show detectable differences between mole-rat status groups. Nevertheless, even if such disparities would eventually be demonstrated, respective traits will be far more subtle than general sex-specific skull characteristics.

The uniformity of cranial characters contrasts with the pronounced differences in postcranial skeletal anatomy of breeding and non-breeding females in this genus (Thorley et al., 2018a) and points to similar functional demands to the skull among reproductive and non-reproductive mole-rats. Although there is evidence that non-reproductive *Fukomys* mole-rats are generally more active (Šklíba et al., 2016; Van Daele et al., 2019; Francioli et al., 2020; Houslay et al., 2020), both breeders and helpers engage in the same set of tasks, resulting in quantitative rather than qualitative differences in behavior, which could explain the lack of cranial divergence between them. Our results suggest that breeders do not develop more formidable weaponry or experience further somatic growth (safe for already documented allometric changes in breeding females' postcranium – Thorley et al., 2018a) after acquiring their status. Still, having more powerful jaws or greater body size might enhance the chances of becoming a breeder in the wild (compare Young and Bennett, 2013).

Field studies reported reproductives (excluding pregnant females) to be significantly heavier than helpers in *F. anselli* (Sichilima et al., 2011). de Bruin et al. (2012) even describe breeders (males: 81.45 ± 13.71 *g*, $n = 18$; females: 63.87 ± 11.39 *g*, $n = 19$) to be on average roughly two times as heavy as helpers (males: 39.80 ± 18.97 *g*, $n = 49$; females: 33.47 ± 11.78 *g*, $n = 64$). However, there are several problems with status assignment

in these studies. First, the authors assume a very early onset of maturity, classifying individuals ≥35 *g* as adults, whereas laboratory results suggest that this species reaches adulthood at approximately two times this body mass (Burda and Begall, 1998). We consider this difference to be too extreme to simply derive from the likely higher growth rates in provisioned captive animals. The assumption of such an early onset of maturity will strongly skew the results toward lower weights in non-reproductive animals. This way, a certain fraction of immature individuals will be counted as adult helpers since diagnostic traits of breeders are missing. Such practices will also bias estimations of SSD (see above). Unfortunately, precise aging of mole-rats in the wild is not possible offhand. Second, typically employed phenotypical criteria to assign breeding status in wild *F. anselli* males (and some congeneric species), such as testes size estimated by palpation or perioral secretions, are ambiguous. The latter frequently occur in non-reproductive males, at least in captivity (pers. obs.) and apart from the difficulty of assessing testes size by touch, contradicting findings have been published on testes volume differences between reproductive groups (de Bruin et al., 2012; Garcia Montero et al., 2016). Therefore, the reliability of available field data on weight distributions, especially for males can be questioned.

In case significant intrasexual differences in both sexes are confirmed, it needs to be clarified whether wild *F. anselli* might attain breeding status because of elevated body mass or develop it after succeeding to do so. Since body size is typically only approximated by mass in field studies, findings of intrasexual size differences might simply result from the allocation of limited resources in combination with lower activity levels of breeders in the wild (compare Francioli et al., 2020). In provisioned captive families, no such deviation in body mass is apparent (pers. obs., see Thorley et al., 2018a for *F. damarensis*) and even the opposite pattern might occur (Schielke et al., 2017). In any case, data on captive animals from this study and others demonstrate that attaining permanent breeding status *per se* does not go along with an isometric increase in body size (which would affect both hindfoot and cranial measurements, see **Figure 5**) or body mass in this species.

On a different note, our findings of uniform skull morphology in *F. anselli* status groups are relevant to *Fukomys* taxonomy, indicating that missing information about a specimen's reproductive history does not bias outcomes of anatomical studies.

Effects of Sex on Skull Morphology in *F. anselli*

In contrast to reproductive status, sex importantly influences both skull size and shape in *F. anselli*. In fact, *F. anselli* was also recovered as one of the more dimorphic small (<200 *g*) *Fukomys* species in regard to body mass, with males being roughly 20% heavier than females in the wild (Sichilima et al., 2011, but see above for problems with this estimate), with data from captive animals indicating even 40% higher body mass in males (Burda and Begall, 1998). Detected cranial difference are profound and not only point to a larger relative skull size in males but also to greater robustness of the male jaw apparatus. In light of this, it is surprising that a previous landmark analysis of the dorsal and ventral skull in sexually dimorphic *F. anselli*, *F. hanangensis* and *F. whytei* (compare **Table 1**) found no sex specific shape patterns (Faulkes et al., 2017). However, this study included immature specimens, which could have led to biased results.

Sex-specific cranial differences in *F. anselli* emerge due to hypertrophy of the facial portion of the skull in males (compare **Figure 7**), while several measurements of the skull base vary little between the sexes (**Table 4**). When scaled to the same size, males display wider as well as thicker zygomatic arches and larger angular processes than females which permit the development of a more voluminous musculus masseter, the most important masticatory muscle (Cox et al., 2020). Shape analysis also revealed a higher coronoid process in male mandibles, which together with more pronounced sagittal crests in males (pers. obs.) indicates a more strongly developed musculus temporalis, another jaw adductor (Cox et al., 2020). We therefore predict higher bite forces in male *F. anselli* compared to females relative to body mass (but see Van Daele et al., 2019 reporting equal bite forces in a small sample of a similar-sized *Fukomys* species). Apart from that, males can be expected to display a wider gape, since their rostra and mandibles are more elongated than the ones of females (**Table 4**). Still, there appears to be no noteworthy sex difference in cheek dentition, indicating that masticatory demands do not drive the sex-specific differentiation in the facial skeleton. The incisors on the other hand, which represent the most important weapons of African mole-rats, are hypertrophied in males, indicating adaptation to combat (Young and Bennett, 2013). This sex-specific trait was not only recovered herein but has already been noticed by Burda (1990) for *F. anselli* and by Young and Bennett (2013) for *F. damarensis*.

Sexual size dimorphism in relative skull size has been seldomly studied in mammals, but its occurrence was reported for *F. damarensis* (Young and Bennett, 2013). Our results agree with the respective study, in which head width (cranium and adhering soft tissues) in anesthetized *F. damarensis* was measured and males were found to have significantly wider heads than females relative to their body size. Unfortunately, head dimensions were only approximated by that single measure and raw data were not communicated, so that no further comparison with our findings on *F. anselli* can be drawn.

Clues to what underlies relative skull size SSD in mole-rats might derive from comparisons with reptiles, since the phenomenon has been intensively researched in lizards, subterranean amphisbaenians, and snakes, where exaggerated relative male head size is a common trait (Shine, 1991; Gienger and Beck, 2007; Martín et al., 2012; Baird, 2013). In fact, squamate reptiles and mole-rats differ little in that jaw and gape dimensions rather than other physical characteristics can decide conflicts with conspecifics. Just as male-biased body mass SSD in general, it is commonly assumed that enlarged heads in reptiles are intrasexually selected traits (Baird, 2013). Alternatively, ecological niche divergence between sexes could underly this anatomical difference (Shine, 1991, but see Baird, 2013), a factor also hypothesized to explain SSD in some monogamous mammals (e.g., Hillis and Mallory, 1996). However, the peculiar

lifestyle and foraging behavior of mole-rats argues against ecological influences on dimorphic head dimensions and again points to males combating competitors. There is also no evidence that male and female *Fukomys* differ in distances or mode of dispersal (above ground or underground) when leaving their natal family (Finn, 2017), so that an influence of this factor can also be ruled out (compare Young and Bennett, 2013). Instead, all the cranial traits discussed beforehand as well as the pattern of SSD scaling in *Fukomys* indicate a pronounced role of male-male combat in the social life of this genus. But how does that comply with the monogamous mating strategy of these mole-rats?

Socioecological Implications of Sexual Dimorphism in *Fukomys* Mole-Rats

Mammals exhibiting social and/or sexual monogamy are not expected to show pronounced SSD (compare Bidau and Martinez, 2016), so that the patterns found in *Fukomys* are surprising. Field studies employing microsatellite data demonstrated that reproductive skew in wild *Fukomys* is indeed extremely high. In *F. anselli*, only a single resident breeder of each sex is present and juveniles are sired almost exclusively by the established breeding male ($n_{families}$ = 13; 96.4% – Patzenhauerová et al., 2013). At the same time, the number of immigrants into established colonies is very small ($n_{non-breeders}$ = 85; 3.5%) but it is yet unclear whether these data are representative for *Fukomys* in general. In *F. damarensis*, a similar field study found the number of immigrants to be higher, at 7.5%, and the rate of extra-pair paternity was increased compared to *F. anselli* [up to 16% (31.9% if two outlier families are included); Patzenhauerová et al., 2013 referring to Burland et al., 2002]. These results do not challenge the assumption that both social and sexual monogamy is predominant in *Fukomys* but suggest that the latter may become compromised under specific circumstances.

However, although mating partners stay mostly faithful to each other and do so over long periods of time, once formed pairs can still be disrupted. This process appears to be mediated by male intrusion into established families. Shorter tenure length in reproductive males compared to females has been demonstrated by several genetic and capture-recapture studies (Young and Bennett, 2013, see below), indicating sex-biased social dynamics compliant with asymmetric reproductive competition and male-male combat. Genetic relatedness levels in males compared to females in wild *F. damarensis* groups also suggest that males do more frequently invade foreign groups and take over a breeding position (Burland et al., 2002). In case a breeder succession happens, or when a foreign male joins a group which lost its male breeder, a "patchwork family" with offspring of mixed descent can be established. Breeding male turnovers by intruders appear not to be rare (but see Torrents-Ticó et al., 2018), as all parentage studies on wild *Fukomys* found evidence for it in at least one family group analyzed (*F. anselli* – Patzenhauerová et al., 2013; *F. damarensis* – Burland et al., 2002; and *F. mechowii* – Šumbera et al., 2012).

Established wild *Fukomys* groups therefore exhibit high reproductive skew, experience at least some degree of immigration pressure and can tolerate the replacement of male breeders. The extreme, cooperatively enacted xenophoby found in laboratory-housed *Fukomys* appears to be an artifact of captivity (compare Bishop et al., 2004 for a similar pattern in *Cryptomys*). We suggest that the increased rate of male compared to female breeder turnover in the wild might be an unappreciated indication for stronger male intrasexual competition in *Fukomys* with major implications for SSD. Given the degree of SSD in *Fukomys* and the cranial adaptations described herein, it is likely that these replacements follow violent attacks. Still, such interactions are not documented so far. The reproductive benefit of securing a reproductive female, a burrow system and even a number of helpers for potentially several years must be extreme and might explain why SSD in *Fukomys* is more strongly expressed than in many polygynous or polygamous rodents, were mating associations are shorter-lived.

But there are several caveats to this hypothesis. First, it must be explained why such reproductive competition would occur in *Fukomys* and not in other cooperatively breeding rodents with slow life histories that occupy self-constructed defendable home ranges, such as mole-voles (*Ellobius* spp.), beavers (*Castor* spp.) and most importantly, *Heterocephalus*. It could also be argued that low documented rates of male group takeovers (Torrents-Ticó et al., 2018) could not create a sufficient selective pressure to explain the observed sexual dimorphism. Besides, faster male turnovers might not be provoked by intrasexual combat but by males facing higher mortality risks, for example increased predation pressure. For this, however, one would expect differences in the activity and ranging patterns of males and females, which is so far not apparent in wild *Fukomys* (*F. damarensis* – Francioli et al., 2020). A final potential caveat is the lack of intraspecific combat adaptations in *Fukomys* that are found in other bathyergids: Even large-bodied *Fukomys* lack defensive dermal shields, a trait found in the dune mole-rats of the genus *Bathyergus* (Jarvis and Bennett, 1991), which might be expected to evolve convergently in both genera in case males commonly experience violent encounters with competitors.

CONCLUSION

Our study provides a comprehensive description of the well-developed sexual dimorphism in the skull of *F. anselli*, which points to a significant role of male competition in the social life of mole-rats belonging to this cooperatively-breeding genus. *Fukomys* might best be characterized as serially monogamous rodent genus, in which males stay faithful to their partners for prolonged time but have to regularly engage in conflicts with same-sex rivals to secure their mate. That assumption is further supported by the recovery of SSD scaling conforming to Rensch's rule among *Fukomys* species, indicating violent monopolization of breeding females by males. At the same time, we show that no morphological differentiation in the cranium of breeders and non-breeders exists, indicating a lack of morpho-functional caste specialization beyond characters relevant to reproduction. The currently available data can only be considered a starting point regarding efforts to understand the evolutionary pressures influencing sexual dimorphism in social mole-rats. Future studies might address the following

questions to characterize the evolutionary pressures behind these morphological findings: How frequent are attempts to take over groups by foreign males in the wild and how do invaded family groups react? Why do helpers accept unrelated male breeders within the "patchwork family" scheme? To which degree do *Fukomys* species differ in respect to this behavior and how do they compare to *Cryptomys* and *Heterocephalus*? What factors underly the varying expression of SSD in the three solitary bathyergid genera?

Solving these questions will importantly contribute to fully unravel the remarkable diversity of social patterns found within the Bathyergidae and the factors that shaped it.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

KRC conceived the study and its methodology with input from SB. KRC and SB prepared the skulls, took linear measurements, and prepared the figures. JM photographed the skulls and compiled landmark datasets of the dorsal and ventral skull. KRC collected landmark data on the mandibles. SB and JM analyzed the data with input from KRC. All authors interpreted the findings. KRC wrote the first draft, which was reviewed by SB and JM. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.638754/full#supplementary-material

Supplementary Table 1 | Linear skull measurements and hindfoot lengths of *Fukomys anselli* specimens used in this study. Yellow mark: Measurement was taken from the left side of the skull. Red mark: Measurement was estimated using the LOST package (Arbour and Brown, 2020).

Supplementary Table 2 | Overview and definitions of point landmarks used to characterize skull traits in *Fukomys anselli*.

Supplementary Table 3 | Variable contributions (%) and eigenvalues of principal components computed for the shape of the dorsal and ventral cranium as well as for the mandible of *Fukomys anselli*.

REFERENCES

Abouheif, E., and Fairbairn, D. J. (1997). A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. *Am. Nat.* 149, 540–562. doi: 10.1086/286004

Adams, D. C., Collyer, M., and Kaliontzopoulou, A. (2020). *Geomorph: Software for Geometric Morphometric Analyses. R package version 3.2.1*.

Adu, E. K., Wallace, P. A., and Ocloo, T. O. (2002). Efficacy of sex determination in the greater cane rat, *Thryonomys swinderianus*, Temminck. *Trop. Anim. Health Product.* 34, 27–33.

Ansell, W. F. H. (1965). Standardisation of field data on mammals. *Zool. Afr.* 1, 97–113. doi: 10.1080/00445096.1965.11447303

Arbour, J., and Brown, C. (2020). *LOST: Missing Morphometric Data Simulation and Estimation. R package version 2.0.2*.

Baird, T. A. (2013). "Lizards and other reptiles as model systems for the study of contest behaviour," in *Animal Contests*, eds C. W. Hardy and M. Briffa (Cambridge: Cambridge University Press), 258–286. doi: 10.1017/cbo9781139051248.014

Bappert, M.-T., Burda, H., and Begall, S. (2012). To mate or not to mate? Mate preference and fidelity in monogamous Ansell's mole-rats, *Fukomys anselli*, Bathyergidae. *Folia Zool.* 61, 71–83. doi: 10.25225/fozo.v61.i1.a11.2012

Barčíová, L., Šumbera, R., and Burda, H. (2009). Variation in the digging apparatus of the subterranean silvery mole-rat, *Heliophobius argenteocinereus* (Rodentia, Bathyergidae): the role of ecology and geography. *Biol. J. Linn. Soc.* 97, 822–831. doi: 10.1111/j.1095-8312.2009.01228.x

Begall, S., Nappe, R., Hohrenk, L., Schmidt, T. C., Burda, H., et al. (2021). Life expectancy, family constellation, and stress in giant mole-rats (*Fukomys mechowii*). *Philos. Trans. R. Soc. B* 376:20200207. doi: 10.1098/rstb.2020.0207

Bennett, N. C., Faulkes, C. G., Hart, L., and Jarvis, J. U. M. (2009). *Bathyergus suillus*. *Mamm. Spec.* 2009, 1–7. doi: 10.1644/828.1

Bennett, N. C., and Jarvis, J. U. M. (2004). *Cryptomys damarensis*. *Mammal. Spec.* 2004, 1–5. doi: 10.1644/756

Bennett, N. C., Jarvis, J. U. M., and Cotterill, F. P. D. (1994). The colony structure and reproductive biology of the afrotropical Mashona mole-rat, *Cryptomys darlingi*. *J. Zool.* 234, 477–487. doi: 10.1111/j.1469-7998.1994.tb04861.x

Bennett, N. C., Maree, S., and Faulkes, C. G. (2006). *Georychus capensis*. *Mamm. Spec.* 2006, 1–4.

Bidau, C. J., and Martinez, P. A. (2016). Sexual size dimorphism and Rensch's rule in Canidae. *Biol. J. Linn. Soc.* 119, 816–830. doi: 10.1111/bij.12848

Bishop, J. M., Jarvis, J. U. M., Spinks, A. C., Bennett, N. C., and O'Ryan, C. (2004). Molecular insight into patterns of colony composition and paternity in the common mole-rat *Cryptomys hottentotus hottentotus*. *Mol. Ecol.* 13, 1217–1229. doi: 10.1111/j.1365-294x.2004.02131.x

Braude, S., Hess, J., and Ingram, C. (2020). Inter-colony invasion between wild naked mole-rat colonies. *J. Zool.* 313:12834.

Bray, T. C., Bloomer, P., O'Riain, M. J., and Bennett, N. C. (2012). How attractive is the girl next door? An assessment of spatial mate acquisition and paternity in the solitary Cape dune mole-rat, *Bathyergus suillus*. *PLoS One* 7:e39866. doi: 10.1371/journal.pone.0039866

Brett, R. A. (1991). "The population structure of naked mole-rat colonies," in *The Biology of the Naked Mole-Rat*, eds P. W. Sherman, J. U. M. Jarvis, and R. D. Alexander (New Jersey: Princeton Univ. Press), 97–136. doi: 10.1515/9781400887132-007

Burda, H. (1990). Constraints of pregnancy and evolution of sociality in mole-rats with special reference to reproductive and social patterns in *Cryptomys*

hottentotus (Bathyergidae, Rodentia). *J. Zool. Syst. Evol. Res.* 28, 26–39. doi: 10.1111/j.1439-0469.1990.tb00362.x

Burda, H., and Begall, S. (1998). Reproductive characteristics and growth in the eusocial Zambian common mole-rat (*Cryptomys* sp., Bathyergidae). *Z. Säugetierkunde* 63, 297–306.

Burda, H., Honeycutt, R. L., Begall, S., Locker-Grütjen, O., and Scharff, A. (2000). Are naked and common mole-rats eusocial and if so, why? *Behav. Ecol. Sociobiol.* 47, 293–303. doi: 10.1007/s002650050669

Burda, H., Šumbera, R., Chitaukali, W. N., and Dryden, G. L. (2005). Taxonomic status and remarks on ecology of the Malawian mole-rat *Cryptomys whytei* (Rodentia, Bathyergidae). *Acta Theriol.* 50, 529–536. doi: 10.1007/bf03192646

Burland, T. M., Bennett, N. C., Jarvis, J. U. M., and Faulkes, C. G. (2002). Eusociality in African mole-rats: new insights from patterns of genetic relatedness in the Damaraland mole-rat (*Cryptomys damarensis*). *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 269, 1025–1030. doi: 10.1098/rspb.2002.1978

Chimimba, C. T., Sichilima, A. M., Faulkes, C. G., and Bennett, N. C. (2010). Ontogenetic variation and craniometric sexual dimorphism in the social giant mole-rat, *Fukomys mechowii* (Rodentia: Bathyergidae), from Zambia. *Afr. Zool.* 45, 160–176. doi: 10.3377/004.045.0218

Cohas, A., and Allainé, D. (2009). Social structure influences extra-pair paternity in socially monogamous mammals. *Biol. Lett.* 5, 313–316. doi: 10.1098/rsbl.2008.0760

Cox, P. G., Faulkes, C. G., and Bennett, N. C. (2020). Masticatory musculature of the African mole-rats (Rodentia: Bathyergidae). *PeerJ* 8:e8847. doi: 10.7717/peerj.8847

Dale, J., Dunn, P. O., Figuerola, J., Lislevand, T., Székely, T., and Whittingham, L. A. (2007). Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. *Proc. R. Soc. B Biol. Sci.* 274, 2971–2979. doi: 10.1098/rspb.2007.1043

Daly, J. C., and Patton, J. L. (1986). Growth, reproduction, and sexual dimorphism in *Thomomys bottae* pocket gophers. *J. Mammal.* 67, 256–265. doi: 10.2307/1380878

Dammann, P., and Burda, H. (2006). Sexual activity and reproduction delay ageing in a mammal. *Curr. Biol.* 16, R117–R118.

de Bruin, P. R., Viljoen, H., Sichilima, A. M., and Bennett, N. C. (2012). Socially induced infertility in Ansell's mole-rat: are there depressed hormone levels in non-reproductive males and females? *J. Zool.* 286, 15–21. doi: 10.1111/j.1469-7998.2011.00841.x

Dengler-Crish, C. M., and Catania, K. C. (2007). Phenotypic plasticity in female naked mole-rats after removal from reproductive suppression. *J. Exp. Biol.* 210:4351. doi: 10.1242/jeb.009399

Fairbairn, D. J. (1997). Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annu. Rev. Ecol. Syst.* 28, 659–687. doi: 10.1146/annurev.ecolsys.28.1.659

Faulkes, C. G., Mgode, G. F., Archer, E. K., and Bennett, N. C. (2017). Relic populations of *Fukomys* mole-rats in Tanzania: description of two new species *F. livingstoni* sp. nov. and *F. hanangensis* sp. nov. *PeerJ* 5:e3214. doi: 10.7717/peerj.3214

Finn, K. T. (2017). *Density-Dependent Effects on Body Size, Philopatry, and Dispersal in the Damaraland Mole-Rat (Fukomys damarensis)*. M.Sc. thesis. Grahamstown: Rhodes University.

Francioli, Y., Thorley, J., Finn, K., Clutton-Brock, T., and Zöttl, M. (2020). Breeders are less active foragers than non-breeders in wild Damaraland mole-rats. *Biol. Lett.* 16:20200475. doi: 10.1098/rsbl.2020.0475

Gabathuler, U., Bennett, N. C., and Jarvis, J. U. M. (1996). The social structure and dominance hierarchy of the Mashona mole-rat, *Cryptomys darlingi* (Rodentia: Bathyergidae) from Zimbabwe. *J. Zool.* 240, 221–231. doi: 10.1111/j.1469-7998.1996.tb05281.x

Garcia Montero, A., Vole, C., Burda, H., Malkemper, E. P., Holtze, S., Morhart, M., et al. (2016). Non-breeding eusocial mole-rats produce viable sperm—spermiogram and functional testicular morphology of *Fukomys anselli*. *PLoS One* 11:e0150112. doi: 10.1371/journal.pone.0150112

Gienger, C. M., and Beck, D. D. (2007). Heads or tails? Sexual dimorphism in helodermatid lizards. *Can. J. Zool.* 85, 92–98. doi: 10.1139/z06-198

Gomes Rodrigues, H., Šumbera, R., and Hautier, L. (2016). Life in burrows channelled the morphological evolution of the skull in rodents: the case of African mole-Rats (Bathyergidae, Rodentia). *J. Mamm. Evol.* 23, 175–189. doi: 10.1007/s10914-015-9305-x

Hart, L., Chimimba, C. T., Jarvis, J. U. M., O'Riain, J., and Bennett, N. C. (2007). Craniometric sexual dimorphism and age variation in the South African Cape dune mole-rat (*Bathyergus suillus*). *J. Mammal.* 88, 657–666. doi: 10.1644/06-mamm-a-058r1.1

Herbst, M., Jarvis, J. U. M., and Bennett, N. C. (2004). A field assessment of reproductive seasonality in the threatened wild Namaqua dune mole-rat (*Bathyergus janetta*). *J. Zool.* 263, 259–268. doi: 10.1017/s0952836904005114

Heske, E. J., and Ostfeld, R. S. (1990). Sexual dimorphism in size, relative size of testes, and mating systems in North American voles. *J. Mammal.* 71, 510–519. doi: 10.2307/1381789

Hillis, T. L., and Mallory, F. F. (1996). Sexual dimorphism in wolves (*Canis lupus*) of the Keewatin District, Northwest Territories, Canada. *Can. J. Zool.* 74, 721–725. doi: 10.1139/z96-081

Houslay, T. M., Vullioud, P., Zöttl, M., and Clutton-Brock, T. H. (2020). Benefits of cooperation in captive Damaraland mole-rats. *Behav. Ecol.* 31, 711–718. doi: 10.1093/beheco/araa015

Ingram, C. M., Burda, H., and Honeycutt, R. L. (2004). Molecular phylogenetics and taxonomy of the African mole-rats, genus *Cryptomys* and the new genus *Coetomys* Gray, 1864. *Mol. Phylogenet. Evol.* 31, 997–1014. doi: 10.1016/j.ympev.2003.11.004

Jarvis, J. U. M., and Bennett, N. C. (1991). "Ecology and behaviour of the family Bathyergidae," in *The Biology of the Naked Mole-Rat*, eds P. W. Sherman, J. U. M. Jarvis, and R. D. Alexander (New Jersey: Princeton Univ. Press), 66–96. doi: 10.1515/9781400887132-006

Lacey, E. A., and Sherman, P. W. (1991). "Social organization of naked-mole rat colonies: evidence for the division of labor," in *The Biology of the Naked Mole-Rat*, eds P. W. Sherman, J. U. M. Jarvis, and R. D. Alexander (New Jersey: Princeton University Press), 275–336. doi: 10.1515/9781400887132-013

Liaw, A., and Wiener, M. (2002). Classification and Regression by randomForest. *R News* 2, 18–22.

Lu, D., Zhou, C. Q., and Liao, W. B. (2014). Sexual size dimorphism lacking in small mammals. *N. W. J. Zool.* 10, 53–59.

Martín, J., Polo-Cavia, N., Gonzalo, A., López, P., and Civantos, E. (2012). Sexual dimorphism in the North African amphisbaenian *Trogonophis wiegmanni*. *J. Herpetol.* 46, 338–341. doi: 10.1670/10-286

Martínez, P. A., and Bidau, C. J. (2016). A re-assessment of Rensch's rule in tuco-tucos (Rodentia: Ctenomyidae: *Ctenomys*) using a phylogenetic approach. *Mamm. Biol.* 81, 66–72. doi: 10.1016/j.mambio.2014.11.008

Mauk, C. L., Houck, M. A., and Bradley, R. D. (1999). Morphometric analysis of seven species of pocket gophers *(Geomys)*. *J. Mammal.* 80, 499–511. doi: 10.2307/1383296

Moshkin, M. P., Novikov, E. A., and Petrovski, D. V. (2001). Seasonal changes of thermoregulation in the mole vole *Ellobius talpinus*. *Physiol. Biochem. Zool.* 74, 869–875. doi: 10.1086/324750

Oosthuizen, M. K. (2008). *The Reproductive Biology of the Solitary Cape Mole-Rat, Georychus capensis and the Social Natal mole-rat, Cryptomys hottentotus Natalensis (Rodentia : Bathyergidae)*. Ph.D. thesis. Pretoria: University of Pretoria.

Patzenhauerová, H., Bryja, J., and Šumbera, R. (2010). Kinship structure and mating system in a solitary subterranean rodent, the silvery mole-rat. *Behav. Ecol. Sociobiol.* 64, 757–767. doi: 10.1007/s00265-009-0893-4

Patzenhauerová, H., Šklíba, J., Bryja, J., and Šumbera, R. (2013). Parentage analysis of Ansell's mole-rat family groups indicates a high reproductive skew despite relatively relaxed ecological constraints on dispersal. *Mol. Ecol.* 22, 4988–5000. doi: 10.1111/mec.12434

Rathbun, G. B., and Rathbun, C. D. (2006). Social monogamy in the noki or dassie-rat (*Petromus typicus*) in Namibia. *Mamm. Biol.* 71, 203–213. doi: 10.1016/j.mambio.2006.01.008

Rensch, B. (1950). Die Abhängigkeit der relativen Sexualdifferenz von der Körpergröße. *Bonner Zool. Beiträge* 1, 58–69.

Rensch, B. (1960). *Evolution Above the Species Level*. New York, NY: Columbia University Press.

Revell, L. J. (2012). phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223. doi: 10.1111/j.2041-210x.2011.00169.x

Rohlf, F. J. (2018). *tpsDig2, Digitize Landmarks and Outlines, Version 2.31*. New York, NY: Department of Ecology and Evolution, State University of New York.

RStudio Team (2020). *RStudio: Integrated Development for R*. Boston, MA: RStudio, PBC.

Scharff, A. (1998). *Systematik und Verhaltensökologie sambischer Sandgräber (Bathyergidae, Rodentia)*. Ph.D. thesis. Duisburg: University of Essen.

Schielke, C. K. M., Burda, H., Henning, Y., Okrouhlík, J., and Begall, S. (2017). Higher resting metabolic rate in long-lived breeding Ansell's mole-rats (*Fukomys anselli*). *Front. Zool.* 14:45. doi: 10.1186/s12983-017-0229-6

Schulte-Hostedde, A. (2007). "Chapter 10 Sexual size dimorphism in rodents," in *Rodent Societies: An Ecological and Evolutionary Perspective*, eds J. O. Wolff and P. W. Sherman (Chicago, IL: The University of Chicago Press), 115–128.

Shine, R. (1991). Intersexual dietary divergence and the evolution of sexual dimorphism in snakes. *Am. Nat.* 138, 103–122. doi: 10.1086/285207

Sichilima, A. M., Bennett, N. C., and Faulkes, C. G. (2011). Field evidence for colony size and aseasonality of breeding and in Ansell's mole-rat, *Fukomys anselli* (Rodentia: Bathyergidae). *Afr. Zool.* 46, 334–339. doi: 10.1080/15627020.2011.11407506

Sichilima, A. M., Faulkes, C. G., and Bennett, N. C. (2008). Field evidence for aseasonality of reproduction and colony size in the Afrotropical giant mole-rat *Fukomys mechowii* (Rodentia: Bathyergidae). *Afr. Zool.* 43, 144–149. doi: 10.3377/1562-7020-43.2.144

Šklíba, J., Lövy, M., Burda, H., and Šumbera, R. (2016). Variability of space-use patterns in a free living eusocial rodent, Ansell's mole-rat indicates age-based rather than caste polyethism. *Sci. Rep.* 6:37497.

Smith, R. J. (1999). Statistics on sexual size dimorphism. *J. Hum. Evol.* 36, 423–459. doi: 10.1006/jhev.1998.0281

Spinks, A. C., Bennett, N. C., and Jarvis, J. U. M. (2000). A comparison of the ecology of two populations of the common mole-rat, *Cryptomys hottentotus hottentotus*: the effect of aridity on food, foraging and body mass. *Oecologia* 125, 341–349. doi: 10.1007/s004420000460

Su, J., Hegab, I. M., Ji, W., and Nan, Z. (2018). Function-related drivers of skull morphometric variation and sexual size dimorphism in a subterranean rodent, plateau zokor (*Eospalax baileyi*). *Ecol. Evol.* 8, 4631–4643. doi: 10.1002/ece3.3986

Šumbera, R., Burda, H., and Chitaukali, W. N. (2003). Reproductive biology of a solitary subterranean bathyergid rodent, the silvery mole-rat (*Heliophobius argenteocinereus*). *J. Mammal.* 84, 278–287. doi: 10.1644/1545-1542(2003)084<0278:rboass>2.0.co;2

Šumbera, R., Mazoch, V., Patzenhauerová, H., Lövy, M., Šklíba, J., Bryja, J., et al. (2012). Burrow architecture, family composition and habitat characteristics of the largest social African mole-rat: the giant mole-rat constructs really giant burrow systems. *Acta Theriol.* 57, 121–130. doi: 10.1007/s13364-011-0059-4

Taylor, P. J., Jarvis, J. U. M., Crowe, T. M., and Davies, K. C. (1985). Age determination in the Cape molerat *Georychus capensis*. *S. Afr. J. Zool.* 20, 261–267.

Thomas, H. G., Bateman, P. W., Scantlebury, M., and Bennett, N. C. (2012). Season but not sex influences burrow length and complexity in the non-sexually dimorphic solitary Cape mole-rat (Rodentia: Bathyergidae). *J. Zool.* 288, 214–221. doi: 10.1111/j.1469-7998.2012.00944.x

Thorley, J., Katlein, N., Goddard, K., Zöttl, M., and Clutton-Brock, T. (2018a). Reproduction triggers adaptive increases in body size in female mole-rats. *Proc. R. Soc. B Biol. Sci.* 285:20180897. doi: 10.1098/rspb.2018.0897

Thorley, J., Mendonça, R., Vullioud, P., Torrents-Ticó, M., Zöttl, M., Gaynor, D., et al. (2018b). No task specialization among helpers in Damaraland mole-rats. *Anim. Behav.* 143, 9–24. doi: 10.1016/j.anbehav.2018.07.004

Torrents-Ticó, M., Bennett, N. C., Jarvis, J. U. M., and Zöttl, M. (2018). Sex differences in timing and context of dispersal in Damaraland mole-rats (*Fukomys damarensis*). *J. Zool.* 306, 252–257. doi: 10.1111/jzo.12602

Van Daele, P. A., Blonde, P., Stjernstedt, R., and Adriaens, D. (2013). A new species of African mole-rat (*Fukomys*, Bathyergidae, Rodentia) from the Zaire-Zambezi watershed. *Zootaxa* 3636, 171–189. doi: 10.11646/zootaxa.3636.1.7

Van Daele, P. A. A. G., Desmet, N., Šumbera, R., and Adriaens, D. (2019). Work behaviour and biting performance in the cooperative breeding Micklem's mole-rat *Fukomys micklemi* (Bathyergidae, Rodentia). *Mamm. Biol.* 95, 69–76. doi: 10.1016/j.mambio.2019.02.002

van Jaarsveld, B., Bennett, N. C., Hart, D. W., and Oosthuizen, M. K. (2019). Locomotor activity and body temperature rhythms in the Mahali mole-rat (*C. h. mahali*): the effect of light and ambient temperature variations. *J. Therm. Biol.* 79, 24–32. doi: 10.1016/j.jtherbio.2018.11.013

van Rensburg, L. J., Chimimba, C. T., van der Merwe, M., Schoeman, A. S., and Bennett, N. C. (2004). Relative age and reproductive status in *Cryptomys hottentotus pretoriae* (Rodentia: Bathyergidae) from South Africa. *J. Mammal.* 85, 1225–1232. doi: 10.1644/ber-113.1

Visser, J. H., Bennett, N. C., and Jansen van Vuuren, B. (2017). Distributional range, ecology, and mating system of the Cape mole-rat (*Georychus capensis*) family Bathyergidae. *Can. J. Zool.* 95, 713–726. doi: 10.1139/cjz-2017-0016

Visser, J. H., Bennett, N. C., and Jansen van Vuuren, B. (2019). Phylogeny and biogeography of the African Bathyergidae: a review of patterns and processes. *PeerJ* 7:e7730. doi: 10.7717/peerj.7730

Webb, T. J., and Freckleton, R. P. (2007). Only half right: species with female-biased sexual size dimorphism consistently break Rensch's rule. *PLoS One* 2:e897. doi: 10.1371/journal.pone.0000897

Yeboah, S., and Dakwa, K. B. (2002). Colony and social structure of the Ghana mole-rat (*Cryptomys zechi*, Matchie) (Rodentia: Bathyergidae). *J. Zool.* 256, 85–91. doi: 10.1017/s0952836902000110

Young, A. J., and Bennett, N. C. (2013). Intra-sexual selection in cooperative mammals and birds: why are females not bigger and better armed? *Philos. Trans. R. Soc. B Biol. Sci.* 368:20130075. doi: 10.1098/rstb.2013.0075

Zöttl, M., Vullioud, P., Mendonça, R., Torrents Ticó, M., Gaynor, D., Mitchell, A., et al. (2016). Differences in cooperative behavior among Damaraland mole rats are consequences of an age-related polyethism. *Proc. Natl. Acad. Sci. U.S.A.* 113:10382. doi: 10.1073/pnas.1607885113

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

Supplementary Table 1 (continues on following page): Linear skull measurements and hindfoot lengths of *Fukomys anselli* specimens used in this study (in mm). Yellow mark: Measurement was taken from the left side of the skull. Red mark: Measurement was estimated using the LOST package (Arbour and Brown, 2020).

Specimen	Sex	Status	Age (years)	DIAS	PALA	UPAL	PREW	BULL	BULW	BASI	SKUL	IORW	ZYPW	ZYAW
9	f	repro	10	10.6	19.23	6.15	2.12	8.26	6.31	27.34	30.96	10.42	4	22.57
12	f	repro	4	12.18	21.71	6.07	2.15	8.55	6.23	29.8	34.51	10.11	4.41	23.83
18	f	repro	12	11.47	21.29	6.84	2.4	8.35	6.49	29.69	35.32	10.65	3.56	23.9
22	f	repro	10.5	13.23	21.49	5.26	1.75	8.69	6.71	30.3	34.35	10.46	3.9	23.92
24	f	repro	5	13.21	22.23	6.17	2.02	9.22	6.97	31.32	36.15	10.65	4.01	25.57
27	f	repro	10	12.23	20.87	5.61	1.92	8.59	6.6	29.07	33.58	9.83	3.7	22.89
32	f	repro	9	12.13	19.78	4.17	1.88	8.2	5.98	28.47	32.25	9.04	3.36	22.72
36	f	repro	5	11.24	19.55	5.87	2	8.41	5.96	27.39	31.37	9.97	3.89	22.94
41	f	repro	14	12.15	21.58	5.79	2.42	9.76	6.5	29.68	34.75	10.89	4.47	25.22
42	f	repro	10.75	13.34	22.03	5.55	1.94	9.47	7.3	30.89	35.6	10.31	4.01	24.88
3	m	repro	11	14.38	24.36	5.92	2.51	10.24	7.32	33.3	37.14	11.93	4.85	27.81
5	m	repro	9	13.72	23.51	5.81	2.22	9.28	6.93	32.17	35.21	11.3	4.72	25.32
10	m	repro	10	14.25	22.95	5.7	1.98	8.96	6.78	31.78	36.29	11.6	3.87	26.22
14	m	repro	4	11.69	21.54	6.6	2.12	8.93	6.7	29.98	34.12	10.92	4.23	26.35
21	m	repro	13	13.68	23.86	7.49	2.24	9.52	6.68	33.32	37.82	11.05	4.23	27.26
23	m	repro	11	12.85	22.91	5.8	2	9.07	6.93	31.06	34.9	11.18	4.11	26.28
26	m	repro	7	13.94	23.25	6.08	2.04	9.55	7.1	32.02	35.63	11.95	4.47	27.4
29	m	repro	16	15.96	25.72	5.67	2.14	10.51	7.5	34.82	38.39	12.89	5.27	29.21
34	m	repro	4	13.54	22.11	5.40	1.96	8.86	6.07	31.02	35.17	10.91	4.43	26.07
43	m	repro	10	15.83	25.31	6.33	2.06	9.45	7.02	34.93	39.05	13.23	4.1	28.14
6	f	non-repro	2.5	11.2	19.6	5.9	2.5	8.17	5.8	27.53	31.7	9.33	3.85	21.19
7	f	non-repro	2.5	11.1	19.93	5.95	2.25	7.49	5.71	27.24	31.94	10.23	3.6	21.32
8	f	non-repro	3	12.43	21.18	5.94	2.17	8.46	5.99	28.83	33.29	9.43	3.5	22.9
13	f	non-repro	3.5	13.05	21.77	6.18	2.06	9.13	7.02	29.75	33.91	10.41	4.26	23.67
16	f	non-repro	2.5	11.92	21.13	5.95	1.93	8.71	6.26	29.12	33.73	10.02	4.06	23.85
25	f	non-repro	4.5	12.48	21.99	6	1.85	8.83	6.61	30.36	34.94	10.42	4.53	24.19
31	f	non-repro	5	11.75	19.79	6.05	1.83	8.59	6.58	28.08	31.94	9.91	3.53	22.81
33	f	non-repro	5	12.64	21.99	6.31	1.81	9.38	7.3	30.64	35.32	12.09	4.02	26.65
40	f	non-repro	3	13.82	23.13	6.51	2.06	9.4	7.6	31.36	36.53	11.14	3.94	25.35
45	f	non-repro	4.5	12.61	21.63	6.52	1.92	8.56	6.51	30.04	34.72	10.83	3.93	23.97
11	m	non-repro	4	13.36	23.37	6.32	2.13	8.89	6.57	32.61	36	12.15	4.09	25.88
15	m	non-repro	3	13.45	23.03	6.41	2.32	8.83	6.61	31.83	35.92	10.91	4.21	26.64
17	m	non-repro	4	13.94	23.44	5.93	2.09	9.14	6.61	32.76	37.16	11.65	4.58	27.35
20	m	non-repro	3	13.87	24.21	6.43	2.38	8.47	6.27	32.49	36.89	11.4	4.32	26.5
28	m	non-repro	13	15.85	25.49	6.05	2.03	9.76	7.44	34.83	39.05	13.43	4.68	28.35
35	m	non-repro	5.5	13.94	23.14	5.19	2.19	9.6	6.93	31.03	35.45	11.35	4.18	25.96
37	m	non-repro	2.75	14.32	24.52	6.67	2.07	9.32	7.12	33.76	37.22	13.35	4.49	27.09
38	m	non-repro	9	13.36	21.94	6.23	2.11	8.95	6.38	31.68	35.69	10.96	4.25	25.48
39	m	non-repro	4.25	14.57	23.84	5.72	1.87	9.39	6.66	32.82	36.82	11.75	4.17	26.85
44	m	non-repro	12	12.82	22.61	5.81	1.9	9.56	7.11	31.44	36.38	12.24	4.29	27

Supplementary Table 1 (continued)

Specimen	Sex	Status	BASW	PAPW	FIOL	PZMW	INCW	ROSL	SKUH	CONW	SOCC	MANL	MANH	CONH	ANGL	Hindfoot
9	f	repro	6.82	13.45	1.54	1.27	5.22	15.54	10.62	7.25	5.34	21.8	10.03	8.38	15.05	18.85
12	f	repro	10.1	14.52	1.53	1.37	5.7	16.31	11.4	7.83	5.86	24.13	11.77	9.03	16.31	22.3
18	f	repro	9.41	14.32	1.49	1.24	6.52	15.58	12.06	7.78	6	23.32	11.86	8.77	15.15	21.4
22	f	repro	7.27	13.41	1.43	1.4	5.79	16.8	11.78	7.45	5.17	23.95	10.94	8.56	16.44	21
24	f	repro	7.7	14.85	1.2	1.41	6.42	17.4	11.96	8.05	6.18	24.72	10.71	9.25	17.44	23.15
27	f	repro	6.65	13.44	1.16	0.96	5.25	16.78	11.01	7.35	5.47	23.01	10.23	8	14.01	21.6
32	f	repro	7.78	12.51	1.28	1.13	5.56	14.01	10.32	7.36	5.68	21.88	10.05	7.8	14.11	21.1
36	f	repro	7.2	13.88	1.28	1.18	5.64	15.34	10.52	7.39	5.49	22.31	9.81	7.64	14.91	19.7
41	f	repro	7.06	14.36	1.49	1.42	6.02	17.64	11.84	8.08	6.2	25.26	11.31	9.76	16.77	22.3
42	f	repro	8.15	14.62	1.25	1.11	6.23	17.12	11.68	8.1	5.83	25.06	10.49	8.22	15.83	22.42
3	m	repro	8.04	15.14	3.42	1.5	6.33	18.44	12.27	8.47	7.1	26.64	12.88	9.55	17.31	22.65
5	m	repro	7.82	14.82	1.94	1.42	6.4	17.48	12.65	7.36	6.32	25.8	11.53	9.9	16.68	21.85
10	m	repro	8.16	14.52	1.91	1.79	6.42	17.32	12.27	7.92	6.06	25.14	13.5	10.43	17.08	24.26
14	m	repro	9.36	14.74	1.82	1.68	6.4	15.49	11.8	7.48	6.72	23.59	12.03	9.81	17.3	23.35
21	m	repro	7.99	14.51	1.48	1.83	7.09	18.55	12.66	8.22	6.21	26.51	11.97	10.23	17.55	23.07
23	m	repro	7.84	14.27	1.18	1.39	6.89	16.92	12.44	7.5	6.75	25.16	11.92	10.14	17.18	21.8
26	m	repro	7.55	14.79	2.07	1.67	6.8	17.14	12.66	8.04	6.25	25.59	12.78	10.93	17.39	23.37
29	m	repro	7.71	16.44	1.89	1.81	7.68	19.21	13.61	7.83	5.81	28.82	15.8	10.43	19.37	22.5
34	m	repro	8.08	14.45	0.98	1.44	6.45	16.31	11.62	7.24	7.27	24.77	11.92	9.96	17.24	21.5
43	m	repro	8.02	15.68	1.06	2.29	7.93	18.59	14.3	8.06	7.35	27.55	13.34	10.43	19.04	24.7
6	f	non-repro	7.76	14.97	1.45	1.31	4.73	14.75	9.93	7.82	5.35	21.93	10.01	8.87	14.4	20.14
7	f	non-repro	7.4	13	1.86	1.49	5.31	15.78	10.64	7.36	5.75	21.44	10.3	8.39	13.46	19.05
8	f	non-repro	7.52	14.81	1.25	1.48	5.34	15.69	10.77	7.62	5.58	22.6	11.46	8.75	16.27	22.4
13	f	non-repro	8.93	15.23	2.18	1.1	5.73	15.12	11.65	8.02	5.31	23.9	11.86	9.4	16.71	21.21
16	f	non-repro	10.7	15.84	1.56	1.28	5.8	15.85	10.81	7.78	5.01	24.21	10.78	9.38	16.79	21.13
25	f	non-repro	7.66	12.96	1.7	1.2	6.05	16.23	11.86	7.94	4.94	24.54	10.67	9.03	15.3	20.3
31	f	non-repro	6.9	13.71	1.19	1.21	5.32	14.48	10.12	7.27	5.68	22.58	10.47	8.43	14.66	20
33	f	non-repro	7.88	14.33	1.61	1.7	5.99	16.07	11.41	8.28	6.29	25.36	12.48	9.62	18.4	21.6
40	f	non-repro	7.84	14.48	1.57	1.41	6.2	16.11	11.35	8.13	6.64	24.48	11.21	8.88	16.42	23.78
45	f	non-repro	7.94	14.22	1.53	1.34	5.89	16.05	11.15	7.63	6.14	23.16	11.08	8.84	15.53	22.6
11	m	non-repro	7.11	14.35	1.49	1.76	6.43	18.22	12.32	7.04	6.62	25.39	11.6	9.36	18	22.03
15	m	non-repro	10.04	13.99	1.41	1.64	6.77	16.62	11.46	7.41	6.37	25.29	12.63	10.67	17.16	20.94
17	m	non-repro	9.13	15.24	2.15	1.97	6.46	18.65	12.98	8.43	6.13	26.55	13.28	11.1	19.43	23.04
20	m	non-repro	7.54	14.78	1.24	1.67	6.62	18.05	11.34	7.91	7.34	25.57	11.78	9.64	17.86	23.47
28	m	non-repro	7.29	15.41	2.12	1.72	7.5	19.17	13.65	8.08	6.98	27.54	13.65	10.38	19.96	24.6
35	m	non-repro	7.08	13.96	1.46	1.31	6.45	17.91	12.05	7.62	6.51	25.61	12.15	9.76	17.55	20
37	m	non-repro	7.6	15.08	1.67	1.81	6.79	18.52	12.54	7.98	7.78	26.14	13.25	9.88	17.22	23
38	m	non-repro	8.46	14.93	1.68	1.7	6.39	16.81	12.14	7.57	5.58	25.11	12.79	9.12	17.62	22.6
39	m	non-repro	8.09	15.75	1.77	1.84	6.74	18.05	12.52	8.45	7.25	25.24	13.37	9.57	17.71	23.45
44	m	non-repro	7.41	15.04	1.4	1.37	6.92	17.4	13.84	7.33	6.6	25.56	12.87	9.42	16.4	21.4

Supplementary Table 2: Overview and definitions of point landmarks used to characterize skull traits in *Fukomys anselli*.

Dorsal cranial landmark	**Description**
1	Posteriormost median point of skull
2	Point of fusion between sagittal crest and nuchal crest
3	Median point of suture between frontals and parietals
4	Median point of suture between frontals and nasals
5	Anteriormost point of intranasal suture
6	Anteriormost visible median point of premaxillary
7	Lateral point of incisor erupting from the premaxillary
8	Anterior rim of infraorbital foramen
9	Deepest indentation of bony orbit
10	Most anterolateral point of nuchal crest
11	Posteriormost point of paroccipital process
12	Foramen for ramus temporalis of the facial cranial nerve
13	Deepest visible indentation of frontals
Ventral cranial landmark	**Description**
1	Posteriormost median point of skull
2	Anteriormost point of foramen magnum
3	Median point of suture between basioccipital and sphenoid
4	Staphylion
5	Posteriormost point of foramen incisivum
6	Anteriormost visible median point of premaxillary
7	Lateral point of incisor erupting from the premaxillary
8	Anteriormost point of dental row
9	Posteriormost point of dental row
10	Lateral most point of bony protrusion at orbit
11	Most lateral point of auditory meatus
12	Posteriormost point of paroccipital process
13	Tip of styliform process of the tympanic bulla

Supplementary Table 2 (continued)

Mandibular landmark	Description
1	Apical tip of coronoid process
2	Ventralmost point of arch connecting the coronoid apex and the glenoid.
3	Anteriormost point of the condyle
4	Highest (dorsal) point of the condyle
5	Point marking the fusion of the corpus mandibulae and the angular process posteriorly.
6	Posterior tip of the angular process
7	Ventral margin of the angular process at the level of the highest point of the condyle
8	Point demarcating the anterior intersection of the angular process and the corpus mandibulae from a lateral view.
9	Ventral anterior point of incisor erupting from the mandible
10	Dorsal anterior point of incisor erupting from the mandible
11	Anteriormost point of bony alveolar margin of the premolar
12	Point demarcating the anterior intersection of the coronoid process and the corpus mandibulae from a lateral view

Supplementary Table 3: Variable contributions (%) and eigenvalues of principal components computed for the shape of the dorsal and ventral cranium as well as for the mandible of *Fukomys anselli*. Individual tables are itemized here for greater clarity.

Dorsal (variable contributions %)									
Landmark	PC 1	PC 2	PC 3	PC 4	Landmark	PC 1	PC 2	PC 3	PC 4
1.X	1.661	4.034	0.225	0.339	23.Y	0.150	1.382	0.067	0.839
1.Y	0.475	0.048	0.307	0.075	24.X	0.547	0.189	1.759	2.912
2.X	0.931	0.844	0.580	4.456	24.Y	0.456	1.646	0.172	0.743
2.Y	0.266	0.000	0.849	0.002	25.X	0.479	0.454	1.687	2.970
3.X	1.846	2.140	1.092	0.406	25.Y	0.993	1.400	0.161	1.092
3.Y	0.013	1.729	1.840	0.237	26.X	0.392	0.886	1.417	3.188
4.X	3.716	2.571	51.221	1.902	26.Y	1.357	1.395	0.098	1.220
4.Y	0.004	1.430	6.597	0.218	27.X	0.276	1.465	1.075	3.345
5.X	0.783	1.219	0.068	1.084	27.Y	1.721	1.475	0.134	1.503
5.Y	1.769	0.248	0.089	0.114	28.X	0.157	2.122	0.732	3.451
6.X	0.209	2.238	1.196	1.292	28.Y	2.083	1.379	0.307	1.862
6.Y	1.216	0.117	2.219	0.055	29.X	0.072	2.913	0.392	3.643
7.X	0.247	5.195	0.003	0.059	29.Y	2.069	1.414	0.478	1.784
7.Y	0.761	0.423	1.901	0.306	30.X	0.018	3.377	0.115	3.815
8.X	0.018	0.001	0.180	1.327	30.Y	1.688	0.777	0.550	1.299
8.Y	0.054	0.867	0.017	0.200	31.X	0.007	3.822	0.007	3.506
9.X	0.000	0.168	1.485	4.600	31.Y	1.765	0.375	0.790	1.011
9.Y	0.808	1.673	0.010	0.806	32.X	0.080	3.700	0.093	2.653
10.X	0.016	0.206	0.406	0.194	32.Y	1.749	0.078	0.600	1.098
10.Y	0.245	0.847	0.024	0.009	33.X	0.253	2.944	0.261	2.068
11.X	1.801	2.469	0.235	0.414	33.Y	1.578	0.016	0.514	0.833
11.Y	0.869	0.014	0.329	0.211	34.X	0.416	2.083	0.404	1.826
12.X	1.558	0.098	0.462	2.044	34.Y	1.111	0.195	0.270	0.251
12.Y	1.637	1.515	2.380	0.003	35.X	0.262	1.046	0.401	1.312
13.X	2.205	0.005	0.002	0.226	35.Y	0.333	0.444	0.055	0.002
13.Y	2.064	3.666	0.481	0.910	36.X	0.032	0.109	0.174	0.507
14.X	0.120	0.248	0.011	2.221	36.Y	0.005	0.500	0.007	0.108
14.Y	1.011	1.916	0.032	0.029	37.X	0.099	0.451	0.321	0.065
15.X	0.560	0.212	0.086	1.912	37.Y	0.234	0.282	0.182	0.446
15.Y	0.773	1.463	0.099	0.010	38.X	0.130	0.400	0.316	0.086
16.X	0.943	0.219	0.158	1.500	38.Y	0.233	0.308	0.178	0.458
16.Y	0.802	1.068	0.135	0.113	39.X	2.362	0.132	0.187	1.961
17.X	1.198	0.254	0.096	0.998	39.Y	0.387	0.180	0.098	0.514
17.Y	0.769	0.721	0.031	0.282	40.X	4.922	1.272	0.001	2.518
18.X	1.681	0.198	0.002	0.306	40.Y	0.346	0.046	0.019	0.342
18.Y	0.428	0.322	0.006	0.540	41.X	7.534	3.219	0.309	2.151
19.X	1.319	0.086	0.042	0.108	41.Y	0.238	0.059	0.001	0.218
19.Y	0.230	0.048	0.072	0.988	42.X	9.131	4.641	1.085	1.401
20.X	1.440	0.072	0.112	0.399	42.Y	0.242	0.129	0.024	0.263
20.Y	0.056	0.000	0.139	0.660	43.X	8.530	4.453	1.815	0.640
21.X	1.312	0.029	0.252	0.921	43.Y	0.287	0.014	0.201	0.560
21.Y	0.003	0.224	0.209	0.763	44.X	5.033	1.993	1.699	0.159
22.X	0.965	0.022	0.629	1.680	44.Y	0.250	0.033	0.714	0.673
22.Y	0.012	0.737	0.178	0.822	45.X	0.018	3.001	0.051	0.032
23.X	0.689	0.050	1.395	2.551	45.Y	0.491	0.143	2.201	0.384

Supplementary Table 3 (continued)

Ventral (variable contributions %)									
Landmark	PC1	PC2	PC3	PC4	Landmark	PC1	PC2	PC3	PC4
1.X	0.049	2.103	4.929	1.355	23.Y	0.760	0.997	0.291	0.790
1.Y	0.311	0.539	0.174	0.813	24.X	0.197	3.987	0.834	0.138
2.X	0.350	2.203	0.008	0.368	24.Y	0.714	1.304	0.736	1.185
2.Y	0.711	0.698	0.000	0.882	25.X	0.631	3.135	0.980	0.000
3.X	0.003	2.737	1.031	1.205	25.Y	0.274	2.068	0.972	1.315
3.Y	0.283	0.705	0.040	1.252	26.X	1.414	2.256	1.056	0.183
4.X	0.001	0.410	1.825	0.563	26.Y	0.113	2.902	0.906	1.629
4.Y	0.232	0.711	0.166	0.958	27.X	2.400	1.578	1.279	0.729
5.X	0.000	0.004	0.780	0.000	27.Y	0.015	3.082	1.579	1.843
5.Y	0.125	0.042	0.245	0.479	28.X	3.518	0.929	1.422	1.456
6.X	0.065	0.033	0.864	1.075	28.Y	0.006	3.335	2.263	2.226
6.Y	0.005	0.821	1.862	0.046	29.X	4.632	0.548	1.299	2.212
7.X	0.000	1.062	1.263	0.111	29.Y	0.087	2.646	2.263	2.422
7.Y	0.065	1.125	0.129	0.002	30.X	6.024	0.269	1.219	2.537
8.X	0.000	0.020	3.797	0.523	30.Y	0.136	1.772	2.606	3.004
8.Y	0.120	0.104	0.629	0.069	31.X	6.834	0.091	1.177	2.559
9.X	0.500	1.394	0.384	0.000	31.Y	0.251	0.960	2.942	2.827
9.Y	0.046	0.249	0.176	0.389	32.X	6.437	0.011	1.033	2.129
10.X	0.759	0.507	0.019	3.954	32.Y	0.365	0.369	2.798	2.458
10.Y	0.321	0.212	0.983	0.969	33.X	4.845	0.000	0.858	1.325
11.X	0.729	0.155	0.528	0.277	33.Y	0.564	0.024	2.396	1.976
11.Y	0.266	0.882	1.619	0.132	34.X	3.689	0.001	0.565	0.656
12.X	0.000	3.110	0.231	3.296	34.Y	0.411	0.117	1.822	1.140
12.Y	2.373	0.085	1.430	1.599	35.X	2.110	0.000	0.204	0.195
13.X	0.100	0.985	0.901	0.198	35.Y	0.231	0.641	1.075	0.377
13.Y	1.273	0.375	0.050	0.153	36.X	0.961	0.059	0.018	0.057
14.X	0.605	0.208	1.563	0.000	36.Y	0.083	1.423	0.361	0.010
14.Y	0.860	0.809	4.529	0.013	37.X	0.042	0.071	0.230	0.000
15.X	0.421	0.923	2.045	0.400	37.Y	0.005	1.837	0.005	0.046
15.Y	0.574	0.586	3.419	0.011	38.X	0.035	0.060	0.158	0.006
16.X	0.166	1.772	2.374	1.100	38.Y	0.004	1.819	0.006	0.057
16.Y	0.414	0.453	2.052	0.146	39.X	0.574	0.013	2.143	3.658
17.X	0.055	2.243	2.375	1.651	39.Y	0.013	1.291	0.081	0.188
17.Y	0.250	0.532	0.986	0.656	40.X	2.847	0.004	4.009	5.621
18.X	0.151	2.735	1.326	2.641	40.Y	0.012	1.474	0.040	0.388
18.Y	0.044	0.590	0.193	1.101	41.X	6.553	0.023	4.126	5.828
19.X	0.418	2.381	0.285	1.390	41.Y	0.013	1.502	0.004	0.189
19.Y	0.013	0.620	0.008	1.784	42.X	10.555	0.174	3.015	4.798
20.X	0.251	2.598	0.333	1.196	42.Y	0.146	1.521	0.185	0.011
20.Y	0.016	0.162	0.013	1.319	43.X	11.254	0.499	1.293	2.862
21.X	0.127	3.231	0.494	0.880	43.Y	0.134	1.313	0.127	0.001
21.Y	0.116	0.039	0.043	1.090	44.X	5.841	0.920	0.030	0.718
22.X	0.041	3.312	0.670	0.505	44.Y	0.414	1.862	0.184	0.005
22.Y	0.458	0.194	0.111	1.061	45.X	0.162	1.110	1.607	0.208
23.X	0.007	4.215	0.771	0.411	45.Y	0.025	1.131	0.152	0.016

Supplementary Table 3 (continued)

Mandible (variable contributions %)				
Landmark	PC1	PC2	PC3	PC4
1.X	7.519	3.849	26.239	2.596
1.Y	6.864	8.108	10.486	0.004
2.X	0.071	0.453	1.838	4.981
2.Y	4.350	1.899	0.025	0.058
3.X	1.195	0.994	0.001	23.035
3.Y	0.306	0.110	0.058	1.690
4.X	0.244	5.312	3.323	5.929
4.Y	0.001	2.615	0.296	0.555
5.X	0.269	2.138	0.059	1.099
5.Y	0.223	1.112	7.133	0.537
6.X	7.662	5.529	1.645	23.943
6.Y	0.179	11.436	1.253	1.885
7.X	39.204	22.203	0.208	1.346
7.Y	1.960	0.478	10.371	1.575
8.X	2.345	1.034	3.952	1.649
8.Y	0.783	2.001	0.697	3.645
9.X	7.397	2.077	7.008	0.001
9.Y	15.762	11.948	2.533	3.030
10.X	0.107	3.069	0.118	1.758
10.Y	0.420	0.018	4.110	0.340
11.X	1.889	0.945	1.752	0.193
11.Y	0.712	5.743	10.732	0.011
12.X	0.010	0.001	5.907	18.859
12.Y	0.528	6.926	0.255	1.281

Supplementary Table 3 (continued)

Dorsal		Ventral		Mandible	
PC	Eigenvalue	PC	Eigenvalue	PC	Eigenvalue
1	0.000399257	1	0.0005157	1	0.0009025
2	0.000299797	2	0.00036743	2	0.0006841
3	0.00022494	3	0.00025115	3	0.0003402
4	0.000178775	4	0.00016959	4	0.0002982
5	0.000127124	5	0.00013257	5	0.0001838
6	0.000108028	6	9.7835E-05	6	0.0001711
7	9.88039E-05	7	8.4783E-05	7	0.0001572
8	5.86901E-05	8	4.6026E-05	8	0.0001063
9	5.39558E-05	9	4.5003E-05	9	0.0000800
10	4.64098E-05	10	4.1754E-05	10	0.0000655
11	3.78483E-05	11	3.4796E-05	11	0.0000611
12	2.79719E-05	12	3.2203E-05	12	0.0000415
13	2.66274E-05	13	2.2825E-05	13	0.0000380
14	2.24459E-05	14	2.2604E-05	14	0.0000246
15	1.96848E-05	15	1.9621E-05	15	0.0000216
16	1.68565E-05	16	1.6994E-05	16	0.0000179
17	1.41245E-05	17	1.4338E-05	17	0.0000134
18	1.05539E-05	18	1.0223E-05	18	0.0000080
19	9.71797E-06	19	8.954E-06	19	0.0000040
20	7.67733E-06	20	8.3284E-06	20	0.0000023
21	7.44031E-06	21	7.4136E-06	21	2.44026E-32
22	5.86161E-06	22	5.9488E-06	22	4.82375E-33
23	5.56262E-06	23	5.3462E-06	23	9.85487E-34
24	4.89217E-06	24	4.5848E-06	24	4.2279E-34
25	4.14218E-06	25	4.1519E-06		
26	2.71173E-06	26	3.1711E-06		
27	2.37066E-06	27	2.5328E-06		
28	2.21535E-06	28	2.5025E-06		
29	1.79988E-06	29	1.8298E-06		
30	1.62062E-06	30	1.4405E-06		
31	1.09804E-06	31	1.0723E-06		
32	1.04782E-06	32	1.0018E-06		
33	9.29485E-07	33	9.3348E-07		
34	7.18156E-07	34	5.8798E-07		
35	6.04846E-07	35	5.5128E-07		
36	5.01097E-07	36	3.8125E-07		
37	4.39844E-07	37	3.127E-07		
38	3.12611E-07	38	1.6774E-07		
39	1.55256E-07	39	1.5639E-07		
40	1.0606E-33	40	9.7255E-34		

Discussion

I feel the need to revisit and discuss two particular issues treated in Chapter 2.5 in greater depth here, given recent publications on social dynamics in common mole-rats and retrospective considerations on the manuscript's content: First, the scaling of sexual size dimorphism (SSD) in *Fukomys* and second, the selective pressures underlying sexual dimorphism in said genus.

We have argued that SSD in *Fukomys*, and potentially other bathyergids, scales conforming to Rensch's rule but we also pointed out an important caveat to our scaling analysis, namely that different authors do not agree about when to classify an individual of a certain species as an adult. To quantify SSD, it is of course crucial to exclusively rely on data from adult individuals to avoid biases. For this study, we have accepted the age group classifications presented in the papers from which we extracted body mass data for our analyses. Due to the fact that raw data were in most cases unavailable, we could not exclude specific information in cases where we considered the determination of adult age classes doubtful.

I want to detail out problems with age group assignments in the literature, using Ansell's mole-rat as an example (but note that the same issue arose also for, e.g., the Mashona mole-rat – Bennett et al., 1994): Body mass data for this species derived from Sichilima et al. (2011), who reported data for a large sample of wild Ansell's mole-rats (n = 173). The authors classified all animals weighing > 35 g as adult and remark that "age-class categories were based on laboratory observations of growth and development in this species" (Sichilima et al., 2011; the same body mass threshold was adopted by de Bruin et al., 2012 without any justification). Unfortunately, these observations have never been published. Their validity appears highly doubtful, since Ansell's mole-rat is a well-researched species for which excellent growth records are available that are at odds with Sichilima et al. (2011).

Captive Ansell's mole-rats typically grow to a body mass of 35 g at an age of 13 weeks already (Begall & Burda, 1998), and instead of reaching sexual maturity, they are weaned when attaining that respective weight (Burda, 1989). Hence, it is obvious that the values of mean male (63 g) and female (53 g) body mass provided by Sichilima et al. (2011) underestimate the actual figures in this species. Bennett & Burda (2013) provide different body mass data for a slightly smaller sample (n = 140) of presumably wild-caught adult Ansell's mole-rats of unknown providence and report a mean weight of 96 g in males and 79 g in females. Our own records on this species from the laboratory (pers. obs.) align well with that. However, the difference in SSD derived from Sichilima et al. (2011) and Bennett & Burda (2013) is rather small (SSD = 1.19 and 1.21, respectively), which could in parts result from the fact that immature males already start to grow faster than females at an age of 20 weeks, after having reached a weight of just 40 g (Begall & Burda, 1998).

This case illustrates that certain biases must be expected for our analyses on SSD scaling in African mole-rats and calls for a replication of our results based on refined datasets. However, in light of the total evidence on SSD in common mole-rats and personal observations on captive populations of the genus *Fukomys*, I expect the findings of such a replication to match the results communicated in Chapter 2.5 and our assumptions about the occurrence of Rensch's rule among *Fukomys* species to remain valid. As detailed out in Chapter 2.5 there are several other issues to address when it comes to quantifying SSD scaling in bathyergids, including species representation and intraspecific variation potentially influenced by local resource availability (Spinks et al., 2000). Interestingly, intra- and interspecific trends in SSD scaling do not necessarily align among mammals (Martínez & Bidau, 2016). A more extensive study on bathyergid SSD thus surely appears to be warranted and might be of interest for researchers concentrating on other taxa as well because pronounced SSD is surprisingly common among cooperatively breeding mammals in general (Young & Bennett, 2013).

Finally, an enlarged species/population sample for *Fukomys* could yield a robust estimate of the phylogenetic signal in SSD data. As remarked on in Chapter 2.5, we recovered a strong phylogenetic signal for SSD among both *Fukomys* and bathyergids in general. This in turn could indicate lineage specific selective pressures maintaining high or low SSD. However, robust inference of phylogenetic signals by means of Pagel's λ (the selected measure in Chapter 2.5) is only possible with datasets encompassing at least 20 operational taxonomic units (Freckleton et al., 2002), so that the presented findings on phylogenetic signals in the data must be considered tentative.

To explain the presence of pronounced SSD and other sexually dimorphic traits in many *Fukomys* species, we have argued that physical male-male intrasexual competition, including the invasion of established family groups by foreign males, is a critical component of social dynamics in these rodents (Chapter 2.5 but see also Chapter 2.4). This idea is not new (Young & Bennett, 2013) but has received only little attention in the literature so far, although an increasing amount of evidence supports it. Since the publication of Chapter 2.5, new evidence in line with our argumentation became available through the study by Mynhardt et al. (2021). Using capture-recapture data collected over 3 years in the Tswalu Nature Reserve in the southern Kalahari, the authors determined that 32 % (6/19) of dispersing Damaraland mole-rat males studied invade functional family groups rather than establish their own. Some males even showed repeated dispersal from one group into another. Females, on the other hand, tend to live alone after dispersal, waiting for a male to eventually arrive (compare Thorley et al., 2021). Only 12 % (2/17) of dispersed females were recaptured in a foreign colony. Furthermore, males were found to disperse greater distances and, accordingly, to exhibit lower levels of genetic relatedness to each other than females (Mynhardt et al., 2021; see also Burland et al., 2002). Interestingly, Leedale et

al. (2021) recently reported that male Damaraland mole-rats tend to respond more strongly to same-sex odors than to female ones in a forced-choice digging assay. This result is compatible with pronounced male-male antagonism but requires further validation. Besides these novel data, there is evidence that female Damaraland mole-rats are more philopatric than their brothers (Torrents-Ticó et al., 2018; likely also the case in Ansell's mole-rats – Šklíba et al., 2012), which would reduce the amount of available partners for dispersed males and increases the pressure on established male breeders to guard their female partner. This potentially important aspect of *Fukomys* social dynamics was overlooked in Chapter 2.5 but further supports the notion that competition from invading males constitutes an important factor selecting for larger body size and more formidable weaponry in these animals.

Needless to say, however, there are also caveats to our hypothesis, some of which I want to stress here since they have been omitted from Chapter 2.5 for reasons of conciseness. For instance, experiments with captive *Fukomys* do not indicate that males behave more aggressively against same-sex opponents than females (Burda, 1995; Begall et al., 2022; but note that the expression of this behavior could be affected by captivity as noted in Chapter 2.5) and it is still unclear how strongly dispersal and group invasion is male-biased in *Fukomys*. Field studies providing most compelling direct evidence for male colony invasion as a driver for the evolution of SSD in *Fukomys* focus on just one species (*Fukomys damarensis*) and derive from just two localities in the Kalahari, the Dordabis area in Namibia and the Tswalu Nature Reserve in South Africa, were capture-recapture studies have been performed (Young & Bennett, 2013; Torrents-Ticó et al., 2018; Mynhardt et al., 2021). To bolster the hypothesis and our understanding of social dynamics among *Fukomys*, data on sex-specific invasion behavior from additional sites and species are required. The line of argument presented in Chapter 2.5 would predict that at the majority of localities, dispersal and family invasion are strongly male-biased. If empirical evidence fails to support this notion, a new hypothesis would need to be formulated to make sense of the selective drivers underlying SSD in *Fukomys*.

Interestingly, Burda (2022) has very recently offered an alternative explanation for sexually dimorphic traits in these animals that does not rely on sex-specific dispersal or foreign male group invasion. Based on personal observations collected on captive families of Ansell's mole-rats, Burda (2022) argues that the otherwise strict incest avoidance in *Fukomys* family groups does not apply to mother-son pairings. Thus, fathers are at risk to be cuckolded by their sons and brothers may have to reproductively compete with each other while staying in the natal colony. He then hypothesizes that SSD as well as male incisor hypertrophy evolved in the context of such intrafamilial reproductive competition. The male overpowering the others in ritualized incisor fencing would gain access to the mother and monopolize the breeding position. Burda (2022) is aware of the limitations of his observations, being collected solely on captive individuals,

but points out that available field data would be consistent with his notion. Indeed, Young & Bennett (2013) also do not exclude male-male intrafamilial competition as a driver for sexual size dimorphism in Damaraland mole-rats, pointing to male non-breeders producing viable sperm while staying in their natal group.

Thus, there are now two testable hypotheses on what might underlay sexual dimorphism in the genus, one suggesting intrafamilial, the other extrafamilial competition as a selective driver. Given that I have argued emphatically for the latter alternative in Chapter 2.5, I want to take the chance here to pledge for its greater plausibility compared to the notion by Burda (2022). Nevertheless, I want to point out that the two hypotheses are not mutually exclusive. There might be intra- as well as extrafamilial competition driving the evolution of larger body size and more formidable weaponry in male mole-rats. Yet, I am not compelled by the arguments of Burda (2022) and list the three main reasons for my skepticism below.

First, an important issue is that a weakened incest taboo between mother and son has never been described by other researchers working on *Fukomys*. I am unaware of mentions in the literature and the mole-rat husbandry records of the University of Duisburg-Essen (which were of course established and long supervised by Burda himself) provide no evidence for this idea: Boll (2016) evaluated husbandry data collected over 30 years on the laboratory kept lineage of Ansell's mole-rats residing in Essen. Within this time frame, only 15 cases of incest were noted to occur among more than 300 monitored individuals. Of these, just two involved mother and son while eleven occurred exclusively between father and daughter (Boll, 2016). Burda (2022) makes no mention about the number of instances he observed mother-son copulations or courtship and papers cited in support of the hypothesis (Burda, 1995) do not contain relevant data. Whether Burda's original observations are representative for *Fukomys* or even captive Ansell's mole-rats must therefore be questioned. If there was physical competition for mating opportunities between fathers and sons in captive *Fukomys* families, one would also expect that adult males would experience greater stress and thus display higher levels of stress hormones. At least in the giant mole-rat, the most dimorphic species of the genus, this is not the case. Instead, male and female non-breeders appear to be equally stressed when being cohabited with their parents (Begall et al., 2021).

Second, one should assume that inbreeding should occur at detectable frequencies if the main competitors of reproductive males were their own sons and if respective competition would be strong enough to drive the evolution of SSD. However, population genetics of wild *Fukomys* are not suggestive of that (Burland et al., 2002; Patzenhauerová et al., 2013).

Third, at least for the strongly sexually dimorphic Damaraland mole-rat, ontogenetic patterns also refute the hypothesis in that males appear to disperse before they reach adult body mass and thus the ability to seriously compete against their father. The mean age at dispersal is

only 371 days (but note a pronounced standard deviation of 257 days) in that species (Torrents-Ticó et al., 2018), while males attain adult body mass at an age of roughly 2 years (Young & Bennett, 2013). This means that a breeding male will, if at all, only witness few of his sons reaching adulthood within the confines of the natal burrow. In fact, breeding male Damaraland mole-rats typically weigh more than twice as much as the next-largest non-breeders of their family group (Young & Bennett, 2013). It is hard to imagine how such conditions could favor the evolution of sexual dimorphism when the intrafamily competition model is adopted. In Ansell's mole-rat, the species on which Burda's (2022) hypothesis is based on, male non-breeders also seem to typically disperse before reaching the body mass of their fathers (Šklíba et al., 2012). However, little data is currently available on social dynamics of Ansell's mole-rat in the wild. It should be pointed out that these dispersal behaviors are compatible with the extrafamilial competition hypothesis but suggest that freshly dispersed males likely cannot challenge fully-grown same sex breeders in invaded family groups immediately. Instead, they may temporarily live alone, spent a few months as "satellite males" in foreign families, or challenge males in newly formed pairs, before having built the capacities to successfully replace an adult breeder (similar ideas were also put forward by Burda, 2022). The sequential male dispersals reported by Mynhardt et al. (2021) could indeed suggest that young males may leave invaded family groups when conditions are unfavorable for them.

To conclude, the hypothesis of Burda (2022) can hardly be reconciled with the available evidence. Instead, the extrafamilial competition-hypothesis, despite the issues outlined above, provides the most coherent explanation for SSD in this genus at the moment and should inform future work on the topic.

References

Aguilar, G. H. (1993). The karyotype and taxonomic status of *Cryptomys hottentotus darlingi* (Rodentia: Bathyergidae). *African Zoology, 28*(4), 201–204.

Al-Aqaba, M. A., Fares, U., Suleman, H., Lowe, J., & Dua, H. S. (2010). Architecture and distribution of human corneal nerves. *British Journal of Ophthalmology*, *94*(6), 784–789.

Arslan, A., Kryštufek, B., Matur, F., & Zima, J. (2016). Review of chromosome races in blind mole rats (*Spalax* and *Nannospalax*). *Folia Zoologica, 65*(4), 249–301.

Begall, S., & Burda, H. (1998). Reproductive characteristics and growth in the eusocial Zambian common mole-rat (*Cryptomys* sp., Bathyergidae). *Zeitschrift für Säugetierkunde, 63*(5), 297–306.

Begall, S., Bottermann, L., & Caspar, K. R. (2022). Self-domestication underground? Testing for social and morphological correlates of animal personality in cooperatively-breeding Ansell's mole-rats (*Fukomys anselli*). *Frontiers in Ecology and Evolution*, *10*, 862082.

Begall, S., Burda, H., & Malkemper, E. P. (2014). Magnetoreception in mammals. In M. Naguib (Ed.), *Advances in the Study of Behavior, Vol. 46* (pp. 45-88). Elsevier.

Begall, S., Nappe, R., Hohrenk, L., Schmidt, T. C., Burda, H., Sahm, A., ... & Henning, Y. (2021). Life expectancy, family constellation and stress in giant mole-rats (*Fukomys mechowii). Philosophical Transactions of the Royal Society B, 376*(1823), 20200207.

Bennett, N.C., & Burda, H. (2013). *Cryptomys anselli*. In M. Happold & D. C. D. Happold (Eds.), *Mammals of Africa. Vol. 3. Rodents, Hares and Rabbits* (pp. 649–650). Bloomsbury Publishing.

Bennett, N. C., Jarvis, J. U. M., & Cotterill, F. P. D. (1994). The colony structure and reproductive biology of the afrotropical Mashona mole-rat, *Cryptomys darlingi*. *Journal of Zoology, 234*(3), 477–487.

Boll, H. (2016). 30 Jahre Graumull-Forschung in Deutschland: Analyse eines umfangreichen Datensatzes. State examination thesis, Universität Duisburg-Essen, Essen, Germany.

Burda, H. (1989). Reproductive biology (behaviour, breeding, and postnatal development) in subterranean mole-rats, *Cryptomys hottentotus* (Bathyergidae). *Zeitschrift für Säugetierkunde, 54*(6), 360–376.

Burda, H. (1995). Individual recognition and incest avoidance in eusocial common mole-rats rather than reproductive suppression by parents. *Experientia, 51*(4), 411–413.

Burda, H. (2022). Zambian mole-rats: 33 years on the scene and what we still do not know and how we could learn it. *Frontiers in Ecology and Evolution*, *10*, 866709.

Burda, H., Chitaukali, W. N., & Dryden, G. L. (2005). Taxonomic status and remarks on ecology of the Malawian mole-rat *Cryptomys whytei* (Rodentia, Bathyergidae). *Acta Theriologica*, *50*(4), 529–536.

Burda, H., Marhold, S., Westenberger, T., Wiltschko, R., & Wiltschko, W. (1990). Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). *Experientia, 46*(5), 528–530.

Burda, H., Zima, J., Scharff, A., Macholan, M., & Kawalika, M. (1999). The karyotypes of *Cryptomys anselli* sp. nova and *Cryptomys kafuensis* sp. nova: new species of the common mole-rat from Zambia (Rodentia, Bathyergidae). *Zeitschrift für Säugetierkunde, 64(1),* 36–50.

Burland, T. M., Bennett, N. C., Jarvis, J. U., & Faulkes, C. G. (2002). Eusociality in African mole-rats: new insights from patterns of genetic relatedness in the Damaraland mole-rat (*Cryptomys damarensis*). *Proceedings of the Royal Society of London. Series B: Biological Sciences, 269*(1495), 1025–1030.

Chubb, E. C. (1909). On mammals from the Upper Zambezi River. *Annals and Magazine of Natural History, 3*(13), 33–36.

Cooper, H. M., Herbin, M., & Nevo, E. (1993). Visual system of a naturally microphthalmic mammal: the blind mole rat, *Spalax ehrenbergi*. *Journal of Comparative Neurology*, *328*(3), 313-350.

Courson, J. A., Smith, I., Do, T., Landry, P. T., Hargrave, A., Behzad, A. R., Hanlon, S. D., Rumbaut, R. E., Smith, C. W., & Burns, A. R. (2019). Serial block-face scanning electron microscopy reveals neuronal-epithelial cell fusion in the mouse cornea. *PLoS One*, *14*(11), e0224434.

Curdt, F., Haase, K., Ziegenbalg, L., Greb, H., Heyers, D., & Winklhofer, M. (2022). Prussian blue technique is prone to yield false negative results in magnetoreception research. *Research Square*, 2022; doi: 10.21203/rs.3.rs-1324083/v1.

de Bruin, P. R., Viljoen, H., Sichilima, A. M., & Bennett, N. C. (2012). Socially induced infertility in Ansell's mole-rat: are there depressed hormone levels in non-reproductive males and females? *Journal of Zoology, 286*(1), 15–21.

Deutschlander, M. E., Freake, M. J., Borland, S. C., Phillips, J. B., Madden, R. C., Anderson, L. E., & Wilson, B. W. (2003). Learned magnetic compass orientation by the Siberian hamster, *Phodopus sungorus*. *Animal Behaviour, 65*(4), 779–786.

Ellerman, J. R., Morrison-Scott, T. C. S., & Hayman, R. W. (1953). *Southern African Mammals 1758–1951: A Reclassification*. Trustees of the British Museum.

Engels, S., Treiber, C. D., Salzer, M. C., Michalik, A., Ushakova, L., Keays, D. A., ... & Heyers, D. (2018). Lidocaine is a nocebo treatment for trigeminally mediated magnetic orientation in birds. *Journal of The Royal Society Interface*, *15*(145), 20180124.

Finn, K. T. (2021). Potential use of a magnetic compass during long-distance dispersal in a subterranean rodent. *Journal of Mammalogy, 102*(1), 250–257.

Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: a test and review of evidence. *American Naturalist, 160*(6), 712–726.

Gerhardt, P., Henning, Y., Begall, S., & Malkemper, E. P. (2017). Audiograms of three subterranean rodent species (genus *Fukomys*) determined by auditory brainstem responses reveal extremely poor high-frequency cut-offs. *Journal of Experimental Biology*, *220*(23), 4377–4382.

Gippoliti, S., & Amori, G. (2011). A new species of mole-rat (Rodentia, Bathyergidae) from the Horn of Africa. *Zootaxa, 2918*(1), 39–46.

Heffner, R. S., Heffner, H. E., Contos, C., & Kearns, D. (1994). Hearing in prairie dogs: transition between surface and subterranean rodents. Hearing Research, *73*(2), 185–189.

Herold, J. (2016). Magnetwahrnehmung bei Ansell-Graumullen (*Fukomys anselli*): Histologie und Verhalten. Master's thesis, Universität Duisburg-Essen, Essen, Germany.

Jackson, L. L., Heffner, H. E., & Heffner, R. S. (1997). Audiogram of the fox squirrel (*Sciurus niger*). *Journal of Comparative Psychology, 111*(1), 100–104.

Jia, S., & He, D. (2005). Motility-associated hair-bundle motion in mammalian outer hair cells. *Nature Neuroscience, 8*(8), 1028–1034.

Kardong, K. V. (2012). *Vertebrates: Comparative Anatomy, Function, Evolution*. 6th edition. McGraw-Hill.

Kavet, R., & Brain, J. (2021). Cryptochromes in mammals and birds: clock or magnetic compass? *Physiology, 36*(3), 183–194.

Kawalika, M., & Burda, H. (2007). Giant mole-rats, *Fukomys mechowii*, 13 years on the stage. In S. Begall, H. Burda, & C. E. Schleich (Eds.), *Subterranean Rodents: News from Underground* (pp. 205–219). Springer.

Keleş, A. İ., Süt, B. B., & Kankiliç, T. (2020). Histopathological analysis of the eye and optic nerve structure in the blind mole rat. *Dicle Tıp Dergisi*, *47*(3), 638–644.

Kimchi, T., & Terkel, J. (2001). Magnetic compass orientation in the blind mole rat *Spalax ehrenbergi*. *Journal of Experimental Biology*, *204*(4), 751–758.

Kishkinev, D. A., & Chernetsov, N. S. (2015). Magnetoreception systems in birds: a review of current research. *Biology Bulletin Reviews, 5*(1), 46–62.

Kitchener, A. C., Breitenmoser-Würsten, C., Eizirik, E., Gentry, A., Werdelin, L., Wilting, A., Yamaguchi, N., Abramov, A. V., Christiansen, P., Driscoll, C., Duckworth, J. W., Johnson, W., Luo, S.-J., Meijaard, E., O'Donoghue, P., Sanderson, J., Seymour, K., Bruford, M., Groves, C., Hoffmann, M., Nowell, K., Timmons, Z., & Tobe, S. (2017). A revised taxonomy of the Felidae. The final report of

the Cat Classification Task Force of the IUCN/ SSC Cat Specialist Group. *Cat News Special Issue, 11*, 1–80.

Kitchener, A. C., Hoffmann, M., Yamaguchi, N., Breitenmoser-Würsten, C., & Wilting, A. (2022). A system for designating taxonomic certainty in mammals and other taxa. *Mammalian Biology, 102*(1), 251–261.

Kössl, M., Frank, G., Burda, H., & Müller, M. (1996). Acoustic distortion products from the cochlea of the blind African mole rat, *Cryptomys* spec. *Journal of Comparative Physiology A*, *178*(3), 427–434.

Kössl, M., Möckel, D., Weber, M., & Seyfarth, E. A. (2008). Otoacoustic emissions from insect ears: evidence of active hearing? *Journal of Comparative Physiology A*, *194*(7), 597–609.

Lange, S. (2006). Sinnesökologie afrikanischer Sandgräber (Bathyergidae) am Beispiel von Hör- und Geruchssinn. Ph.D. dissertation, Universität Duisburg-Essen, Essen, Germany.

Leedale, A. E., Thorley, J., & Clutton-Brock, T. (2021). Odour-based social recognition in Damaraland mole-rats, *Fukomys damarensis*. *Animal Behaviour, 179*, 83–96.

Lindecke, O., Holland, R. A., Pētersons, G., & Voigt, C. C. (2021). Corneal sensitivity is required for orientation in free-flying migratory bats. *Communications Biology*, *4*(522), 1–7.

Malewski, S., Begall, S., Schleich, C. E., Antenucci, C. D., & Burda, H. (2018). Do subterranean mammals use the Earth's magnetic field as a heading indicator to dig straight tunnels? *PeerJ, 6*, e5819.

Manley, G. A. (2001). Evidence for an active process and a cochlear amplifier in nonmammals. *Journal of Neurophysiology, 86*(2), 541–549.

Manley, G. A., & Kraus, J. E. (2010). Exceptional high-frequency hearing and matched vocalizations in Australian pygopod geckos. *Journal of Experimental Biology, 213*(11), 1876–1885.

Marfurt, C. F., & Del Toro, D. R. (1987). Corneal sensory pathway in the rat: a horseradish peroxidase tracing study. *Journal of Comparative Neurology, 261*(3), 450–459.

Marhold, S., Wiltschko, W., & Burda, H. (1997). A magnetic polarity compass for direction finding in a subterranean mammal. *Naturwissenschaften 84*(9),421–423.

Martínez, P. A., & Bidau, C. J. (2016). A re-assessment of Rensch's rule in tuco-tucos (Rodentia: Ctenomyidae: *Ctenomys*) using a phylogenetic approach. *Mammalian Biology, 81*(1), 66–72.

Mason, M. J. (2013). Of mice, moles and guinea pigs: functional morphology of the middle ear in living mammals. *Hearing Research*, *301*, 4–18.

Meier, J.-L. (2002). Diversität und Artbildung der Graumulle (*Cryptomys*, Bathyergidae) entlang des Oberen Sambesi. Diploma thesis, Universität-GH Essen, Essen, Germany.

Müller, J. (2021). Schädelmorphologie bei *Fukomys anselli* und *Fukomys micklemi*. Bachelor's thesis, Universität Duisburg-Essen, Essen, Germany.

Mynhardt, S., Harris-Barnes, L., Bloomer, P., & Bennett, N. C. (2021). Spatial population genetic structure and colony dynamics in Damaraland mole-rats (*Fukomys damarensis*) from the southern Kalahari. *BMC Ecology and Evolution, 21*(1), 1–17.

Němec, P., Altmann, J., Marhold, S., Burda, H., & Oelschlager, H. H. (2001). Neuroanatomy of magnetoreception: the superior colliculus involved in magnetic orientation in a mammal. *Science*, *294*(5541), 366-368.

Nevo, E., Capanna, E., Corti, M., Jarvis, J. M., & Hickman, G. C. (1986). Karyotype differentiation in the endemic subterranean mole rats of South Africa (Rodentia, Bathyergidae). *Zeitschrift für Säugetierkunde, 51*(1), 36–49.

Nevo, E., Filippucci, M. G., Redi, C., Korol, A., & Beiles, A. (1994). Chromosomal speciation and adaptive radiation of mole rats in Asia Minor correlated with increased ecological stress. *Proceedings of the National Academy of Sciences, 91*(17), 8160–8164.

Oliveriusová, L., Němec, P., Králová, Z., & Sedláček, F. (2012). Magnetic compass orientation in two strictly subterranean rodents: learned or species-specific innate directional preference? *Journal of Experimental Biology*, *215*(20), 3649–3654.

Painter, M. S., Davis, M., Ganesh, S., Rak, E., Brumet, K., Bayne, H., ... & Phillips, J. B. (2018). Evidence for plasticity in magnetic nest-building orientation in laboratory mice. *Animal Behaviour*, *138*, 93–100.

Peddie, C. J., & Collinson, L. M. (2014). Exploring the third dimension: volume electron microscopy comes of age. *Micron*, *61*, 9–19.

Pyott, S. J., van Tuinen, M., Screven, L. A., Schrode, K. M., Bai, J. P., Barone, C. M., ... & Park, T. J. (2020). Functional, morphological, and evolutionary characterization of hearing in subterranean, eusocial African mole-rats. *Current Biology, 30*(22), 4329–4341.

Semm, P., Schneider, T., & Vollrath, L. (1980). Effects of an earth-strength magnetic field on electrical activity of pineal cells. *Nature, 288*(5791), 607-608.

Sichilima, A. M., Bennett, N. C., & Faulkes, C. G. (2011). Field evidence for colony size and aseasonality of breeding and in Ansell's mole-rat, *Fukomys anselli* (Rodentia: Bathyergidae). *African Zoology, 46*(2), 334–339.

Šklíba, J., Mazoch, V., Patzenhauerová, H., Hrouzková, E., Lövy, M., Kott, O., & Šumbera, R. (2012). A maze-lover's dream: burrow architecture, natural history and habitat characteristics of Ansell's mole-rat (*Fukomys anselli*). *Mammalian Biology, 77*(6), 420–427.

Spinks, A. C., Bennett, N. C., & Jarvis, J. U. M. (2000). A comparison of the ecology of two populations of the common mole-rat, *Cryptomys hottentotus hottentotus*: the effect of aridity on food, foraging and body mass. *Oecologia, 125*(3), 341–349.

Taschenberger, G., Gallo, L., & Manley, G. A. (1995). Filtering of distortion-product otoacoustic emissions in the inner ear of birds and lizards. *Hearing research*, *91*(1-2), 87–92.

Thalau, P., Ritz, T., Burda, H., Wegner, R. E., & Wiltschko, R. (2006). The magnetic compass mechanisms of birds and rodents are based on different physical principles. *Journal of the Royal Society Interface*, *3*(9), 583–587.

Thorley, J., Bensch, H., Finn, K., Clutton-Brock, T., & Zöttl, M. (2021). Damaraland mole-rats are not obligate cooperative breeders. *bioRxiv,* 2021.12.08.471794; doi: https://doi.org/10.1101/2021.12.08.471794

Torrents-Ticó, M., Bennett, N. C., Jarvis, J. U., & Zöttl, M. (2018). Sex differences in timing and context of dispersal in Damaraland mole-rats (*Fukomys damarensis*). *Journal of Zoology, 306*(4), 252–257.

Treiber, C. D., Salzer, M. C., Riegler, J., Edelman, N., Sugar, C., Breuss, M., ... & Keays, D. A. (2012). Clusters of iron-rich cells in the upper beak of pigeons are macrophages not magnetosensitive neurons. *Nature, 484*(7394), 367–370.

Van Daele, P. A. A. G., Blonde, P., Stjernstedt, R., & Adriaens, D. (2013). A new species of African mole-rat (*Fukomys*, Bathyergidae, Rodentia) from the Zaire-Zambezi watershed. *Zootaxa, 3636*(1), 171–189.

Van Daele, P. A. A. G., Dammann, P., Meier, J.-L., Kawalika, M., Van De Woestijne, C., & Burda, H. (2004). Chromosomal diversity in mole-rats of the genus *Cryptomys* (Rodentia: Bathyergidae) from the Zambezian region: with descriptions of new karyotypes. *Journal of Zoology, 264*(3), 317–326.

Van Daele, P. A. A. G, Desmet, N., Šumbera, R., & Adriaens, D. (2019). Work behaviour and biting performance in the cooperative breeding Micklem's mole-rat *Fukomys micklemi* (Bathyergidae, Rodentia). *Mammalian Biology, 95*(1), 69–76.

Van Daele, P. A. A. G, Verheyen, E., Brunain, M., & Adriaens, D. (2007). Cytochrome b sequence analysis reveals differential molecular evolution in African mole-rats of the chromosomally

hyperdiverse genus *Fukomys* (Bathyergidae, Rodentia) from the Zambezian region. *Molecular Phylogenetics and Evolution, 45*(1), 142–157.

van Dijk, P., Mason, M. J., & Narins, P. M. (2002). Distortion product otoacoustic emissions in frogs: correlation with middle and inner ear properties. *Hearing Research*, *173*(1-2), 100–108.

Visser, J. H., Bennett, N. C., & Jansen van Vuuren, B. (2019). Phylogeny and biogeography of the African Bathyergidae: a review of patterns and processes. *PeerJ*, *7*, 7730.

Walker, M. M., Diebel, C. E., Haugh, C. V., Pankhurst, P. M., Montgomery, J. C., & Green, C. R. (1997). Structure and function of the vertebrate magnetic sense. *Nature*, *390*(6658), 371–376.

Wang, Y., Pan, Y., Parsons, S., Walker, M., & Zhang, S. (2007). Bats respond to polarity of a magnetic field. *Proceedings of the Royal Society B: Biological Sciences, 274*(1627), 2901–2905.

Wegner, R. E., Begall, S., & Burda, H. (2006). Magnetic compass in the cornea: local anaesthesia impairs orientation in a mammal. *Journal of Experimental Biology*, *209*(23), 4747–4750.

Williams, S. L., Schlitter, D. A., & Robbins, L. W. (1983). Morphological variation in a natural population of *Cryptomys* (Rodentia, Bathyergidae) from Cameroon. *Annales du Musee Royal de l'Afrique Centrale Tervuren: Sciences Zoologiques*, *237*(1), 159–172.

Young, A. J., & Bennett, N. C. (2013). Intra-sexual selection in cooperative mammals and birds: why are females not bigger and better armed? *Philosophical Transactions of the Royal Society B: Biological Sciences, 368*(1631), 20130075.

Chapter 3 – Synopsis and Outlook

This thesis aimed to investigate selected aspects of sensory physiology and sociality in subterranean rodents. The main contributions to these fields of study encompass novel behavioral data suggestive of ocular magnetoreception in a burrowing mammal, ABR-derived audiograms of three species of subterranean rodents, multiple lines of evidence demonstrating sex- and reproductive status-dependent olfactory signaling via perioral glands in African mole-rats, and finally a thorough assessment of sexual dimorphism in Ansell's mole-rat as well as an analysis of sexual size dimorphism scaling in the family Bathyergidae.

The results on eye-based magnetic sensing in Ansell's mole-rat (Chapter 2.2) represent a key step to understand this enigmatic sensory modality in mammals by narrowing down the search space for receptors responding to the geomagnetic field. Only once this has been achieved, the physiology and cellular architecture of these structures can be directly studied to arrive at a holistic understanding of mammalian and, more generally speaking, vertebrate magnetoreception.

New data on hearing sensitivity in African mole-rats and coruros (Chapter 2.3) presented here point out that both degenerative and adaptive evolutionary trajectories have likely shaped the auditory capacities of subterranean rodents instead of just one of these alternatives. Additionally, this Chapter stresses the value of comparative studies on species differing in the degree of their subterranean adaptation to retrace the mode and pacing of sensory systems evolution in underground habitats.

The results on olfactory signaling in African mole-rats (Chapter 2.4) provide a first impression of how the so far neglected perioral gland secretions impact communication in these animals. As shown here, these secretions convey complex social information and thus may be importantly involved in various behavioral contexts. This discovery emphasizes the relevance of olfaction to subterranean mammal communication and will hopefully stimulate further research on perioral gland functions in African mole-rats but also other rodent species, including readily available laboratory mice and rats, which also exhibit these little-known peculiar structures.

Finally, findings on sexual dimorphism in Ansell's mole-rat and the scaling of body mass dimorphism in bathyergids in general point to a so far underappreciated role of male intrasexual competition in this group or at least within the Northern common mole-rats of the genus *Fukomys* (Chapter 2.5). Related behaviors might well be mediated by aforementioned sexually dimorphic olfactory signals, so that the results of Chapters 2.4 and 2.5 complement each other. The reported data will help to inform hypotheses on social dynamics and reproductive strategies in these rodents and might stimulate analogous research on other sexually dimorphic cooperatively-breeding species.

All these manuscripts offer numerous questions and potential starting points for follow-up studies to build on, as I have pointed out in the respective discussion sections. These perspectives will not be reiterated here among the closing remarks of this thesis. Instead, I want to emphasize one further general issue that will be crucial to address in order to advance our understanding of subterranean rodent biology in the future.

A factor that severely limits our ability to retrace evolutionary patterns in burrowing rodents is a lack of data on epigeic relatives on which to model ancestral states. By now, several groups of subterranean rodents have become exceptionally well characterized compared to most other small mammals, both at the organismic and the molecular level (compare Chapter 2.1). First and foremost, this includes the naked mole-rat (Buffenstein et al., 2021), but it also holds true for common mole-rats (Begall et al., 2018), Mediterranean blind mole-rats (e.g., Malik et al., 2012; Burda, 2021) and tuco-tucos (Freitas et al., 2021). In contrast, knowledge on the closest living epigeic relatives of these animals has often remained scarce, which can create critical biases. For instance, the closest living relatives of African mole-rats are the dassie-rats (Petromuridae) of Western Namibia and adjacent regions, and the cane-rats or grass cutters (Thryonomyidae) that occur throughout sub-Saharan Africa (Lacher et al., 2016). In dassie-rats, only three field studies and little complementary research have been conducted, rendering their biology enigmatic (Rathbun & Rathbun, 2006). More data are available on cane-rats, particularly the greater cane-rat (*Thryonomys swinderianus*). These large rodents are commercially farmed and represent important livestock in West Africa. A total of just 146 scientific studies have been published on this species over the last 60 years, despite of its economical and zoological significance (Mustapha et al., 2020). More than half of this body of literature is concerned with captive management, breeding, and the reproductive physiology of this species. Its ecology and behavior in the wild remain little known. Yet, as greater cane-rats have become subjects of notably increasing research efforts in recent years, this situation can be expected to change for the better soon (Mustapha et al., 2020).

By missing data on these close outgroups and due to the virtual absence of fossils (Chapter 1.2.1), we can hardly reconstruct traits of the ancient epigeic ancestor of African mole-rats. This greatly hinders our evolutionary interpretations of bathyergid biology since we can often only speculate about whether specific traits evolved over the course of subterranean adaptation or represent plesiomorphies. Our understanding of the evolution of hearing, sexual dimorphism and olfaction in bathyergids, as discussed in this thesis, greatly suffers from that lack of reference. African mole-rats, particularly naked mole-rats, are often compared to laboratory murids regarding their sensory biology, physiology, and senescence patterns (e.g., Lindenlaub et al., 1995; Viltard et al., 2019). Obviously, this situation emerged mostly due to a limited access to alternative reference species, but it nevertheless created problematic narratives that continue to linger on.

Given that murids exhibit an array of highly derived traits, including specialized high-frequency hearing, secondarily shortened life spans and related cancer susceptibility (compare Weigl, 2005; Argyle & Mason, 2008; Sahm et al., 2018), these unfitting comparisons have led researchers to characterize various traits of bathyergids, or just the naked mole-rat, as extremely deviant and/or unique although they are similarly found in other subterranean rodents or even hystricomorph rodents in general (e.g., Braude et al., 2021). These issues emphasize the need for proper outgroups in studies that make assumptions about the evolution of African mole-rats. I will refrain from discussing the situation for other burrowing rodent groups here but one could often sketch a similar picture for them.

Hence, we require more research on related epigeic species to better characterize our subterranean focus groups to put their traits into a phylogenetic context. In addition to that, crucial insights might also be gained from including geologically young subterranean rodents such as coruros, mole-voles, and long-clawed mole-mice into comparative studies, as I have argued in Chapter 2.3. These forms are typically far less extensively studied than more derived taxa such as bathergids or spalacids and might offer important insights into how and when specific subterranean adaptations emerge in burrowing lineages.

To conclude, although we have gained substantial knowledge about various groups of subterranean rodents over the last decades, the biology of mole-rats and their ecological analogues continue to remain puzzling in many respects. I hope that this thesis has done its bit to elucidate the sensory ecology and social behaviors of these fascinating animals and will provide impulses for future research. There is much left to explore.

References

Argyle, E. C., & Mason, M. J. (2008). Middle ear structures of *Octodon degus* (Rodentia: Octodontidae), in comparison with those of subterranean caviomorphs. *Journal of Mammalogy, 89*(6), 1447–1455.

Begall, S., Burda, H., & Šumbera, R. (2018). *Graumulle – Cryptomys und Fukomys*. Neue Brehm-Bücherei – VerlagsKG Wolf.

Braude, S., Holtze, S., Begall, S., Brenmoehl, J., Burda, H., Dammann, P., ... & Hildebrandt, T. B. (2021). Surprisingly long survival of premature conclusions about naked mole-rat biology. *Biological Reviews*, *96*(2), 376–393.

Buffenstein, R., Park, T. J., & Holmes, M. (2021). *The Extraordinary Biology of the Naked Mole-Rat*. Springer.

Burda, H. (2021). Sensory perception of mole-rats and mole rats: assessment of a complex natural global evolutionary "experiment". In S. P. Wasser & M. Frenkel-Morgenstern (Eds.), *New Horizons in Evolution* (pp. 161–191). Academic Press.

Freitas, T., Gonçalves, G., & Maestri, R. (2021). *Tuco-Tucos: An Evolutionary Approach to the Diversity of a Neotropical Subterranean Rodent*. Springer.

Lacher, T. E., Mittermeier, R. A., & Wilson, D. E. (Eds.). (2016). *Handbook of the Mammals of the World: Lagomorphs and Rodents I*. Lynx Edicions.

Lindenlaub, T., Burda, H., & Nevo, E. (1995). Convergent evolution of the vestibular organ in the subterranean mole-rats, *Cryptomys* and *Spalax*, as compared with the aboveground rat, *Rattus*. *Journal of Morphology, 224*(3), 303–311.

Malik, A., Korol, A., Weber, M., Hankeln, T., Avivi, A., & Band, M. (2012). Transcriptome analysis of the *Spalax* hypoxia survival response includes suppression of apoptosis and tight control of angiogenesis. *BMC Genomics, 13*(1), 615.

Mustapha, O. A., Teriba, E. E., Ezekiel, O. S., Olude, A. M., Akinloye, A. K., & Olopade, J. O. (2020). A study of scientific publications on the greater cane rat (*Thryonomys swinderianus*, Temminck 1827). *Animal Models and Experimental Medicine, 3*(1), 40–46.

Rathbun, G. B., & Rathbun, C. D. (2006). Social monogamy in the noki or dassie-rat (*Petromus typicus*) in Namibia. *Mammalian Biology, 71*(4), 203–213.

Sahm, A., Bens, M., Szafranski, K., Holtze, S., Groth, M., Goerlach, M., ... & Platzer, M. (2018). Long-lived rodents reveal signatures of positive selection in genes associated with lifespan. *PLoS Genetics*, *14*(3), e1007272.

Viltard, M., Durand, S., Pérez-Lanzón, M., Aprahamian, F., Lefevre, D., Leroy, C., ... & Friedlander, G. (2019). The metabolomic signature of extreme longevity: naked mole rats versus mice. *Aging (Albany NY), 11*(14), 4783.

Weigl, R. (2005). *Longevity of Mammals in Captivity; from the Living Collections of the World*. Kleine Senckenberg-Reihe.

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Finally, I want to acknowledge my parents and family for their support and patience over the last three years. As this Chapter of my life has been completed, perhaps now I can go out and search for "something that has nothing to do with mole-rats, pays, and that people actually care about".

Abbreviations

Abbreviations that exclusively appear in the manuscripts included in Chapter 2 are not included here but are spelled out and clarified in the respective papers.

ABR	auditory brainstem response
ca.	approximately
dB	decibel
DPOAE	distortion product otoacoustic emissions
e.g.	for example
et al.	and others
etc.	et cetera; and so on
Fig.	figure
g	gram
Hz, kHz	Hertz, kilohertz
i.e.	that is
m, mm	meter, milimeter
p.	page
pers. com.	personal communication
pers. obs.	personal observation
s, ms	second, milisecond
SPL	sound pressure level
SSD	sexual size dimorphism
Tab	table
vs.	versus; in opposition to

List of figures

Figures that appear in the manuscripts included in Chapter 2 are not considered here.

Curriculum vitae

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Appendix

I. Author contribution statement – Bestätigung des Betreuers

Bestätigung:

Hiermit bestätige ich die Darstellung zu den Anteilen von Herrn Caspar an Konzeption, Durchführung und Abfassung jeder Publikation (Chapter 2) gemäß der Promotionsordnung der Fakultät für Biologie zur Erlangung des Dr. rer. nat.

Essen, den _____ _____

Prof. Dr. Sabine Begall

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Hiermit erkläre ich, gem. § 7 Abs. (2) d) + f) der Promotionsordnung der Fakultät für Biologie zur Erlangung des Dr. rer. nat., dass ich die vorliegende Dissertation selbständig verfasst und mich keiner anderen als der angegebenen Hilfsmittel bedient, bei der Abfassung der Dissertation nur die angegebenen Hilfsmittel benutzt und alle wörtlich oder inhaltlich übernommenen Stellen als solche gekennzeichnet habe.

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Erklärung der Befürwortung der Promotion:

Hiermit erkläre ich, gem. § 6 Abs. 2, g der Promotionsordnung der Fakultät für Biologie zur Erlangung der Dr. rer. nat., dass ich das Arbeitsgebiet, dem das Thema „Sensory ecology and correlates of sociality in common mole-rats (*Fukomys* spp.) and other subterranean rodents“ zuzuordnen ist, in Forschung und Lehre vertrete und den Antrag von Kai Robert Caspar befürworte.

Essen, den _____ _____

Prof. Dr. Sabine Begall