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ORIGINAL ARTICLE

The biology of an isolated Mashona mole-rat population from southern Malawi, with implications for the diversity and biogeography of the genus *Fukomys*

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Abstract

The Mashona mole-rat, *Fukomys darlingi* (Thomas, 1895), is a little studied social African mole-rat (Bathyergidae) from south-astern Africa. Here, we present an integrative study characterizing the genetic diversity of populations assigned to *F. darlingi* with special focus on animals from Nsanje, southern Malawi. These mole-rats show pronounced differences in body mass and general appearance compared to nominate *F. darlingi* from Zimbabwe and Mozambique, but their taxonomic status has so far remained unclear. A genetic analysis encompassing all major lineages of the genus *Fukomys* suggests that this population indeed represents a deeply nested lineage within the *F. darlingi* clade. The karyotype of the Nsanje mole-rats also corresponds to that of the nominate form, being 2n = 54. While both nuclear and mitochondrial data agree about the assignment of the Nsanje mole-rats to *F. darlingi*, our analyses revealed substantial mitonuclear discordance for other branches within the *Fukomys* phylogenetic tree. Nsanje mole-rats are significantly larger than nominate *F. darlingi* and their ontogeny and reproduction closely resemble similar-sized congeneric species rather than the nominate population. The somatic growth of the Nsanje form is the slowest of all African mole-rats. The maximum life span of *F. darlingi* is at least 19 years. The observed differences between nominate *F. darlingi* and mole-rats from Nsanje may be attributed mainly to their different body mass. Our study highlights the advantages of an integrative approach for understanding the diversity of African mole-rats and emphasizes the great intraspecific variability that may be encountered in these underground-dwelling rodents.

Keywords Bathyergidae · Diversity · Subterranean rodent · Life history · Inter-population differences · Reproduction

Introduction

The African mole-rats (Bathyergidae), a family of strictly subterranean rodents, have attracted great scientific attention due to many peculiar aspects of their biology. This group comprises six genera, *Cryptomys* (southern common mole-rats), *Fukomys* (northern common mole-rats), *Heterocephalus* (naked mole-rat), *Georychus* (Cape mole-rat), *Bathyergus* (dune mole-rats), and *Heliophobius* (silvery mole-rat), which predominately occur in the savannah habitats of

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sub-Saharan Africa. All of them have been subjected to multiple physiological, behavioural, and phylogenetic studies (reviewed in Begall et al., 2018; Bennett & Faulkes, 2000). For many reasons, however, the life histories, species diversity, and geographic distribution of many taxa are still little known. Considering general biology and life history characteristics, we only possess relatively complete information for a few species such as the Damaraland mole-rat ((*Fukomys damarensis* (Ogilby, 1838)) of the Kalahari sands, the Zambian Ansell's mole-rat ((*Fukomys anselli* (Burda et al., 1999)), and the naked mole-rat (*Heterocephalus glaber,* Rüppel, 1842) from the Horn of Africa. For most other bathyergid taxa, such data is still lacking or largely anecdotal. Estimates of bathyergid species diversity are hindered by the fact that many taxa were described or established based on singular lines of evidence from either morphological studies (e.g., Gippoliti & Amori, 2011; Thomas, 1895), karyological differences (e.g., Aguilar, 1993; Burda et al., 1999), or single-gene phylogenies (e.g., Faulkes et al., 2011). Currently, integrative approaches to bathyergid taxonomy are lacking (but see Van Daele et al., 2013).

For a long time, common mole-rats, the most diverse and widespread bathyergid radiation, were all classified within the genus *Cryptomys* (Gray, 1864; Thomas, 1917; Wilson & Reeder, 2005). Molecular methods revealed that the morphologically rather uniform species, which were traditionally comprised by *Cryptomys*, form in fact two deeply diverging cryptic lineages, which are now differentiated at the generic level as *Cryptomys* and *Fukomys* (Faulkes et al., 2004; Ingram et al., 2004; Kock et al., 2006). Both *Cryptomys* and *Fukomys* are social and live in cooperatively breeding groups, typically structured around a breeding pair. However, while there is evidence for low male reproductive skew and the regular occurrence of genetic polyandry in *Cryptomys*, *Fukomys* is predominately monogamous (Bishop et al., 2004; Burland et al., 2002; Patzenhauerová et al., 2013). Furthermore, offspring dispersal is more delayed in most *Fukomys* species compared to *Cryptomys*, leading to a greater group size in the former (Begall et al., 2018).

Whereas *Cryptomys* is exclusively found in southern Africa, *Fukomys* ranges from the north of the Republic of South Africa to the northern, Sahelo-Sudanian, savannahs. *Fukomys* is the most speciose bathyergid genus, encompassing at least 14 (Kock et al., 2006; Monadjem et al., 2015) but most likely more species (Faulkes & Bennett, 2013; Mammal Diversity Database, 2022). Although there were notable efforts to elucidate *Fukomys* genetic diversity and taxonomy (Faulkes et al., 2017; Van Daele et al., 2004, 2007, 2013), many phylogenetic lineages within this genus remain severely understudied.

One such lineage of *Fukomys* occurs in the southernmost part of Malawi, in the Nsanje district, where it is especially common on the escarpment of Chididi (Ansell & Dowsett, 1988). Albeit relatively small, Malawi is a geomorphologically heterogenous country and its *Fukomys* populations show a disjunct distribution. Between the Nsanje district in the south and Kasungu near to the Viphya plateau in the north, there are no confirmed records of mole-rats (Ansell & Dowsett, 1988). The mole-rats living in northern Malawi have long been classified as *Fukomys whytei* (previously *Cryptomys hottentotus whytei* Thomas, 1897) and their distinctiveness has been repeatedly confirmed by genetic studies (Ingram et al., 2004; Van Daele et al., 2007; Visser et al., 2020). However, not all *Fukomys* in northern Malawi belong to that species. Surprisingly, a mole-rat population near Mzuzu was found to form a deeply divergent lineage within the genus (Faulkes et al., 2010). In one of three alternative phylogenies, these Mzuzu mole-rats shared a recent common ancestor with animals from Hanang in northern Tanzania (Faulkes et al., 2010) which were later on described as a distinct species, *F. hanangensis* (Faulkes et al., 2017). Without any proper justification, Visser et al. (2020) grouped the Mzuzu mole-rats into *F. hanangensis*, despite the long branch lengths and genetic distances separating the two lineages. All northern Malawian mole-rats live usually at altitudes above 1000 m a.s.l.

Common mole-rats in southern Malawi, in the Nsanje region, occurr at much lower altitudes along the Shire River. They are generally supposed to be isolated from both the mole-rats of northern Malawi and those ranging south of the Zambezi River in central Mozambique (Smithers & Tello, 1976). Their phylogenetic affinities have remained obscure. Apart from a potential relationship to *F. whytei*, there is also the possibility that the southern Malawi mole-rats are closely related or belong to the species *Fukomys darlingi* (Thomas, 1895), the Mashona mole-rat, which is distributed primarily in the highlands of eastern Zimbabwe and central Mozambique (Bennett & Faulkes, 2000). Finally, these animals could represent an undescribed species, given striking differences between southern Malawi mole-rats and both *F. whytei* from northern Malawi and *F. darlingi* from Zimbabwe. When compared to nominate *F. darlingi* in particular, southern Malawi mole-rats are notably larger and more homeothermic, i.e., exhibiting a more stable core body temperature across different ambient temperatures (see Bennett et al., 1994 and Zemanová et al., 2012 for comparison).

In this study, we use one mitochondrial and five nuclear gene sequences to determine the phylogenetic position of mole-rats from the Nsanje district of southern Malawi. Clarifying the taxonomic status of Nsanje mole-rats is important, because descendants of these animals breed in the labs of the University of South Bohemia and the University of Duisburg-Essen and are used for various behavioural and physiological studies (see Dvořáková et al., 2016; Wiedenová et al., 2018; Vejmělka et al., 2021; Caspar et al., 2021a). So far, we

used the provisional name *F.* "Nsanje" for these animals or denoted them as closely related to *F. darlingi* based on very preliminary trees constructed from mitochondrial cytochrome b data. Finally, we provide information on reproductive and life history parameters of Nsanje mole-rats obtained from the two aforementioned labs and compare them to data on the nominate form of *F. darlingi* from Zimbabwe, as well as to other *Fukomys* species.

Methods

Study animals

Mole-rats were trapped in the vicinity of Nsanje town, southern Malawi (16° 55′S, 35° 15′E, altitude 53 m a.s.l.), in August 2005. Altogether, we obtained 16 individuals (5 males, 11 females) from different family groups (Supplementary Information 1). Ten individuals were exported to the Czech Republic, being the later founders of our captive breeding stock in the vivarium at the University of South Bohemia in České Budějovice. In July 2019, descendants of this lab lineage were transferred to the vivarium at the University of Duisburg-Essen.

Molecular data

Phylogenetic inference was based on sequences of the mitochondrial gene cytochrome b (*CYTB*) and five nuclear loci: recombination activating gene 1 (*RAG1*), intron 7 of the β-fibrinogen gene (*FGB*), intron 7 of 24-dehydrocholesterol reductase precursor (*DHCR*), intron 9 of smoothened homolog precursor (*SMO*), and intron 7 of transient receptor potential cation channel, subfamily V, member 4 (*TRPV*).

The *CYTB* tree included 59 sequences, 706–1140 base pairs (bp) long. Out of them, 27 sequences were newly obtained from fresh tissue samples (GenBank accessions: OQ559424–OQ559450) and 32 sequences, including three *Cryptomys* as outgroups, were taken from published data (Faulkes et al., 1997, 2004, 2010; 2017; Van Daele et al., 2007; Visser et al., 2018; Krásová et al., 2021). For molecular barcoding, 25 additional short (189 bp) *CYTB* sequences (GenBank accession: OQ559451–OQ559475) were obtained from specimens of the National History Museum of Zimbabwe (NMZB). The nuclear tree was based on 122 sequences from 25 individuals of *Fukomys;* (30 sequences published by Uhrová et al. (2022), 92 newly generated with GenBank accessions for *RAG1*: OQ442881–OQ442896, *FGB*: OQ442843–OQ442861, *DHCR*: OQ559483–OQ559501, *TRPV*: OQ559505–OQ559523, *SMO*: OQ442862–OQ442880) and as an outgroup we used 15 sequences of three individuals of *Georychus capensis* published by Uhrová et al. (2022). The nuclear data matrix was almost complete, only three ingroup sequences of *RAG*1 were missing. The length of the loci was 1066 bp (*RAG1*), 823 bp (*FGB*), 377 bp (*DHCR*), 433 bp (*SMO*), and 320 bp (*TRPV*).

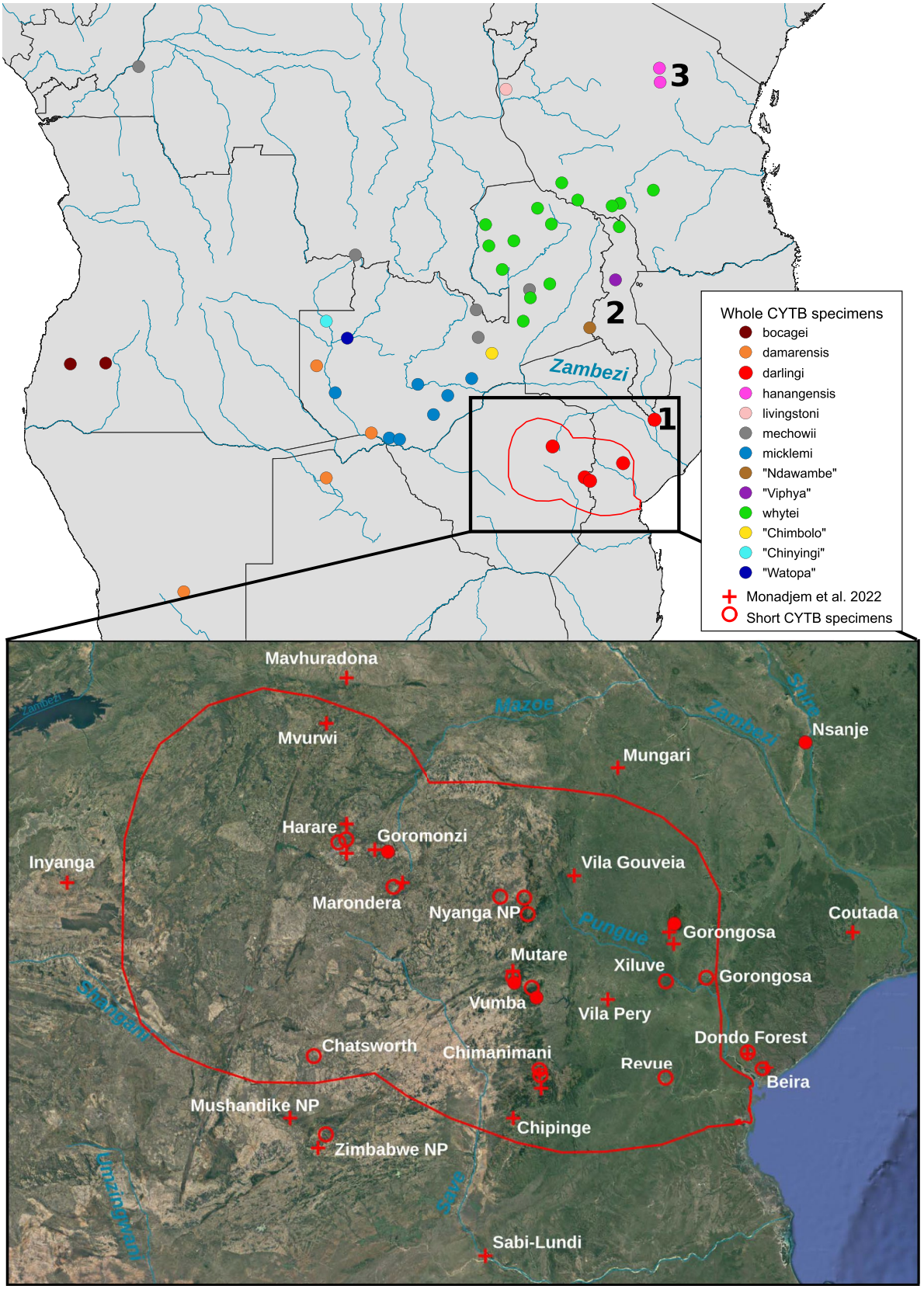
DNA from fresh tissue samples was extracted using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen) following the manufacturer's protocol. Nucleotide sequences were amplified by polymerase chain reaction using the primers and protocols specified in Uhrová et al. (2022). The short *CYTB* sequences were obtained from museum material by amplicon sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA), using primers designed by Galan et al. (2012).

The *CYTB*-sequenced individuals cover almost the full geographic range of the genus *Fukomys* (Fig. 1), but special focus was put on the population from Nsanje (represented by two individuals) and mole-rats from adjacent regions, including several putative representatives of *F. whytei* and *F. darlingi*. The individuals with nuclear sequence data represented all main *CYTB* lineages in the genus. The complete list of the used material is provided in the Supplementary Information 2, which includes also GenBank accession numbers of the published sequences and geographical coordinates of the sampling sites, if available. Finally, Fig. 1 and Supplementary Information 2 also contain 30 additional records of *F. darlingi* for which GPS coordinates were obtained from Monadjem et al. (2015).

Phylogenetic methods

The *CYTB* tree was reconstructed by means of Bayesian inference as implemented in MrBayes 3.2.6 (Ronquist et al., 2012). The alignment was partitioned according to the codon position with GTR + F + G4 nucleotide substitution model (Tavaré, 1986; Yang, 1994) in each partition, as suggested by ModelFinder tool of IQ-TREE v1.6 (Kalyaanamoorthy et al., 2017; Nguyen et al., 2015), with AIC (Akaike, 1974) specified as the search criterion. The Markov chain Monte Carlo (MCMC) was run for 2×10^6 generations with sampling every 1000 generations and its convergence to posterior examined by comparison of four independent runs in Tracer 1.7 (Rambaut et al., 2018). From all four posterior samples, the first 20% of trees were discarded as burn-in and the remaining were pooled and outgroup-rooted, and the outgroups were removed. The sample of 6400 trees was represented by the maximum clade credibility (MCC) tree with the mean common ancestor node heights (Drummond & Bouckaert, 2015), which was calculated in R (R Core Team, 2022) using the function *find_mcct* available at https://github.com/onmikula/bpptools.

The *CYTB* lineages, representing putative species, were determined using the branch-cutting method (https://www.biorxiv.org/content/10.1101/419887v1.full) whose R


3
2
1
Zambezi
Whole CYTB specimens
bocagei
damarensis
darlingi
hanangensis
livingstoni
mechowii
micklemi
"Ndawambe"
"Viphya"
whytei
"Chimbolo"
"Chinyingi"
"Watopa"
Monadjem et al. 2022
Short CYTB specimens
Mavhuradona
Mvurwi
Mazoe
Zambezi
Shire
Nsanje
Mungari
Harare
Goromonzi
Inyanga
Vila Gouveia
Marondera
Nyanga NP
Pungue
Gorongosa
Coutada
Mutare
Xiluve
Gorongosa
Vumba
Vila Pery
Save
Chatsworth
Chimanimani
Dondo Forest
Revue
Beira
Mushandike NP
Chipinge
Zimbabwe NP
Umzingwani
Sabi-Lundi

◂**Fig. 1** The geographic distribution of localities where mole-rats were sampled for this study. In the inset, full circles indicate localities represented by whole *CYTB* sequences, while empty symbols denote short *CYTB* sequences of museum samples. Red crosses show records of *Fukomys darlingi* individuals published in Monadjem et al. (2015). The symbol colours match those of the *CYTB* lineages illustrated in the trees shown in Figs. 4 and 5. Red outlines show the estimated range of *F. darlingi* according to the IUCN Red List (IUCN 2022). The samples from Ghana (*Fukomys zechi*: Atebubu: 7.75N, 1W) and Nigeria (*Fukomys foxi*: Panyam: 9.4104411N, 9.21362E) are not depicted. 1, Nsanje + Chididi; 2, Kasungu + Mzuzu; 3, Hanang

implementation is available at https://github.com/onmikula/phyloeda. This method partitions a tree into lineages according to the importance of branches and assuming that speciation is such a unique process that deletion of any branch representing speciation would significantly change the structure of a given phylogeny. The partitioning was constrained by an assumption that individuals previously classified as *F. bocagei* and *F. mechowii* are heterospecific and the lineages were also required to be monophyletic with posterior probability (PP) ≥ 0.50. For better understanding of differentiation between the putative species, we calculated average pairwise Kimura two-parameter (K2P) distances (Kimura, 1980) separating them.

The short sequences of museum specimens, not included in the *CYTB* tree, were classified into the *CYTB* lineages using an evolutionary placement algorithm (EPA; Berger et al., 2011) implemented in RaxML v8.2.10 (Stamatakis, 2014). In short, the algorithm attempts to place query sequences one by one on the pre-specified phylogenetic tree and outputs a table containing the potential placements (i.e., tree branches) with their likelihood weights. We set EPA to place every short *CYTB* sequence separately on the MCC tree from MrBayes analysis and for each of them we calculated relative probabilities of belonging to different lineages. The probabilities are sums of likelihood weights of placements on branches belonging to the lineages. R functions used for post-processing of EPA outputs are available at https://github.com/onmikula/epatools.

The inference of the nuclear phylogenetic tree was also accomplished in MrBayes using concatenated sequences of all five loci. Each locus was set as a separate partition with its own nucleotide substitution model selected by ModelFinder as in the *CYTB* analysis. The models selected were HKY + F for *DHCR* and *SMO*, HKY + F + G4 for *FGB*, GTR + F for *RAG1*, and GTR + F + G4 for *TRPV* (Hasegawa et al., 1985; Tavaré, 1986; Yang, 1994). The nuclear sequences were analysed as unphased with heterozygous states treated as ambiguous. MrBayes was run four times independently for 5×10^6 generations with sampling every 5000th generation. The convergence of MCMC was checked and the posterior samples were summarized as in the *CYTB* analysis.

Karyotype of *F. darlingi* from Nsanje

The karyotype of *F. darlingi* from the Nsanje population was established from a blood sample taken from one male kept at the University of Duisburg-Essen (F2 offspring of wild-caught mole-rats). Lymphocytes in whole blood cultures were stimulated to enter the cell cycle using phytohemagglutinin L (PAN Biotech, 3 µg/mL) for 2 days at 37 °C. Mitotic cells were arrested using colcemid (Ciba, 80 ng/mL). Preparation and Giemsa staining followed standard protocols. Chromosomes in C-metaphases were examined. Altogether, 12 mitotic cells were analysed.

Reproduction, postnatal development, and sexual dimorphism of *F. darlingi* from Nsanje

Families of *F. darlingi* from the Nsanje population were monitored in the vivaria of the University of South Bohemia in České Budějovice and the University of Duisburg-Essen. The mole-rats were kept in glass terraria of varying sizes with horticultural peat (České Budějovice) or saw dust (Essen) as substrate. Ceramic flowerpots were offered as nest chambers, and filter paper as nesting material. Room temperature was 25 ± 1 °C, relative air humidity 40–60%, and light regime 12D/12L. The animals were fed with potatoes, sweet potatoes, carrots, apples, and commercial rodent pellets thrice a week.

Data on reproduction and postnatal development were collected for individuals from 14 families. We recorded litter size and inter-birth intervals as a proxy for the length of gestation (c.f. Burda, 1989, 1990). For some pups, we specifically monitored their development after birth, including body mass (g, measured every 3 days), the presence of fur and externally visible incisors, and the opening of eyes, as well as specific behaviours (eating solids, leaving the nest for the first time, starting to spar with other family members).

We calculated linear mixed models with the body mass of pups (g) as the dependent variable, litter size as a fixed factor, and litter number as a random factor with the aid of the package lme4 (Bates et al., 2022) in Rstudio (Rstudio Team, 2020). Gaussian distribution of the dependent variable and the model residuals were both tested with a Shapiro–Wilk test for normality ($p > 0.05$).

Body weight W at time t and growth parameters fitted to a Gompertz growth curve were estimated in a total of 73 individuals (32 males, 41 females) for which approximately adult body mass was reached (i.e., at least 90 g and/or not gaining weight over a period of 4 weeks) using the following formula:

$$W(t) = A \bullet e^{-e^{-K(t-I)}}$$

with A being the asymptotic body mass (g), K the growth constant (days^{-1}), and I the inflection point. The maximum growth rate (g × days^{-1}) was calculated as $K \times A \times e^{-1}$

(Begall, 1997). The mean age of individuals for which growth parameters have been calculated was 8.1 ± 4.5 years (range: 1–16.4 years). Growth parameters including maximum growth rate were analysed with generalized linear regression models with family as a random factor and sex and breeding status as explanatory variables.

We visualized growth trajectories of males and females of both status groups (breeders and non-breeders) over the course of 10 years (520 weeks). Weekly mean values were taken into account for a group according to sex and breeding status, if body weights for at least three individuals of the respective status group had been collected.

Results

Wild-caught *F. darlingi* from Nsanje

Wild-caught mole-rats were of dark grey colour and displayed relatively large white head spots of different sizes and shapes (see Fig. 2). Males were larger than females (Table 1) and one of the females was pregnant with three foetuses. Other information on morphology and reproductive condition of free-living individuals can be found in Supplementary Information 1.

Karyotype of *F. darlingi* from Nsanje

The diploid chromosome number of *F. darlingi* from Nsanje was 2n = 54 with 23 metacentric chromosomes (including the single X chromosome in males) and 31 acro/telocentric chromosomes (including the single Y chromosome in males). The fundamental number was 78 with the autosomal fundamental number (aFN) being 76 (Fig. 3).

Phylogenetic position of *F. darlingi* from Nsanje and phylogeny of the genus *Fukomys*

The *CYTB* tree (Fig. 4) contained 15 lineages delimited by the branch-cutting method, which represent already recognized species of *Fukomys* (*F. foxi*, *F. zechi*, *F. mechowii*, *F. bocagei*, *F. livingstoni*, *F. hanangensis*, *F. whytei*, *F. darlingi*, *F. damarensis*, and *F. micklemi*), but also new putative species ("Chimbolo" from central Zambia, "Chinyingi" and "Watopa" from western Zambia, "Ndawambe" from the border of Zambia and Malawi, and "Viphya" from northern Malawi). The topology of the MCC tree showed the pair of haplotypes from Nsanje belong to *F. darlingi* lineage with PP = 1.00. Mole-rats from Nsanje together with mole-rats from Gorongosa in Mozambique (PP = 1.00) formed an internal lineage within *F. darlingi*. This is in accordance

Fig. 2 Selected *Fukomys* mole-rats from Malawi and adjacent regions. Shown are representatives of *F. whytei* from southern Tanzania (top left), nominate *F. darlingi* from Zimbabwe (top right), and *F. darlingi* from Nsanje in southern Malawi (below left: in the field on the day of capture; below right: captive family at the University of South Bohemia in České Budějovice). Photos by Tim Jackson and R. Šumbera

Table 1 Ecological, physiological, reproductive, and behavioural characteristics of populations of *Fukomys darlingi* from Nsanje (Malawi) and Goromonzi (Zimbabwe). If respective information is available, the results are presented as mean ± s.d

Parameter	Zimbabwe	Malawi	Reference
Locality	Goromonzi	Nsanje	
Altitude (m a.s.l.)	1323	53	
Rainfall (mm/year)	738.1 ± 240.4	949.2 ± 332.5	Rainfall station: Kwenda Mission, Zimbabwe (1890–1989); Vila Fontes, Malawi (1959–1977)
Mean family size (range)	7 (5–9)	n.a	Bennett et al. (1994), this study
Male field body mass (g)	65.3 ± 14.1 (n = 18)	138.2 ± 32.4 (n = 5)	Bennett et al. (1994), this study
Female field body mass (g)	62.9 ± 14.9 (n = 15)	101.4 ± 25 (n = 11)	Bennett et al. (1994), this study
Male captive body mass (g)	91.0 ± 12.1 (n = 12)	153.6 ± 22.6 (n = 45)	Herbst & Bennett (2001), this study
Captive body mass females (g)	72.9 ± 9.4 (n = 11)	114.0 ± 15.4 (n = 51)	Herbst & Bennett (2001), this study
Pup body mass at birth (g)	6.9–8.2 (n = 4)	9.8 ± 1.7 (6.2–13.9, n = 108)	Bennett et al. (1994), this study
Pup length at birth (cm)	3 (n = 4)	6.1 ± 0.4 (5.5–6.8, n = 11)	Bennett et al. (1994), this study
Incisors present at birth	yes	yes	Bennett et al. (1994), this study
Eyes open (days)	14	36 ± 9 (21–54, n = 14)	Bennett et al. (1994), this study
Ear meatus open (days)	12	n.a	Bennett et al. (1994), this study
Pups out of nest (days)	10	14	Bennett et al. (1994), this study
Pups eating solid food (days)	14	14	Bennett et al. (1994), this study
Age weaned (days)	± 36	n.a	Bennett et al. (1994), this study
Sparring among siblings (days)	36–40	34	Bennett et al. (1994), this study
Pregnancy length (days)	56–62 (n = 2), 63*	111 ± 4.7 (92–120, n = 80)	Bennett et al. (1994), *Herbst and Bennett (2001), this study
Litter size	1.7 ± 0.5 (1–2, n = 6)	2.3 ± 1.1 (1–5, n = 138), 3.0 ± 1.0 (1–5, n = 17) *	Bennett et al. (1994), this study, * Jerkovičová (2010)
Resting metabolic rate (RMR)	0.98 ± 0.14/110%[a]	0.76 ± 0.20/106%[a]	Bennett et al. (1993), Zemanová et al. (2012)
Width of Thermoneutral zone (TNZ, °C)	28–31.5	27–34	Bennett et al. (1993), Zemanová et al. (2012)
Conductance (C)	0.19 ± 0.03/135%[b]	0.12 ± 0.06/137%[b]	Bennett et al. (1993), Zemanová et al. (2012)
T_b at TNZ	33.0 ± 0.5	34.8 ± 1.1	Bennett et al. (1993), Zemanová et al. (2012)

[a]% RMR as predicted by curve for bathyergids: $RMR = 2.4w - 0.243$ (Zelová et al., 2007)

[b]% C as predicted by curve for a subterranean rodent: $C = 1.34w - 0.55$ (Contreras & McNab, 1990)

with their geographical distribution, as Gorongosa is the nearest locality to Nsanje. Average K2P distances between *F. darlingi* and other *CYTB* lineages ranged from 0.0688 (*F. damarensis*) to 0.1742 (*F. foxi*). The average K2P distances observed within the *F. darlingi* lineage were lower: 0.0299 in *F. darlingi* as a whole, 0.0029 within the Nsanje population, and 0.0285 between the Nsanje population and the remaining individuals assigned to *F. darlingi*. Such internal variation is comparable to other lineages with larger geographical distributions, e.g., *F. micklemi* (0.0179) or *F. mechowii* (0.0374). For the complete matrices of pairwise distances between *CYTB* lineages and between individuals of *F. darlingi* lineage, see Supplementary Information 3. All short *CYTB* sequences of museum samples were placed with very high probability (> 0.99) into *F. darlingi*. This data, together with other museum records of mole-rats from the area, indicate that this species is mainly distributed across the Mashonaland plateau in Zimbabwe and the lowland regions of Mozambique between the Zambezi and Save rivers (see Fig. 1).

The nuclear phylogenetic tree only partially matched its *CYTB* counterpart (Fig. 5). Most of the *CYTB* lineages are found also in the nuclear tree, but some of them were intermixed, namely those identified with species *F. damarensis* and *F. micklemi*. The monophyly of major *CYTB* clades, including *damarensis-micklemi*-"Chimbolo", was largely supported by nuclear data, but their relationships sometimes differed between the trees. For instance, the *darlingi* lineage has different closest relatives in both trees: the clade *damarensis-micklemi*- "Chimbolo"- "Chinyingi"- "Watopa" in *CYTB* tree, but the clade *whytei-hanagensis*-"Viphya"- "Ndawambe" in the nuclear phylogeny. Nevertheless, the mole-rats from Nsanje are again recovered as a part of the *F. darlingi* with a high support (PP = 1.00). Although they occupy the periphery of the species' range, they belong to a lineage deeply nested within *F. darlingi* (PP = 0.98).

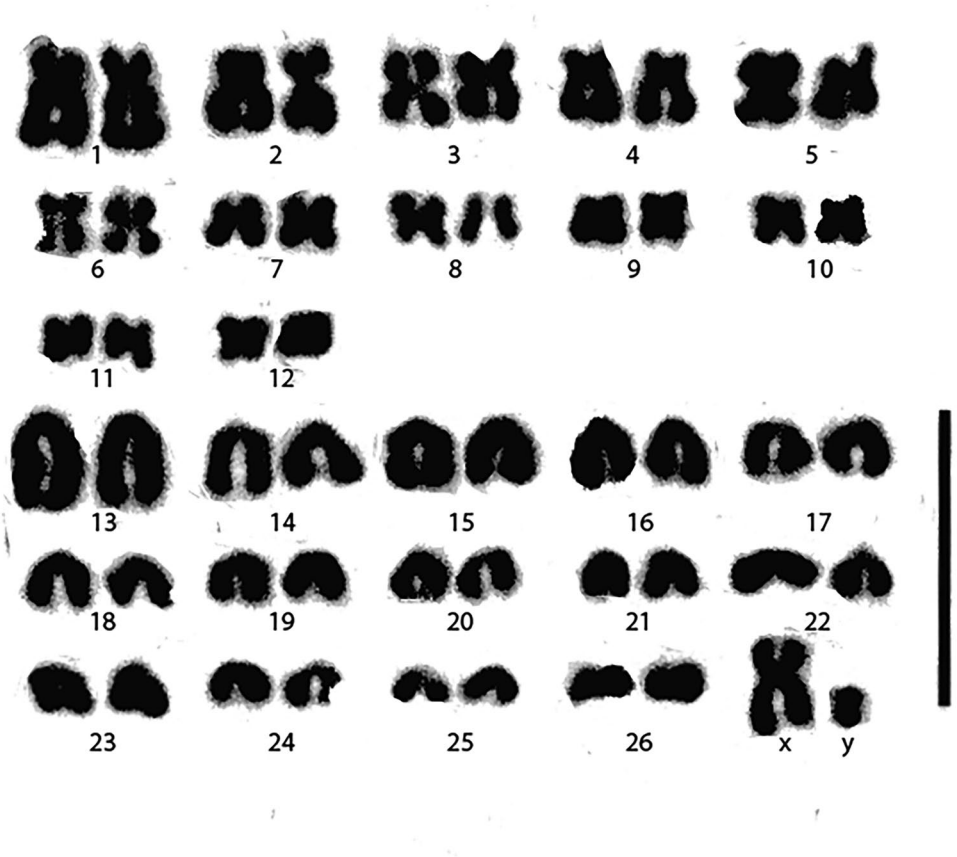


Fig. 3 Karyogram of a male *Fukomys darlingi* from Nsanje, Malawi; 2n = 54; aFN = 76. The chromosomes were paired by eye and ordered according to the position of the centromere and chromosome size. The X chromosome was arbitrarily designated. The vertical bar represents 10 µm

Reproduction, postnatal development, and sexual dimorphism of *F. darlingi* from Nsanje

Fukomys darlingi from Nsanje bred throughout the year in both labs. Based on observations from regularly breeding families, the mean inter-birth interval was 111 ± 4.7 days ($n = 80$ pregnancies of 19 females) and mean litter size 2.3 ± 1.1 ($n = 138$ litters) (Table 1). Nevertheless, average litter size was larger (3.0 ± 1.0, $n = 17$) in the first years after the establishment of the species in České Budějovice (Jerkovičová 2010).

On the day of birth, the pups were of pink colour and, if at all, only sparsely haired with a clear whitish spot on the head, which allowed for individual identification. The eyes were closed while the protruding extrabuccal incisors were already apparent. The mean body mass of neonates was 9.8 ± 1.7 ($n = 108$ pups from 50 litters) (Table 1). Body mass of pups was not influenced by litter size ($t = -1.003$; d.f. = 1; $P = 0.318$; $n = 108$ pups, 50 litters).

Pups left the nest at an age of about 2 weeks and also started to consume solid food at that time. Eyes opened at an age of 7 weeks and at the same age pups started sparring with other juveniles. Their postnatal development was slow; see Fig. 6 for the growth trajectories of 32 males (13 breeders, 19 non-breeders) and 40 females (13 breeders, 27 non-breeders) over the course of 520 weeks. Sex was found to have a significant effect on asymptotic body mass *A*, inflection point *I*, and maximum growth rate, but not on the growth constant *K* (Fig. 7, Table 2). Breeding status was shown to have an influence on the asymptotic body mass, but not on any other parameter. The combined effect of sex and breeding status exerted statistically significant effects on all parameters (*K*, *I*, and maximum growth rate), but not on the asymptotic body mass. The mean growth during the first 80 days of life was on average 0.37 ± 0.08 g/day with significant differences between the four status groups (one-way ANOVA, $F_{3,64} = 5.47$; $p = 0.002$). A Tukey HSD test revealed a significant difference between male breeders (0.43 ± 0.06 g/day) and female non-breeders (0.33 ± 0.07 g/day; $p_{adj} < 0.0015$); differences between the other multiple comparisons were not significant. Adult (≥ 18 months) body mass was determined to be 114 ± 15.4 g ($n = 51$) in captive females and 153.6 ± 22.6 ($n = 45$) in captive males and hence somewhat higher than in wild Nsanje mole-rats (Table 1).

Breeding females reproduced up to an age of at least 14 years, but the respective animals were still breeding at the time this manuscript was written. Nsanje mole-rats can still be successfully mated for the first time at an age of 8–10 years and are generally long-lived, as is typical for the genus *Fukomys*. The maximum life span is at least 19 years,

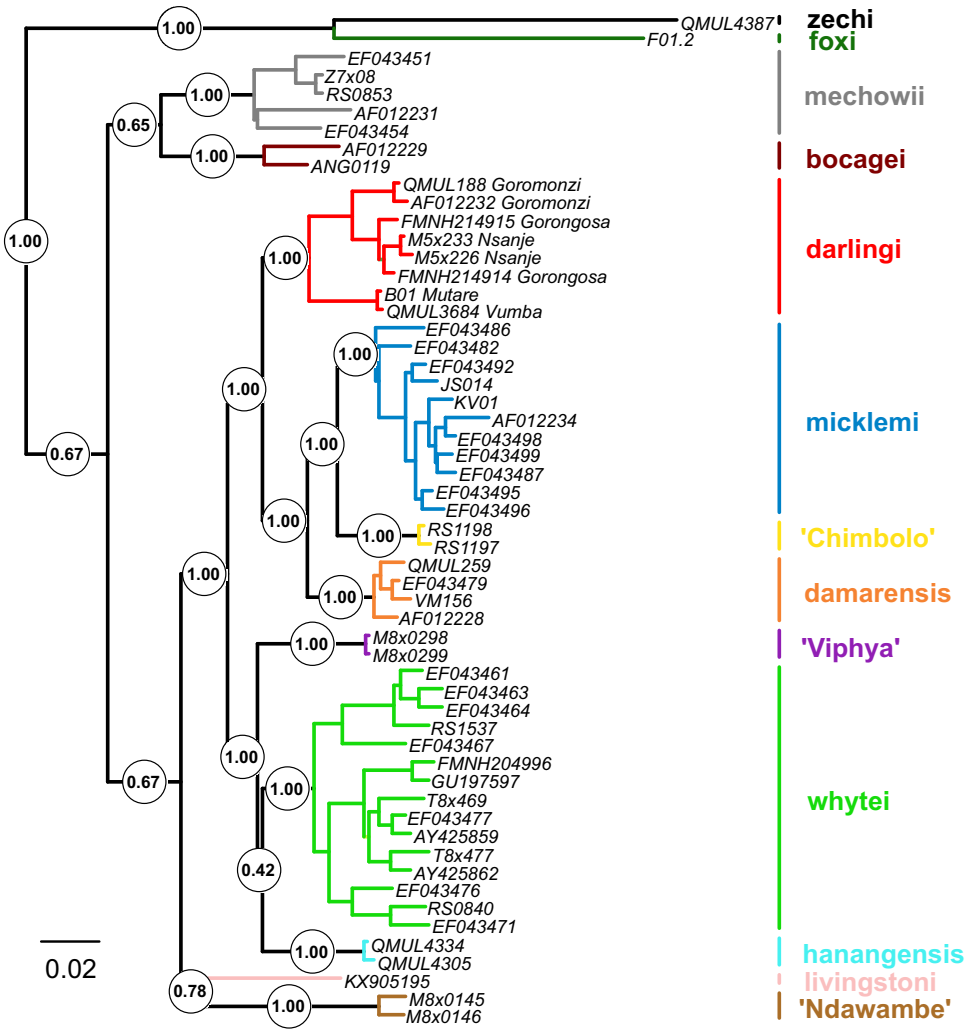


Fig. 4 Mitochondrial phylogeny of *Fukomys* mole-rats. MCC tree estimated from the *CYTB* dataset with coloured lineages delimited by the branch-cutting method. The colours of the *CYTB* lineages match those shown in the range map and the nuclear tree (Figs. 1 and 5). The tip labels contain specimen IDs and those belonging to *F. darlingi* also locality of origin

because a female trapped in the field in 2005, at an estimated age of 1 year, died in January 2023 in the vivarium at the University of South Bohemia.

Discussion

Phylogeny, diversity, and biogeography of *F. darlingi*

We demonstrate that mole-rats from Nsanje form a nested lineage within *F. darlingi* and that a species or subspecific status for these animals is unwarranted considering the genetic and karyological data presented. The Nsanje population was not even found to be monophyletic in the concatenated nuclear tree and based on *CYTB* data its average distance from the rest of *F. darlingi* is comparable to the overall variability in the species. The remarkable morphological and reproductive differences between *F. darlingi* populations thus do not align with phylogenetic distance, and instead correspond to intraspecific variation. We thus recommend retaining *F. darlingi* as a monotypic species, as is the current consensus (Honeycutt, 2016; Monadjem et al., 2015).

In the nuclear phylogeny, *F. darlingi* forms the sister lineage to the *whytei-hanangensis*-"Viphya"-"Ndawambe" clade (PP = 0.99) (Fig. 4). This relationship would make the species a part of an eastern-African radiation of *Fukomys*, which is nested in the major south-eastern African clade and whose species occupy savannah environments from Tanzania to north-eastern Zambia, Malawi, and Zimbabwe. At the same time, this inferred sister group relationship makes *F. darlingi* a clear example of mitonuclear discordance, because in the *CYTB* tree, the lineage is supported unambiguously (PP = 1.00) as a sister of the clade formed by five lineages corresponding to *F. damarensis* and *F. micklemi* s.l. (Fig. 4). The discordance could be caused by incomplete lineage sorting, which is especially common in radiations that experienced a rapid succession of ancestral speciation events. It could also be caused by introgression from the ancestor of the *micklemi-damarensis* clade into *F. darlingi*

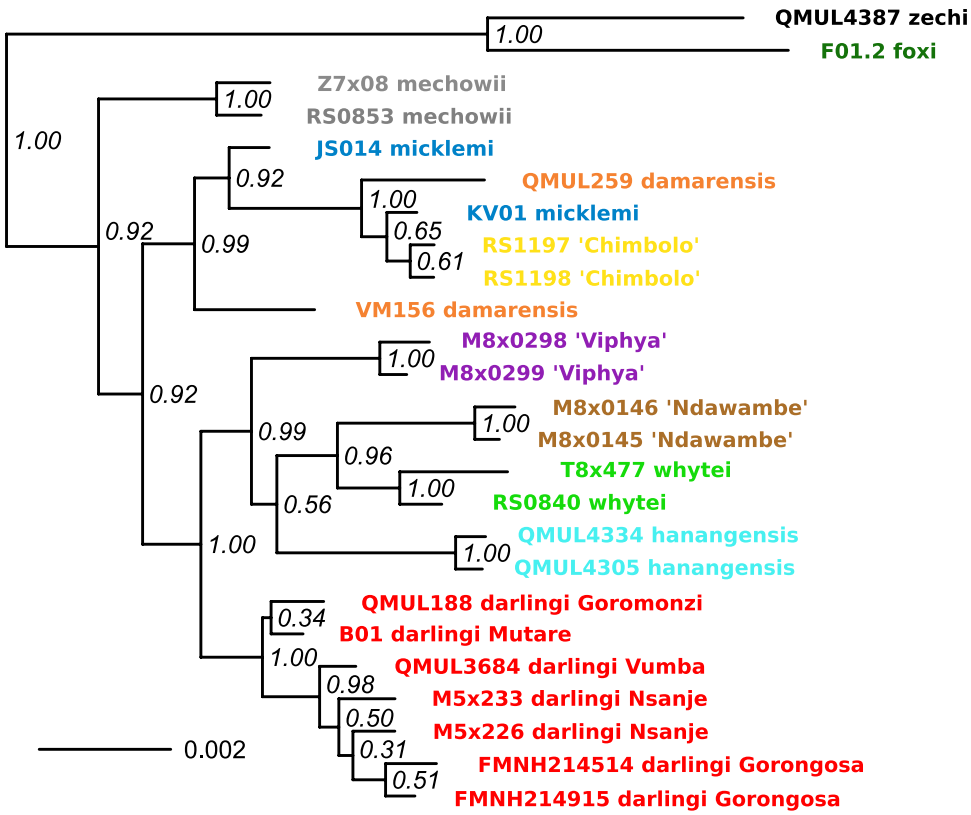


Fig. 5 Nuclear phylogeny of *Fukomys* mole-rats. MCC tree estimated from nuclear datasets of five genes. Tip labels are colour-matched with those shown in the range map and the mitochondrial tree (Figs. 1 and 4). Each tip name contains the sample ID, the name of the respective *CYTB* lineage, and in the case of samples from *F. darlingi* clade also the name of the collection locality

(or vice versa). Both, *F. darlingi* and *F. damarensis* are distributed mainly south of the Zambezi River. If assuming the same for their common ancestor and in case their split post-dated the branching of *F. darlingi* from the *whytei-hanangensis*-"Viphya"-"Ndawambe" clade, such an introgression scenario appears feasible.

Given their nested position among *F. darlingi* lineages and because all other records of *F. darlingi* are from south of the Zambezi River in eastern Zimbabwe and adjacent

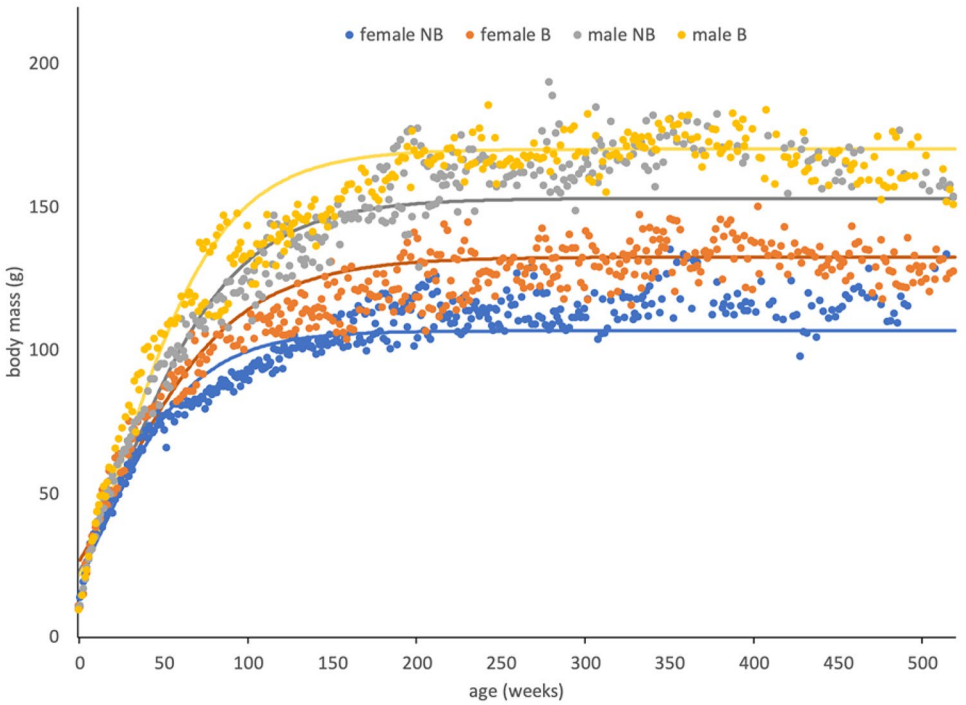


Fig. 6 Growth in captive *Fukomys darlingi* from the Nsanje population. Mean body mass (g) calculated weekly for individuals belonging to one of four groups based on sex and breeding status (non-breeding female NB, $n = 27$; breeding female B, $n = 13$; non-breeding male NB, $n = 19$; breeding male B, $n = 13$) over a period of 10 years. Gompertz growth parameters have been estimated for each individual separately, and thereafter, mean values have been calculated for each group. These parameters have been used for modelling the solid lines

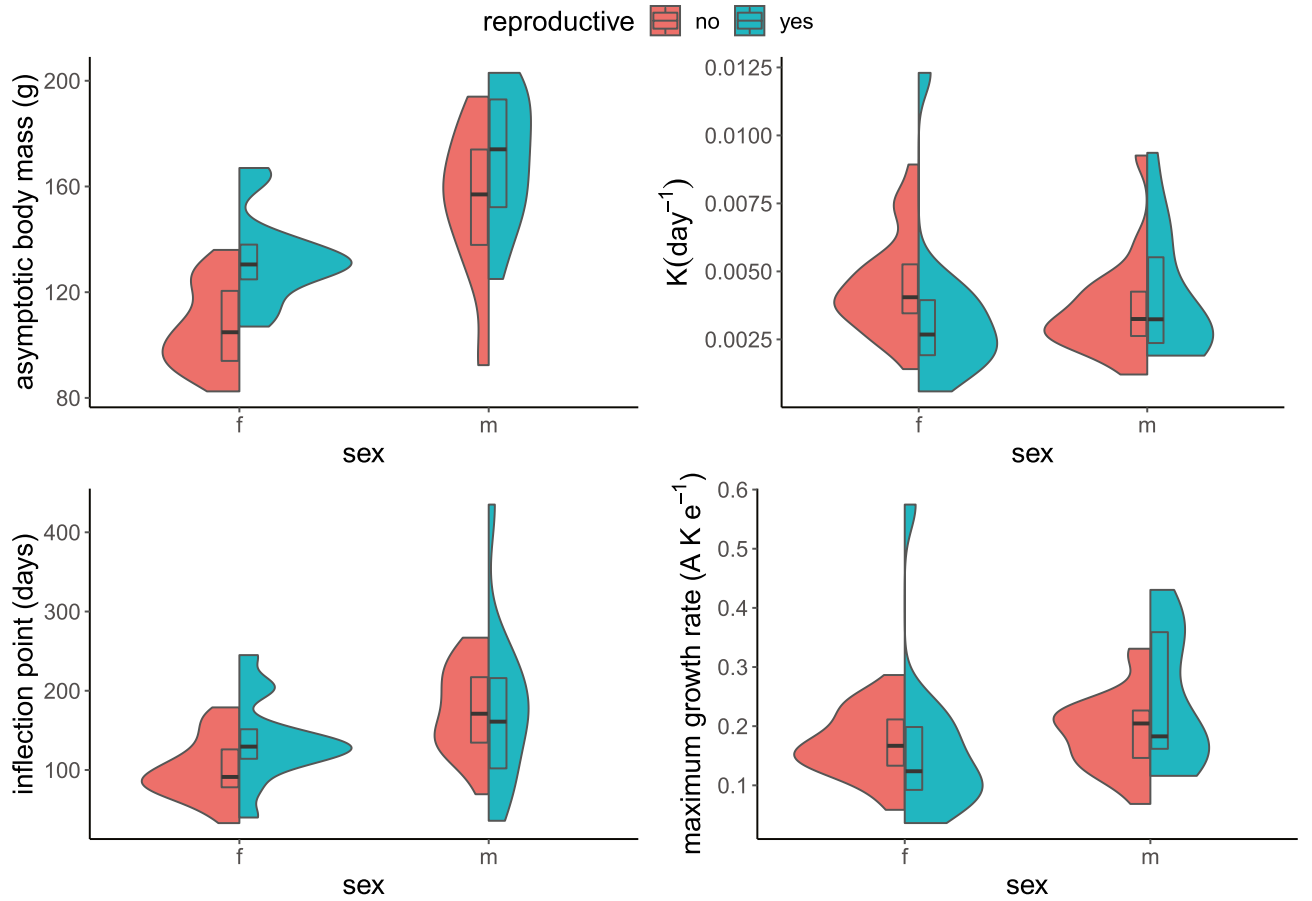


Fig. 7 Violin plots showing the distribution of growth parameters (*A*, *K*, *I*, and maximum growth rate) calculated separately for 73 individuals of *Fukomys darlingi* from the Nsanje population

parts of Mozambique, it appears likely that the ancestors of the Nsanje form of *F. darlingi* crossed the Zambezi River to colonize southern Malawi. Although mole-rats generally can swim well due to their cylindrical body and broad hands and feet (Hickman, 1978), we do not expect that they were able to actively cross such a large river, especially at its lower reaches. Alternatively, changes of the river course, which might have repeatedly isolated and released parts of mole-rat populations, could have enabled the ancestors of the Nsanje mole-rats to successively overcome this barrier. Indeed, the Zambezi and its tributaries are known for the occurrence of marked ox bows in the river course. A similar type of dispersal has been suggested to explain how annual killifish of the genus *Nothobranchius*, i.e., fishes which do not inhabit streams and are very poor swimmers, can cross the lower reaches of large rivers in the lowland flood plains of Mozambique (Bartáková et al., 2015).

We would like to briefly touch on the cases of two purported species of social mole-rats that are no longer recognized by taxonomists. Both were described from the vicinity of Beira in the lowland areas of Mozambique, namely *Georychus beirae* Thomas & Wroughton, 1907 (later *Cryptomys beirae* – see Roberts, 1951) and *Cryptomys zimbitiensis* (Roberts, 1946). Animals originally assigned to these

Table 2 Summary of linear mixed models calculated to determine the influence of sex, breeding status, and their combined effect on *F. darlingi* growth parameters *A* (asymptotic body weight), *K* (growth constant), *I* (time at inflection point, and maximum growth rate ($K \times A \times e^{-1}$)

	A			***K***			***I***			$K \times A \times e^{-1}$		
	F	d.f	*p*	*F*	d.f	*p*	*F*	d.f	*p*	*F*	d.f	*p*
Sex	67.0	63.6	$<10^{-9}$	0.1	59.2	0.77	11.4	69	0.001	11.2	58.3	0.001
Status	17.1	68.5	$<10^{-4}$	2.3	68.9	0.13	0.7	69	0.398	0.04	68.6	0.84
Sex:status	0.7	66.5	0.417	4.7	62.2	0.03	3.2	69	0.079	4.0	61.3	0.049

species are virtually indistinguishable from one another (De Graaff, 1964) and both are currently synonymized with *F. darlingi* (Happold, 2013; Mammal Diversity Database, 2022). These mole-rats have been described as being slightly larger than nominate *F. darlingi* with a somewhat lighter, more yellowish fur colouration (De Graaff, 1964). Apart from their occurrence around Beira, they were also reported from Gorongosa (Roberts, 1951; see De Graaff, 1964). Our results clearly demonstrate that mole-rats from Gorongosa are closely related to those from Nsanje. Concordantly, the description of *beirae* by Roberts (1951) matches the body measurements (head and body length, hind foot) of mole-rats from Nsanje including the presence of a white patch on the head. Both populations are larger than nominate *F. darlingi* from the Zimbabwean highlands (Roberts, 1951; Thomas, 1895). Inspection of the holotypes of *F. darlingi* (NHMUK 1895.7.16.4) and the *beirae* form (NHMUK 1907.6.2.98) did not reveal any notable differences in their coat colouration (see Supplementary Information 4). Considering these data, it seems appropriate to retain all specimens denoted as *beirae* and *zimbitiensis* within *F. darlingi*.

How many *Fukomys* species inhabit Malawi?

Although it was originally suggested that common mole-rats in Malawi belong to a single species, which was referred to as *Cryptomys hottentotus* (Ansell & Dowsett, 1988), it becomes clear that the country is home to at least four well-defined lineages of *Fukomys*, most likely distinct species (Figs. 1, 4, and 5). The northern parts of Malawi are occupied by *F. whytei*, whereas in the southernmost regions of Malawi, *F. darlingi* is present. Two other lineages provisionally named "Ndawambe" and "Viphya" occur in the central part of the country. Whereas mole-rats from Viphya seem to be related to *F. whytei* in both the *CYTB* and nuclear dataset, the position of those from Ndawambe is not clear (Figs. 4 and 5). Mole-rats from Mzuzu in northern Malawi (see Faulkes et al., 2010) seem to be almost identical to the animals from Viphya according to our unpublished *CYTB* data. For sure, these relatively isolated lineages deserve further study. Ansell and Dowsett (1988) proposed that *F. mechowii* (Peters, 1881), the giant mole-rat, which is abundant in northern Zambia, also occurs in northern Malawi. However, the single specimen that this assumption was based on exhibited a head spot, which is typically absent in this species (Caspar et al., 2021b). No further records of *F. mechowii* from Malawi have been noted since then, and it appears that the species' eastern range is limited by the Zambian Muchinga Escarpment and the Luangwa River system (see Caspar et al., 2021b). Thus, its range should not extend into Malawi. In case *F. mechowii* is indeed absent from Malawi and the mole-rat populations from Ndawambe and Viphya are recognised as species in future analyses, the bathyergid fauna of the country will consist of five species: four social *Fukomys* and the solitary silvery mole-rat *Heliophobius argenteocinereus*.

Karyotypic differentiation within *F. darlingi*

The karyotype of *F. darlingi* from Nsanje greatly resembles that of the nominate form sampled at Goromonzi (Zimbabwe). Both possess a diploid number of 2n = 54 (Aguilar, 1993, this study), but there are deviations in the autosomal fundamental number (aFN = 76 in this study, aFN = 80 in Aguilar's study). Aguilar (1993) reports three pairs of submetacentric chromosomes that could not be identified in our sample. Because it is difficult to differentiate the submetacentric from the acrocentric chromosomes in the karyogram presented by Aguilar (1993), it might well be that there is no difference at all in the karyotypes of Nsanje and nominate *F. darlingi.*

Variation in body size in *F. darlingi*

One of the most remarkable differences between populations of *F. darlingi* from Nsanje and the nominate form studied at Goromonzi lies in their body mass. Wild-caught mole-rats from Nsanje are almost twice as large as individuals from Goromonzi used to establish a captive breeding group at the University of Pretoria (Bennett et al., 1994; this study, Table 1, Fig. 8). Adults of the nominate form from Goromonzi exhibit a mean body mass of 70.6 g (±7.5 g; $n = 8$) in females and 92.6 g (±12.4 g; $n = 10$) in males (Bennett et al., 1994; Gabathuler et al., 1996; Herbst & Bennett, 2001), while it was

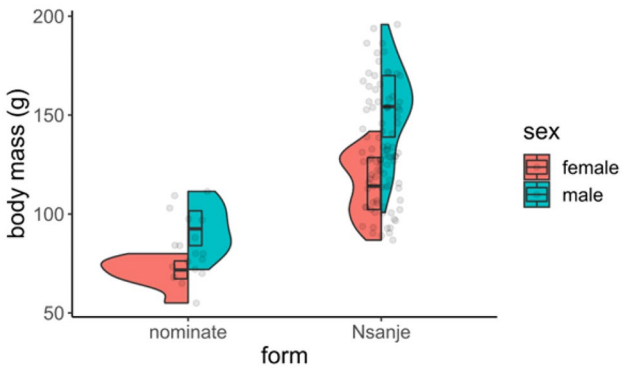


Fig. 8 Adult body mass distribution of *Fukomys darlingi* from Zimbabwe (nominate form, $n = 18$) and southern Malawi (Nsanje form, $n = 96$). Note the larger body mass and greater sexual dimorphism in animals from Nsanje. Data on *F. darlingi* individuals from Goromonzi were compiled from Bennett et al. (1994), Gabathuler et al. (1996), and Herbst and Bennett (2001); animals were deemed adult when identified as breeders or when being heavier as the same-sex breeder in the respective family. Nsanje mole-rats were classified as adults at an age of 18 months

found to be 114 g (± 15.4 g; $n = 51$) in Nsanje females and 153.6 g (± 22.6, $n = 45$) in Nsanje males older than 18 months (Table 1). This is also the case for Zimbabwean *F. darlingi* (Bennett et al., 1997). Interestingly, Genelly (1965) reported body mass of males 110 g (80–156, $n = 16$) and females 87 g (50–114, $n = 13$) from a mole-rat population in Harare, the type locality of *F. darlingi* (and not far from Goromonzi). It seems that mole-rats from Goromonzi are the smallest representatives of *F. darlingi,* and in fact among the smallest of all *Fukomys* currently known.

Pelage colouration

Happold (2013) described the nominate form of *F. darlingi* as an animal with a longitudinal white stripe on the ventrum which may extend along the full medial axis of the body. Other authors have not mentioned such stripes, nor did we find them in museum specimens of the species, including the holotype. It is also absent in the Nsanje form, but some individuals had white markings stretching from the chin down to the throat. In other species of *Fukomys*, such as *F. damarensis*, *F. anselli*, and *F. micklemi*, the presence of a medial abdominal stripe is also a polymorphic trait (pers. obs.). Size and shape of the head spots in the Nsanje mole-rats correspond well to detailed descriptions of this characteristic available for *F. darlingi* from Harare (Genelly, 1965). Animals from Goromonzi also exhibit a large head spot (see Fig. 2). Compared to the slate grey animals from Nsanje, *F. darlingi* from Goromonzi typically have a more brownish colour (Supplementary Information 4). Nevertheless, we may assume that fur colouration bears no taxonomic value because large variation in pelage colour ranging from light brown to dark grey was found among different families caught at the same locality and even within families of *F. darlingi* from Harare (Genelly, 1965). In addition, brownish colored juveniles born to grey-furred parents were observed in two breeding groups at the lab at the University Duisburg-Essen (pers. obs.). The brown colouration of these juveniles turns to slate grey at an age of about 18 months. In other captive families of Nsanje mole-rats, such a pattern is not apparent. While some species of *Fukomys*, such as *F. anselli* and *F. mechowii*, exhibit a well-documented ontogenetic colour change (Burda, 1989; Scharff et al. 1999), this trait has so far not been described in *F. darlingi*. In old animals (> 12 years), we noticed that the fur, particularly on the head, progressively turns pale.

Ontogeny and reproduction

In several reproductive parameters, mole-rats from Nsanje resemble similarly sized *Fukomys* species, especially *F. damarensis*, more than nominate conspecifics. This is the case, for instance, for neonate body mass which is 8–9 g in *F. damarensis* (Bennett & Jarvis, 2004; Bennett et al., 1991), but 6.9–8.2 g in *F. darlingi* from Goromonzi. The difference is also remarkable in mean litter size, which is 3.0 (1–6) in *F. damarensis* (Bennett & Jarvis, 2004) and 2.3 pups in the Nsanje *F. darlingi* studied here, but only 1.7 in *F. darlingi* from Goromonzi (Bennett et al., 1994). Whereas smaller litter size in *F. darlingi* from Goromonzi could be attributed to their smaller body size it may also be an artefact created by the low sample size of just $n = 2$ (Table 1) for this population. Although litter size in Nsanje mole-rats based on our long-term records is relatively small (2.3), it was larger (3.0) in the beginning of the history of our breeding in České Budějovice when a detailed study on reproduction was carried out (Jerkovičová, 2010). We assume that the smaller average litter sizes recovered in this study are caused by including a large number of usually smaller litters of young breeding females from recently established families in Essen. It can be hypothesized that average litter size may increase with parity in regularly breeding females similarly to, for instance, *F. mechowii* (Scharff et al., 1999). Behavioural parameters such as leaving the nest, starting to consume solid food, and the emergence of agonistic interactions between pups are comparable to nominate *F. darlingi* (see Table 1).

The most pronounced inter-population difference within *F. darlingi* seems to be the length of pregnancy (i.e., inter-birth interval). Assuming that the minimum inter-birth interval corresponds to the maximum length of pregnancy (as in *F. anselli*, cf. Burda, 1989, 1990), we estimate the length of gestation in *F. darlingi* from Nsanje to be about 111 days. This fits the typical *Fukomys* pattern: 98 days in *F. anselli* (Begall & Burda, 1998; Burda, 1989); 112 days in *F. mechowii* (Scharff et al., 1999); 78–92 days in *F. damarensis* (Bennett & Jarvis, 1988, 2004). We found much longer pregnancies in the Nsanje form than were reported for *F. darlingi* from Zimbabwe. For these animals, Bennett et al. (1994) and Herbst and Bennett (2001) reported gestation lengths of just about 60 days (Table 1). Due to very low sample sizes reported, it is hard to evaluate if this is a real inter-population difference or, alternatively, an observational artefact. Observed mating events do not necessarily lead to successful fertilisation and in addition, it is known that females of *Fukomys* engage in copulations even when already pregnant (Burda, 1989), making estimates based on this behavioural measure dubious. It should also be mentioned that such great intraspecific differences in the length of pregnancy would be very unusual for small mammals (Kiltie, 1982). Interestingly, the reported lowest values for intervals between litters or timespans from pairing to parturition in *F. darlingi* from Goromonzi correspond well to mean inter-birth interval in our study (Bennett et al., 1997; Greeff & Bennett, 2000). This may indicate that the length of pregnancy has been underestimated for the Zimbabwean population of the species. Nevertheless, this topic surely deserves further study.

Table 3 Comparison of growth parameters estimated according to the Gompertz model for different bathyergid species. *A*, asymptotic body weight (g); *I*, age at inflection point (days); *K*, growth constant (days^{-1}); $K \times A \times e^{-1}$, maximum growth rate (g/day)

Species	*A*	*I*	*K*	$K \times A \times e^{-1}$	Source
Bathyergus suillus	217.5	22.3	0.042	3.34	Bennett et al. (1991)
Bathyergus janetta	90.8	14.8	0.052	1.68	Bennett et al. (1991)
Georychus capensis	74.6	16.9	0.044	1.23	Bennett et al. (1991)
Heliophobius argenteocinereus	138	64	0.01	0.53	Šumbera et al. (2003)
Cryptomys h. hottentotus	42	12.6	0.015	0.23	Bennett et al. (1991)
Fukomys anselli	106	114.6	0.006	0.24	Begall and Burda (1998)
F. damarensis	42.5	15.6	0.015	0.23	Bennett et al. (1991)
F. darlingi (Zimbabwe)	92.6	94.1	0.008	0.27	Bennett et al. (1994)
F. darlingi (Malawi)	135.2	141.5	0.004	0.19	This work
F. mechowii	341.9	186.8	0.006	0.7	Scharff et al. (1999)
Heterocephalus glaber			0.017	0.21	O'Riain et al. (1998)

A comparison of Gompertz growth parameters (Table 3) reveals that *F. darlingi* originating from Nsanje exhibit the lowest growth constants and the lowest maximum growth rates (0.19 g/day) of all bathyergids. This is consistent with our observations from the laboratory, indicating that these animals may still grow at an age of 2 years, when congeneric species are considered to be fully adult. Since many of the reported species growth parameters have asymptotic values much lower than the actual body masses, it is obvious that the calculations were based on incomplete records (i.e., animals not fully grown) which leads to an overestimation of the growth constants (see Begall, 1997, for details). The growth constants obtained for *F. darlingi* from Nsanje are slightly lower than those of other *Fukomys* species for which reliable data are available (e.g. *F. anselli* – Begall & Burda, 1998, *F. mechowii* – Scharff et al., 1999), but still in the same order of magnitude (Table 3).

Differences in growth parameters between the sexes (as well as between breeders and non-breeders) are evident also in animals older than 80 days reflecting sexual size dimorphism typical for *Fukomys* mole-rats (Caspar et al., 2021c). The difference between the mean growth rates during the first 80 postnatal days of (future) male breeders compared to female non-breeders might be an artefact of the selection regime for breeders. As the initial male population was very small ($n = 4$), rather large fast-growing males of the first generation of offspring might have been preferentially picked as founders of new families.

The only previously available estimate for the life span of *F. darlingi* was 7 years (Greeff & Bennett, 2000). Our data suggest that the maximum life span of the species is much longer, i.e., at least 19 years. This corresponds better to data recorded of other *Fukomys* species, which may live to an age beyond 20 years (Begall et al., 2021; Dammann pers. com.; Fang et al., 2014).

What causes inter-population differences in *F. darlingi*?

Both inter- and intraspecific differences in various morphological parameters can frequently be related to the ecological conditions the respective populations face. One of the best-known ecological rules explaining differences in body mass is Bergmann's rule, proclaiming that within clades of endothermic vertebrates, those living in colder environments are larger than those deriving from warmer habitats (Bergmann, 1847). The range of *F. darlingi* indeed covers areas differing markedly in ambient temperatures, which are chiefly coupled to altitude. For instance, Nsanje is situated close to sea level, while Goromonzi is located over 1000 m a.s.l. Thus, the intraspecific body mass pattern fits the inverse to Bergmann's rule, with smaller mole-rats occupying higher altitudes (Table 1). Among subterranean mammals, the inverse to the Bergman rule has been described already in the South American tuco-tucos of the genus *Ctenomys* (Medina et al., 2007). Unfortunately, due to lack of data, it is hard to estimate which other factor such as different soil or food characteristics may play a role in shaping such dramatic differences in body mass.

In *F. darlingi*, it appears that the variability in body mass is indeed responsible for many inter-population differences that have been characterized in various studies. Apart from parameters related to reproduction, another example is the ability to keep a stable core body temperature. Whereas smaller *F. darlingi* from the nominate population lack this capacity at low ambient temperatures and show signs of heterothermy (Bennett et al., 1993), their conspecifics from Nsanje are truly homeothermic keeping their body temperature stable across a substantial range of experimental ambient temperatures (Zemanová et al., 2012; but see another study on *F. darlingi* from Goromonzi in which mole-rats do not show such remarkable decrease of T_b in lowest measured $T_a = 18$ °C, Boyles et al., 2012). Although Zemanová et al. (2012) speculated that the animals tested by Bennett

et al. (1993) were not fully grown, the fact that some of them were already able to reproduce demonstrates that deviations in body mass cannot be attributed to ontogeny. This remarkable intraspecific difference in thermal biology has been explained by the overall lowered ability to defend stable T_b in smaller animals with worse surface body mass ratio (Zemanová et al., 2012).

Conclusion

In our study, we took an integrative approach to analyse the phylogeny and biology of an isolated lowland population of social African mole-rats from southern Malawi. Our multilocus phylogenetic analysis based on samples of both fresh and museum-conserved tissues convincingly demonstrated that the mole-rats from Nsanje are Mashona mole-rats, *Fukomys darlingi*, a species otherwise distributed across the highlands of Zimbabwe and lowland regions of Mozambique. Karyological data align with this diagnosis. Overall, the bathyergid mole-rat fauna of Malawi was shown to likely encompass at least four species of *Fukomys* besides one of the genus *Heliophobius*. We also document an instance of mitonuclear discordance in major lineages of *Fukomys*.

We further present information on various life history parameters of the Nsanje population of *F. darlingi*. Although there are some remarkable inter-population differences between the Nsanje and the nominate form of these mole-rats in reproductive and physiological characteristics, these deviations can largely be attributed to dramatic differences in body mass between animals from different localities. Future studies should address why lowland populations of *F. darlingi* from warm humid areas are notably larger than those from cooler highland environments.

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Author contribution RS, HB, and PVD conceived and designed the study. RS provided funding. RS and WNC collected animals in the field. RS, DJ, KRC, and SB collected and analysed morphological, reproductive, and postnatal development data. RS, WNC, PVD, CGF, and NCB provided material for genetic analysis. CJ and SB performed karyotyping. MU performed genotyping. MU and OM performed phylogenetic analyses. RS, OM, KRC, SB, and MU wrote the first draft of the manuscript that was complemented by all authors. All authors approved the final version of the manuscript.

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Availability of data and materials GPS of localities, museum voucher numbers, GenBank Accession numbers, and other details about specimens included in analyses are provided in Supplementary Information 2. New DNA sequences were uploaded to GenBank and their accession numbers (OQ559424-OQ559475, OQ442881–OQ442896, OQ442843–OQ442861, OQ559483–OQ559501, OQ559505–OQ559523, OQ442862–OQ442880) are in Supplementary Information 2.

Code availability Not applicable.

Declarations

Ethics approval This work complies with all current laws governing research in the Czech Republic, Germany, and Malawi. GRBC, the Technical Committee of the National Research Council of Malawi, permitted to carry out our research in Malawi and export the mole-rats.

Consent to participate All authors gave consent to participate.

Consent for publication All authors gave consent for publication.

Conflict of interest The authors declare no competing interests.

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