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Multiple radiations of spiny mice (Rodentia: *Acomys*) in dry open habitats of Afro-Arabia: evidence from a multi-locus phylogeny

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Abstract

Background: Spiny mice of the genus *Acomys* are distributed mainly in dry open habitats in Africa and the Middle East, and they are widely used as model taxa for various biological disciplines (e.g. ecology, physiology and evolutionary biology). Despite their importance, large distribution and abundance in local communities, the phylogeny and the species limits in the genus are poorly resolved, and this is especially true for sub-Saharan taxa. The main aims of this study are (1) to reconstruct phylogenetic relationships of *Acomys* based on the largest available multilocus dataset (700 genotyped individuals from 282 localities), (2) to identify the main biogeographical divides in the distribution of *Acomys* diversity in dry open habitats in Afro-Arabia, (3) to reconstruct the historical biogeography of the genus, and finally (4) to estimate the species richness of the genus by application of the phylogenetic species concept.

Results: The multilocus phylogeny based on four genetic markers shows presence of five major groups of *Acomys* called here *subspinosus*, *spinosissimus*, *russatus*, *wilsoni* and *cahirinus* groups. Three of these major groups (*spinosissimus*, *wilsoni* and *cahirinus*) are further sub-structured to phylogenetic lineages with predominantly parapatric distributions. Combination of alternative species delimitation methods suggests the existence of 26 molecular operational taxonomic units (MOTUs), potentially corresponding to separate species. The highest genetic diversity was found in Eastern Africa. The origin of the genus *Acomys* is dated to late Miocene (ca. 8.7 Ma), when the first split occurred between spiny mice of eastern (Somali-Masai) and south-eastern (Zambezi) savannas. Further diversification, mostly in Plio-Pleistocene, and the current distribution of *Acomys* were influenced by the interplay of global climatic factors (e.g., Messinian salinity crisis, intensification of Northern Hemisphere glaciation) with local geomorphology (mountain chains, aridity belts, water bodies). Combination of divergence dating, species distribution modelling and historical biogeography analysis suggests repeated “out-of-East-Africa” dispersal events into western Africa, the Mediterranean region and Arabia.

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Conclusions: The genus *Acomys* is very suitable model for historical phylogeographic and biogeographic reconstructions of dry non-forested environments in Afro-Arabia. We provide the most thorough phylogenetic reconstruction of the genus and identify major factors that influenced its evolutionary history since the late Miocene. We also highlight the urgent need of integrative taxonomic revision of east African taxa.

Keywords: *Acomys*, Savanna, Biogeography, Africa, Arabia, Sahara, Somali-Masai, Zambeziian savanna, Plio-Pleistocene

Background

The Old-World savanna biome spans the tropical grasslands, scrublands and wooded savannas of sub-Saharan Africa [1]. These open non-forested habitats represent the most widespread terrestrial environment in Africa [2] and they harbour one of the most abundant and diverse mammalian communities on Earth [3]. In Africa, four major biogeographic regions can be distinguished, which are defined by the geographical distribution of vascular plants and terrestrial vertebrates, where savanna-like ecosystems predominate (Zambeziian, Somali, Sudanian and South African [3]).

Numerous geological and climatic events have affected the biological diversity of contemporary savanna-like ecosystems in Africa. In Eastern Africa, the East African Rift System (EARS) started to develop *ca.* 45–33 Ma [4], which led to a change in the region's topography and the consequent aridification of East Africa, most intensively since late Miocene [5–8]. These climatic changes are best documented by the shift from C3 (moisture-adapted plants) to C4 (tropical arid-adapted grasses) plants [9–11]. The climatically turbulent Pliocene and especially Pleistocene periods, when arid and humid conditions alternated, resulted in a series of expansions and contractions of climatic zones [12, 13] that influenced the distribution and diversification of biodiversity in this region [14, 15].

Small mammals, especially rodents, are very good model organisms for phylogeographic reconstructions. Most of them are usually habitat specialists, exhibit low dispersal ability and have relatively high substitution rates, at least at mitochondrial DNA (mtDNA). Spiny mice of the genus *Acomys* I. Geoffroy Saint-Hilaire, 1838 inhabit seasonally dry open habitats in large regions of sub-Saharan Africa, the Eastern Mediterranean and the Arabian Peninsula [16]. Because they usually constitute abundant parts of local small mammal communities and their samples are easy to collect, they potentially represent a suitable group for testing hypotheses pertaining to the biogeography of dry open habitats in Africa and Arabia. *Acomys* belongs to a handful of rodent taxa that have been extensively studied for decades, and they have been used in several fields of study (e.g. ecology [17–22], physiology [23–26] and evolutionary biology [27–29]).

Nevertheless, the vast majority of these studies was performed on taxa from Israel and neighbouring areas of the Middle East, representing only small fragment of the phylogenetic diversity of the genus [30–33].

The genus *Acomys* was described as a separate taxon at the beginning of the nineteenth century, but there is still no synthesis of diversity across the genus, even though species names and descriptions abound [16, 34–38] (see Additional file 1). There were repeated attempts for systematic classification of *Acomys* using morphological characters [34, 39] and chromosomes [38, 40] (see Additional file 1). However, many currently recognized species cannot be easily distinguished using morphological characters due to significant intraspecific variability and generalized morphology. Available molecular studies [30–33, 41–46], are all based on limited taxon sampling and/or geographic coverage. Furthermore, earlier studies largely relied only on sequences of mitochondrial genes, which can be misleading in species delimitations (e.g., [47, 48]). To conclude, the estimation of the total number and delimitation of extant *Acomys* species and their biogeographical history would benefit from a more extensive study based on multiple molecular markers.

Aims

In this study, we focus on phylogeography and biogeography of the genus *Acomys* by phylogenetic analysis of the largest available dataset and substantially improved geographic and taxon sampling. The aims of this study are (1) to reconstruct the phylogeny of the genus using multilocus dataset; (2) to identify the main biogeographical divides in the distribution of *Acomys* diversity in seasonally dry open habitats in Afro-Arabia; (3) to test proposed hypotheses of historical biogeography of the genus *Acomys* (i.e. to disentangle the role of geomorphology and climate changes on their diversification), with particular focus on dispersal events among major dry regions in Africa and between Africa, the Arabian Peninsula and the Eastern Mediterranean; (4) to estimate the species richness of the genus by applying of the phylogenetic species concept to identify the genetic groups and geographical regions that are worth further integrative taxonomic studies.

Results

Phylogeny, species delimitation, and distribution of genetic variability of *Acomys*

Both Bayesian inference (BI) and Maximum Likelihood (ML) analyses of concatenated multilocus data provided very similar phylogenetic reconstructions and revealed five major groups that we will hereafter call *subspinosus*, *spinosissimus*, *russatus*, *wilsoni* and *cahirinus* (Fig. 1a). If the number of nodes that are supported by $PP \geq 0.95$ or $BP \geq 70$ are considered, BI analyses yielded a slightly more robust topology (79 supported nodes; topology

shown at Fig. 1a) compared to the ML tree (64 supported nodes; topology not shown). Five major groups are also supported in separate mitochondrial (not shown) and nuclear (Additional files 2 and 3) gene trees.

Three of these major groups (*spinosissimus*, *wilsoni*, and *cahirinus* groups) are further sub-structured to phylogenetic lineages (Fig. 1, Additional files 2 and 3) with predominantly parapatric distributions (Fig. 2). Based on the topology and the shape of the phylogenetic trees and the geographical distribution of genetic variability, we identified 26 distinct genetic lineages as our

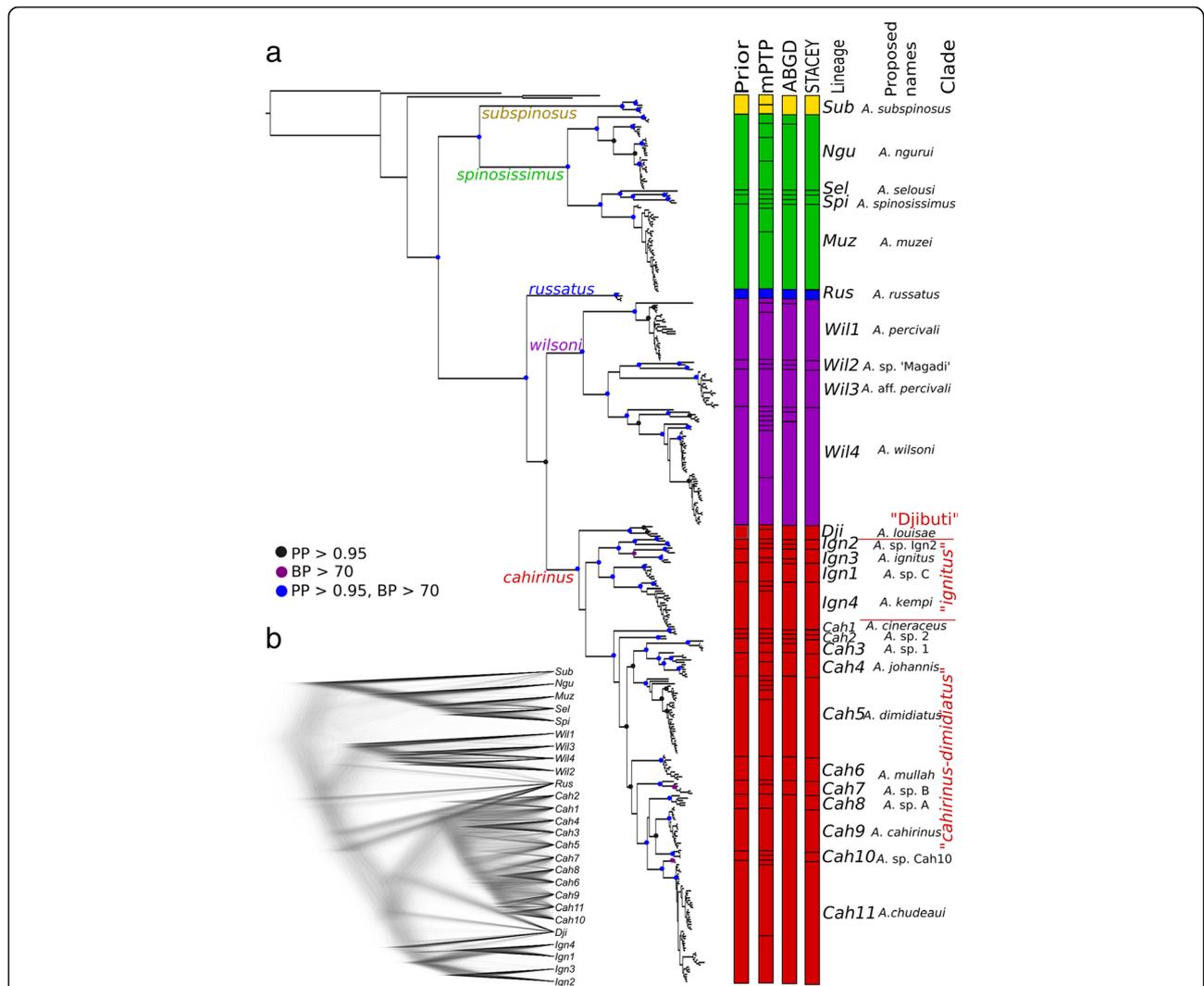
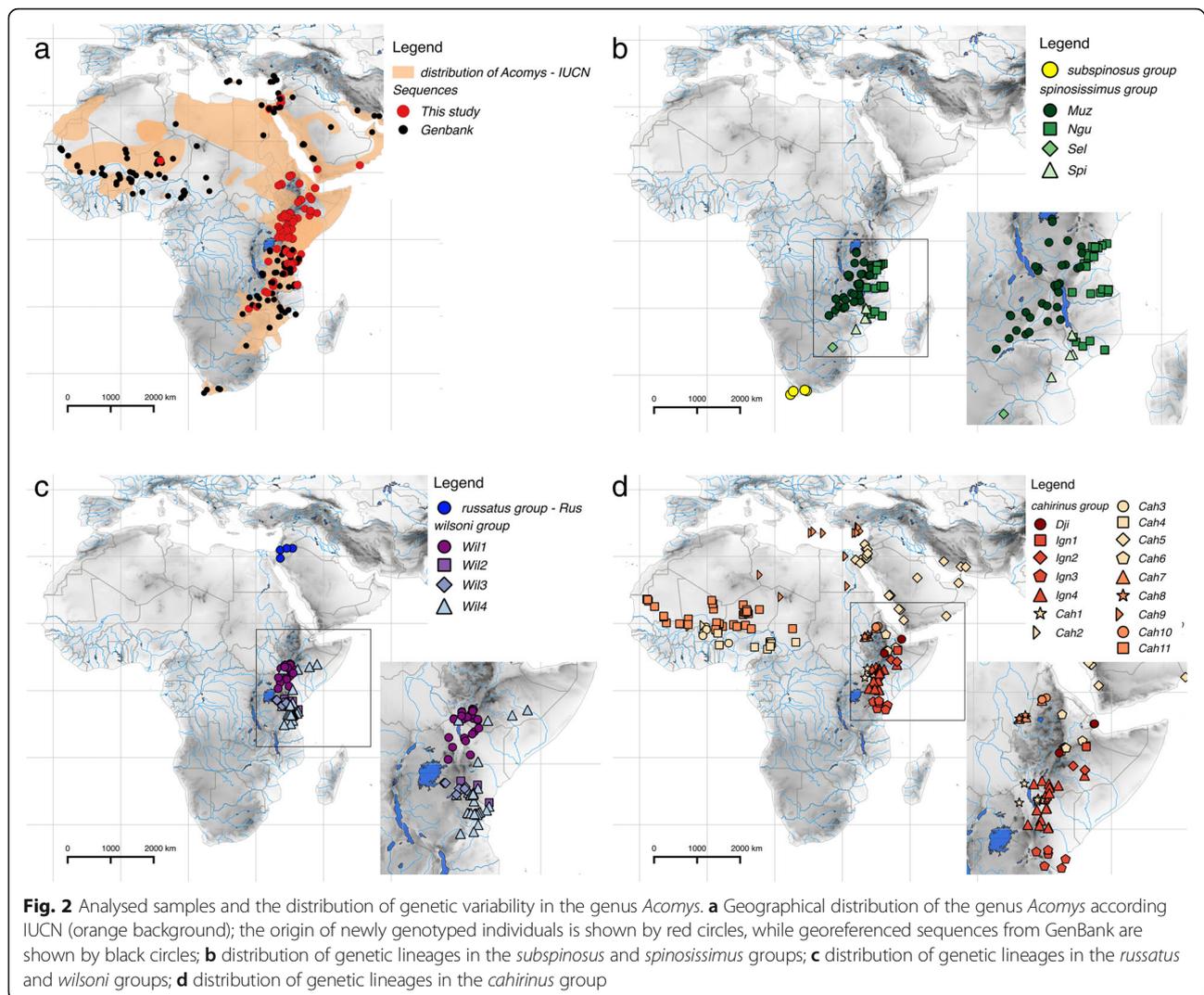


Fig. 1 Multilocus phylogeny of the genus *Acomys*. **a** Bayesian phylogeny of the concatenated multi-locus matrix calculated in MrBayes. The support from Bayesian analysis in MrBayes (posterior probability, PP) and maximum likelihood analysis in RAXML (bootstrap probability, BP) is indicated by different colours on the nodes (black $PP > 0.95$, $BP < 70$; violet $PP < 0.95$, $BP > 70$; blue $PP > 0.95$, $BP > 70$). Five main *Acomys* groups (*subspinosus*, *spinosissimus*, *russatus*, *wilsoni* and *cahirinus*) are shown by different colours. The results of four different species delimitation approaches (“by-eye” prior; two delimitation approaches based on mtDNA: mPTP, ABGD; and a multilocus species delimitation in STACEY - see more details in the text) are shown in columns on the right, where individual “species” are separated by black lines. Additional information for 26 delimited taxa are provided, abbreviation of the lineages, and previously used taxonomic assignments. **b** DensiTree cloudogram of coalescent species trees from STACEY (for MCC species tree with PP see Additional file 4)



prior candidate species for next steps of genetic species delimitation (Fig. 1a). Their mutual relationships are relatively well resolved (with three well-defined clades: “Djibuti”, “ignitus” and “cahirinus-dimidiatus”, in the *cahirinus* group), with the important exception of lineages *Cah1-Cah11* representing probably a rapid radiation of the “cahirinus-dimidiatus” clade (Fig. 1a). The multispecies coalescent species tree from Species Tree And Classification Estimation, Yarely (STACEY) also revealed very similar topology to that reconstructed from the concatenated super-matrix (Fig. 1b, Additional file 4). The main differences are in unresolved positions of the *russatus* group and the “Djibuti” clade and a weakly resolved topology within the three major groups (*spinosissimus*, *wilsoni*, *cahirinus*; Additional file 4).

Species discovery approaches based on mitochondrial cytochrome *b* gene (*CYTB*) variability split the genus *Acomys* into 57 putative species in multi-rate Poisson Tree Process (mPTP) and 32 putative species

in Automatic Barcode Gap Discovery (ABGD; Fig. 1), but ABGD did not find any clear gap between intra- and interspecific distances (“barcoding gap”; not shown). On the other hand, results from multispecies coalescent analysis in STACEY supported all 26 a priori defined species as separate gene pools (Fig. 2a). Taking into account the fact that multispecies coalescent does not statistically distinguish structure associated with population isolation vs. species boundaries [49], we will therefore use the term “species” for genetically distinct lineages or molecular operational taxonomic units (MOTUs).

The *subspinosus* group with only one lineage (*Sub*) is limited to South Africa (Fig. 2b). The separate species status was confirmed by all species delimitation analyses, and mPTP even suggested two different mitochondrial sublineages as two separate species (Fig. 1a). Mean intra-specific genetic distance is 1.5%, the distance to the nearest lineage is 21.3% (Table 1).

Table 1 Genetic distances calculated from BI phylogenetic tree by the Species Delimitation algorithm in Geneious. Intraspecific distances and interspecific distances from the nearest lineage in percents (%)

Group	Lineage	Nearest lineage	Intra Dist	Inter Dist - Closest
<i>subspinosus</i>	<i>Sub</i>	<i>Ngu</i>	1.5	21.3
<i>spinosissimus</i>	<i>Ngu</i>	<i>Muz</i>	2.9	10.7
	<i>Sel</i>	<i>Spi</i>	0.0	7.1
	<i>Spi</i>	<i>Sel</i>	3.2	7.1
	<i>Muz</i>	<i>Spi</i>	1.1	8.0
<i>russatus</i>	<i>Rus</i>	<i>Ign1</i>	0.5	14.4
<i>wilsoni</i>	<i>Wil1</i>	<i>Wil2</i>	1.5	12.3
	<i>Wil2</i>	<i>Wil3</i>	4.7	10.5
	<i>Wil3</i>	<i>Wil2</i>	1.0	10.5
	<i>Wil4</i>	<i>Wil2</i>	3.1	9.6
<i>cahirinus</i>	<i>Dji</i>	<i>Ign1</i>	2.2	9.6
	<i>Ign2</i>	<i>Ign3</i>	2.9	6.4
	<i>Ign3</i>	<i>Ign2</i>	2.9	6.4
	<i>Ign1</i>	<i>Ign4</i>	0.7	5.9
	<i>Ign4</i>	<i>Ign1</i>	1.6	5.9
	<i>Cah1</i>	<i>Cah2</i>	0.6	7.5
	<i>Cah2</i>	<i>Cah6</i>	0.8	6.5
	<i>Cah3</i>	<i>Cah4</i>	0.8	6.1
	<i>Cah4</i>	<i>Cah5</i>	2.1	6.0
	<i>Cah5</i>	<i>Cah4</i>	1.4	6.0
	<i>Cah6</i>	<i>Cah9</i>	0.8	5.5
<i>Cah7</i>	<i>Cah9</i>	1.3	5.5	
<i>Cah8</i>	<i>Cah9</i>	1.2	4.0	
<i>Cah10</i>	<i>Cah9</i>	0.6	3.0	
<i>Cah9</i>	<i>Cah10</i>	0.6	3.0	
<i>Cah11</i>	<i>Cah9</i>	1.2	4.5	

The *spinosissimus* group inhabits the eastern part of the Zambezi bioregion (Fig. 2b). The STACEY approach confirmed four distinct species (*Muz*, *Ngu*, *Spi*, *Sel*), while mPTP and ABGD suggested 10 and six species, respectively (Fig. 1a). The intraspecific *CYTB* distances in four species range from 1.1 to 3.2% (excluding *Sel*, where only a single sequence was available). Interspecific distances to the nearest neighbour are 7.1–27.0% (Table 1).

The distribution of sequenced samples from the *russatus* group is restricted to arid regions of the Levant (Jordan, Israel; Fig. 2c). A single species (*Rus*) was supported by all species delimitation analyses. The mean intraspecific distance is low (0.5%), while interspecific distance to the nearest neighbour is 14.4% (Table 1).

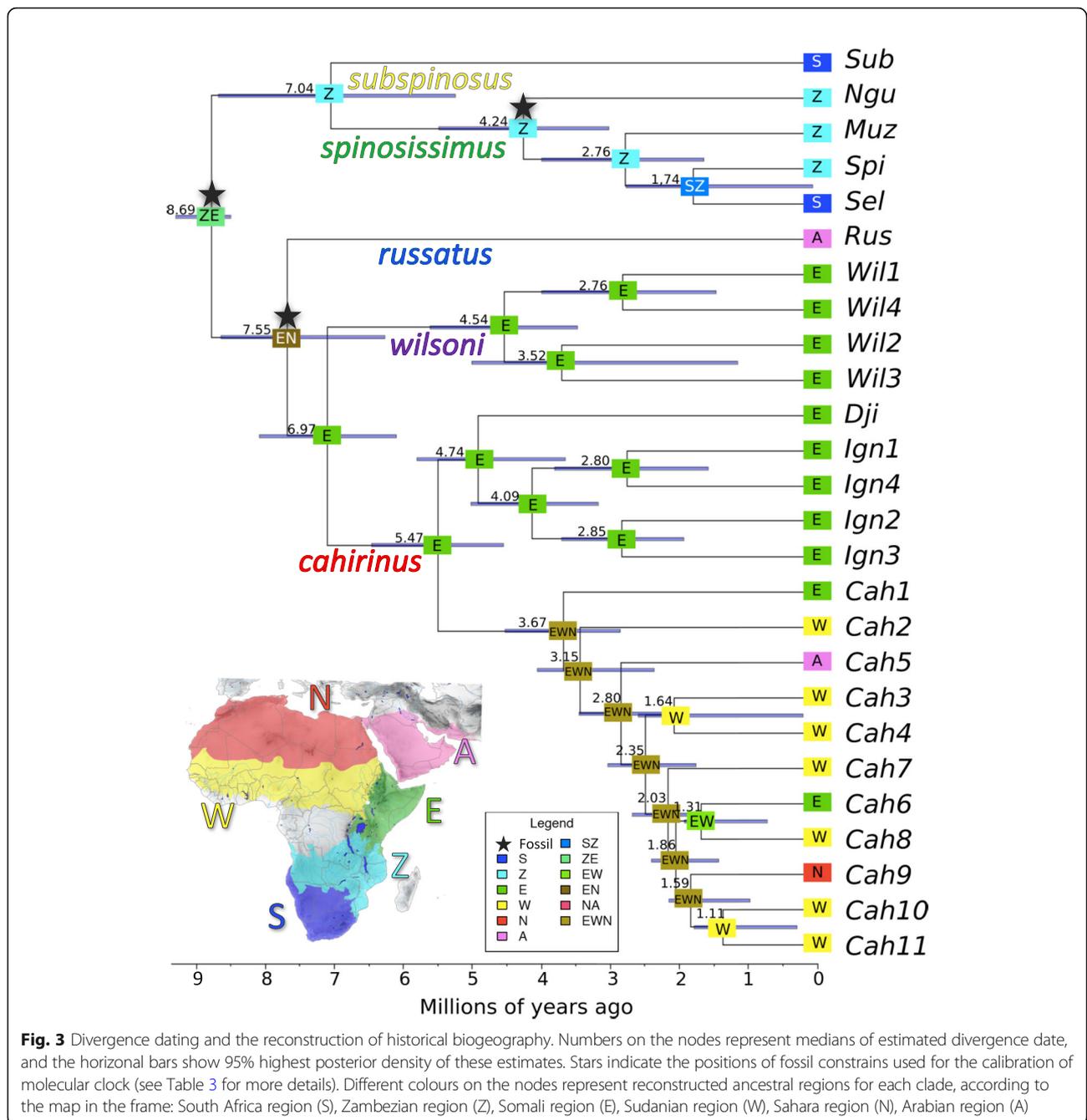
The *wilsoni* group is divided into four well supported lineages, suggested as separate species by STACEY, and

they are predominately distributed in the Somali-Masai savanna (Fig. 2c): *Wil1* lives on both sides of the Great Rift Valley (GRV) in Kenya and Ethiopia, *Wil2* and *Wil3* are two lineages with parapatric distribution in southern Kenya and northern Tanzania, and, finally, *Wil4* was found east of GRV from north-eastern Tanzania to southern Ethiopia, where it overlaps with *Wil1*. The mPTP split the *wilsoni* group into 13 putative species, and ABGD into eight species. The intraspecific distances of four STACEY species ranged from 1.0 to 4.7%, interspecific distances among them are from 10.5 to 12.3% (Table 1).

The highest genetic diversity was found within the *cahirinus* group (16 lineages delimited as species by STACEY). The group is composed of three significantly supported clades (with unresolved relationships among them), distributed parapatrically, with only small overlap (Fig. 2d): (i) the clade “Djibuti” with a single species (*Dji*) recorded from Djibuti and Dera National Park in Ethiopia, geographically neighbouring the Ethiopian Afar province; (ii) the clade “*ignitus*” distributed south-east of GRV with four lineages (*Ign1–Ign4*); (iii) the clade “*cahirinus-dimidiatus*” widespread north-west of GRV, including Sahel and Sudanian savanna, eastern Mediterranean, Middle East and Arabian peninsula, with 11 species (*Cah1–Cah11*) delimited by STACEY (Fig. 1). Intraspecific distances within the *cahirinus* group range from 0.6 to 2.9%, interspecific from 3.0 to 9.6% (Table 1).

Historical biogeography and divergence dating

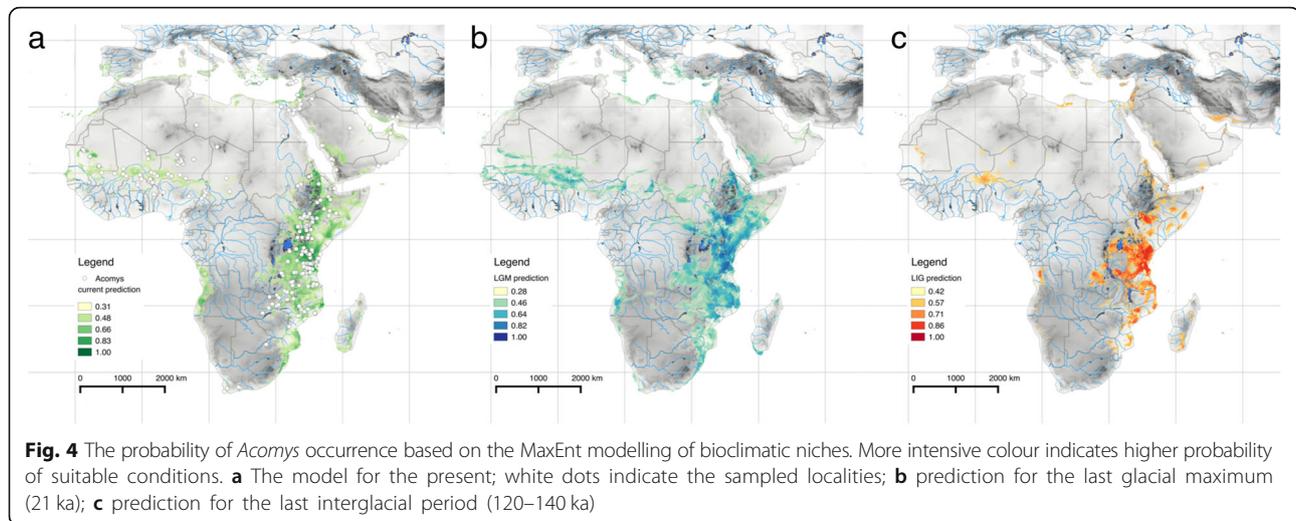
Based on the Dispersal-Extinction Cladogenesis model and the time-calibrated tree, the TMRCA of the genus *Acomys* is dated to 8.69 Ma (95% HPD = 8.51–9.29 Ma). The ancestral area is predicted to be in Eastern Africa, but it is not resolved whether in the Zambezi or the Somali region (Fig. 3). The first split in the Late Miocene separated southern groups (*subspinosus* + *spinosissimus*) from northern groups (*russatus* + *wilsoni* + *cahirinus*). Around 7.04 Ma (95% HPD = 5.25–8.67 Ma) the ancestor of the *subspinosus* group in South Africa diverged from the ancestor of the *spinosissimus* group in the Zambezi region, where the latter group started to diverge around 4.24 Ma (95% HPD = 3.02–5.48 Ma). The ancestor of the *russatus* group in Arabian region separated from *Acomys* in Somali region around 7.55 Ma (95% HPD = 6.27–8.64 Ma). The split between *cahirinus* and *wilsoni* groups occurred in Somali region around 6.97 Ma (HPD = 6.10–8.08 Ma). The *wilsoni* group started to diversify around 4.54 Ma (95% HPD = 3.48–5.61 Ma), while the first split in the *cahirinus* group is dated to 5.47 Ma (95% HPD = 4.55–6.46 Ma), in both cases the beginning of radiation is predicted in the Somali-Masai savanna. Subsequent splits in the *cahirinus* group occurred around 5 Ma, when the ancestors of clades “Djibuti”, “*ignitus*” and



“cahirinus-dimidiatus” diverged most likely in Eastern Africa. The biogeographic history of the “cahirinus-dimidiatus” clade was more complex. While *Cah1* stayed in Somali region, the ancestor of lineages *Cah2-Cah11* likely dispersed to the north-west (especially Sudanian savanna), where most genetic lineages currently occur. The results suggest one Pleistocene migration back to the Somali region (*Cah6* in Afar region, ~1.31 Ma) and two independent dispersals to the north, either to the Arabian region (*Cah5* dated at 2.80 Ma) and to the Sahara region (*Cah9* dated at 1.59 Ma).

Species distribution modelling

The bioclimatic MaxEnt model for the present shows that the sampling in this study covers almost the complete range of suitable climatic conditions for genus *Acomys* (Fig. 4a). The most important variables predicting the modelled geographic distribution of spiny mice were the annual range of temperature and annual precipitation. The predicted distribution is relatively continuous in eastern Africa, mainly in the Somali-Masai and eastern Zambezi savanna. On the contrary, climatically unsuitable are moist mountains in Ethiopia,



Kenya and Albertine Rift, as well as very arid regions of Horn of Africa and Masai xeric scrublands in north-eastern Kenya. The belt of suitable climatic conditions occurs in West Africa, on the boundary between Sudanian savanna and Sahel, as well as along Mediterranean sea and Arabian Peninsula and southern Iran. Isolated suitable areas are predicted in western Angola and northern Namibia, despite the absence of present-day occurrence of *Acomys* species. The model for LGM predicts very similar distribution of spiny mice, with more continuous belt of suitable conditions in Sudanian region (Fig. 4b). The predicted distribution during LIG was more fragmented, with highly suitable conditions in southern Ethiopia, Kenya and Tanzania, and isolated patches in the Horn of Africa, southern part of the Sahara desert, and Mozambique (Fig. 4c). Altogether, it seems very likely that the climatic conditions in the core of the present day distribution, in open seasonally dry habitats in East Africa, were apparently favourable at least during the last glacial cycle.

Discussion

Spiny mice of the genus *Acomys* represent a speciose group of rodents, widely distributed in seasonally dry savanna of Africa, Arabia and Middle East. They often are a dominant component of small mammal assemblages and in some habitats, e.g. in rocky outcrops, they can be even the only rodents captured (our unpubl. data). They were able to colonize wide spectrum of non-forested habitats, from miombo woodlands to rocks in the middle of Sahara, from the sea coast up to 2500 m above sea level (a.s.l.) [e.g., 36]. Despite *Acomys* abundance and practical importance (e.g., as model taxa in behavioural or biomedical research; [50]), the knowledge of their evolutionary history, taxonomy and biogeography has been very limited and biased to particular regions or

intrageneric clades [28, 30, 31, 33, 41, 43, 45]. Here we compiled the largest multilocus genetic dataset to date for the genus *Acomys* (genotypes of 699 individuals from more than 280 localities covering a majority of the distribution of this genus), reconstructed phylogenetic relationships, described biogeographical patterns and evolutionary history, and estimated the spiny mice species diversity.

Phylogeny and biogeographical patterns in the genus *Acomys*

Phylogenetic analysis revealed clear evidence for the existence of five major groups, *subspinosus*, *spinosissimus*, *russatus*, *wilsoni* and *cahirinus* (Figs. 1 and 2, Additional files 2 and 3), which diverged in the late Miocene (Fig. 3). For the first time we provide nuclear genetic data for the *subspinosus* group and we refer its sister position with the *spinosissimus* group, which has never been confirmed [31, 41, 45]. This sister relationship is supported also by the fact that *subspinosus* and *spinosissimus* groups share the same triplicate-type X chromosome that is very rare among mammals [51]. For the first time we also sequenced the nuclear markers of the *russatus* group, but its phylogenetic relationships with the *wilsoni* and *cahirinus* groups remained unresolved (Fig. 1), which suggests fast divergence in the late Miocene (see similar results in [45]). Using multilocus genetic data, we unequivocally identified that the first split within the genus occurred between south-eastern and eastern Africa, i.e. (*subspinosus* + *spinosissimus*) vs. (*russatus* + *wilsoni* + *cahirinus*) (Fig. 3). Further diversification of the five major groups is dated to Plio-Pleistocene and, interestingly, the diversification rate within them is very unequal. While *subspinosus* and *russatus* remained monotypic at the margins of *Acomys* distribution (in the Cape Region and Middle East, respectively), *spinosissimus*

and *wilsoni* clades diversified by a comparable rate in Eastern Africa, either in Zambezan or Somali-Masai savannas. The most intensive spatial and cladogenetic expansion occurred in the *cahirinus* group that colonized large areas of Africa, Arabia and eastern Mediterranean region, even if the most genetic variation is still observed in eastern Africa.

Our phylogenetic analysis revealed at least three independent “out-of-Africa” events, differing significantly by the date of divergence from their African counterparts. The oldest disperser was the ancestor of the *russatus* group, currently distributed only in the Sinai Peninsula and the Middle East, which split from the ancestors of the *wilsoni* and *cahirinus* groups already in late Miocene (Fig. 3, see also [30]). Much more recently, at the beginning of Pleistocene, the ancestors of *Cah5* colonized the Arabian Peninsula (and South Iran). It is unclear whether it was through the Bab al-Mandab connection between Africa and Yemen [33, 52] or through Sinai and around the Red Sea coastline [53]. Lastly, the western Mediterranean islands and south coast of Turkey were colonized during Antiquity by possibly commensal populations from the lineage *Cah9*, likely from Egypt [31, 33, 54].

Spiny mice of the *cahirinus* group (specifically “*cahirinus-dimidiatus*” clade) are widely distributed also in the Sudanian savanna. Results of our biogeographical analysis suggest multiple colonization and diversification waves, directed usually from eastern to western Africa. The first split between *Cah1* (which stayed restricted in Somali region along the Lake Turkana) and remaining lineages occurred in the late Pliocene/early Pleistocene. The ancestors of lineages *Cah2*, *Cah3*, and *Cah4* diverged in southern (more humid) part of Sudanian savanna probably simultaneously with the colonization of Arabian Peninsula by *Cah5*. Much later (ca. 1–1.5 Mya), the split between northern (*Cah9*) and southern (*Cah10* and *Cah11*) resulted in current diversity in large arid areas in northern part of the Sudanian region, Sahel and Sahara desert. At the same time the Ethiopian highlands caused the diversification of *Cah8* (living in easternmost Sudanian region) and *Cah6* (currently limited to Afar triangle). Faunal exchange between Somali-Masai and Sudanian savanna in the Plio-Pleistocene have been described also in other rodents and very often the oldest lineages of Sudanian taxa are found in Eastern Africa [55–57], which well corresponds with the pattern found in spiny mice.

The current distribution of genetic diversity of *Acomys* is affected by major biogeographic divides, represented by (1) extremely dry open habitats, (2) mountain blocks and (3) large water bodies, e.g. Rift Valley lakes or rivers. Even if the spiny mice are considered as typical inhabitants of (semi-)arid environments [58], they avoid

extremely dry habitats. Distribution modelling based on bioclimatic data (Fig. 4) suggests low suitability of habitats in most of Saharan and Arabian deserts, as well as in Masai xeric bushland and shrublands in northernmost Kenya and Horn of Africa. However, even in such habitats the spiny mice can occur, but they have very patchy distribution with isolated populations in rocky areas, considered as remains of more continuous distribution in past humid periods, when savanna-like habitats prevailed [43, 59–63]. Sahara desert seems to work as a barrier between clades *Cah9* and *Cah11* (albeit the data are missing e.g. from southern Algeria).

The expansion of forests during interglacials [64, 65], known as Pleistocene breathing model [66], repeatedly fragmented savanna biome, especially in eastern Africa. The forested mountain blocks thus formed an important biogeographical divide for savanna-dwelling organisms and supported allopatric diversification in this nowadays relatively continuous ecosystem [67]. In agreement with this hypothesis, we can see the coincidence of genetic structure in the *spinosissimus* group with Eastern Arc Mountains (EAM) and Southern Rift Mountains (SRM). These mountain ranges, even if permeable today for taxa living in open habitats, clearly separate *Ngu* and *Muz* lineages (Fig. 2b; see more details in [44]). Similar structure was recently observed in numerous other taxa of sympatric murid and bathyergid rodents living in open habitats of Zambezan region [67–70]. Similarly, the mountains in north-eastern Tanzania (Kilimanjaro, Pare, Usambara) currently delimit distribution for some *Acomys* lineages (*Wil2* vs. *Wil3* or *cahirinus* vs. *spinosissimus* groups), and, again, this pattern was found in other savanna rodents (e.g. *Saccostomus* [71], *Gerbilliscus* [72]). Further to the north, Kenyan highlands could have worked in similar way as they seem to limit distribution of several *Acomys* lineages (*Wil1* vs. *Wil2* + *Wil3*, *Ign3* vs. *Ign4*). Lastly, Ethiopian Highlands, delimit distributions of lineages especially in the *cahirinus* group. For example lineages *Dji* and *Cah6* are restricted to the Afar province in north-eastern Ethiopia (see also very similar pattern in gerbils of the genus *Gerbilliscus*, often sympatric with spiny mice; [72]).

Rivers and other more or less linear water bodies (e.g., contemporary rift lakes, or palaeolakes; [73]) are known to play an important role in shaping genetic structure and diversification patterns in organisms living in open habitats. For example the Zambezi-Kafue river system delimits southern border of distribution of *Ngu* and *Muz* lineages (Fig. 2b; [44]) and have been implicated in forming genetic diversity in savanna-dwelling organisms as diverse as killifishes [74], gerbils [75], African pouched mouse [71], baboons [76] and several species of antelopes [77]. The largest river in Sudanian savanna is the Niger River, which makes a border between *Cah11*

and other West African *Acomys* lineages, is also dominant biogeographical divide in many other rodents (e.g., [43, 55, 67, 78]). On the other hand, the Nile Valley seems to serve rather as a suitable migration corridor for northward spreading of savanna taxa from eastern Africa (see [56] for grass rats, or [79] for shrews). In *Acomys*, it very likely allowed the lineages *Cah5*, *Cah9* and *Rus* to colonize the northern Africa and Arabian Peninsula. Trauth et al. [73] suggested that during the humid periods of Pleistocene, the bottom of GRV was filled by water (much more than today), and the so-called palaeolakes formed an important biogeographic barrier causing allopatric diversification or even speciation. The phylogeographic pattern concordant with this hypothesis was recently described in Eastern African gerbils [72] and the role of GRV on genetic structure is visible also in *Acomys*. For example, *Cah1* is separated from “*ignitus*” clade by GRV and, similarly, more records of *Wil1* occur west of GRV, while other lineages of the *wilsoni* group were found predominantly east of GRV.

Evolutionary scenario of the genus *Acomys* – interplay of geomorphology and climatic changes

The origin of *Acomys* is dated in late Miocene, which is in concordance with the first occurrence of savannas that appeared as a result of rifting activity in eastern Africa and climatic changes [4]. Spiny mice belong to the subfamily Deomyiinae, whose other members occur mostly in forest or forest margins (i.e. genera *Deomys* and *Lophuromys*) or moist savanna (*Uranomys*) [16]. The phylogenetic relationships among genera of Deomyiinae are not sufficiently resolved [46, 80, 81], but it seems likely that the ancestor of *Acomys* colonized dry open habitats early after their late Miocene appearance profiting from empty niches in this ecosystem. Baromet et al. [31] proposed the origin of *Acomys* ca. 13.7 Ma in East Africa or in South Africa, while Alhajeri et al. [46] placed it more generally to sub-Saharan Africa at 10 Ma. Other authors [40, 82] suggested the origin for the genus in Eastern Africa, mainly in the Ethiopian region [82]. Our historical biogeography reconstruction is in partial agreement with previous studies as the origin of the genus is placed either in Somali and/or Zambezian regions (Fig. 3), i.e. the regions with the highest contemporary genetic diversity of spiny mice. Even if the oldest fossil records of *Acomys* ancestors are found in Zambezian savanna (*†Preacomys griffini* and *†Preacomys karsticus* ca. 9 Ma from Namibia; [83]), they occurred in the late Miocene also in East Africa (*†Preacomys kikiae* 8.5 Ma from Chorora, Ethiopia; [84]).

Periods of warm humid climate in Late Miocene caused the last occurrence of coast-to-coast belt of tropical forest, which is evidenced by phylogenetic analyses of plants and animals living in nowadays fragmented

forests of Congo basin and eastern African montane and coastal forests [85, 86]. This continuous forest was likely one of the most important factors in early evolution of savanna inhabitants, because it separated northern (= Somali-Masai) and southern (= Zambezian) savannas. In *Acomys*, this resulted in allopatric divergence between the ancestor of *subspinosus* + *spinosissimus* group in the Zambezian savanna and the ancestor of *wilsoni* + *russatus* + *cahirinus* in the Somali-Masai savanna (estimated in this study at 8.7 Ma). The same geographical and temporal pattern, i.e. late Miocene split of northern and southern taxa, was observed also in other savanna mammals, e.g. gerbils [57], pouched mice [71], several genera of antelopes [87], warthogs [88] and giraffes [89, 90].

Later on, but still in Late Miocene, the evolution of savanna's fauna was significantly influenced mainly by the Messinian salinity crisis (MSC, [91]), dated to 6.0–5.3 Ma. Very little is known about the effect of the MSC on eastern African climate [6], but it is generally held that overall aridification at the Miocene/Pliocene boundary promoted the expansion of very dry habitats [92]. Inhospitable very arid (desert) areas in north-eastern Africa expected at MSC period thus likely interrupted the connection of *Acomys* populations between Somali-Masai and eastern Mediterranean area. This period corresponds to the split of the *russatus* group, which remained effectively isolated in the north.

Plio-Pleistocene period (starting 5.3 Ma) is characterized by intensive climatic oscillations. There are several well-known climatic transitions, like the intensification of Northern Hemisphere glaciation (iNHG; 3.2–2.5 Ma, [93, 94]), the development of the Walker circulation (2.0–1.7 Ma; [95]) and the early-middle Pleistocene transition (1.2–0.8 Ma; [96]). These periods of pronounced climate variability significantly affected the distribution of forests, palaeolakes and savannas [97]. For example during more humid periods the currently fragmented montane forests in Eastern Arc Mountains and Kenyan highlands probably expanded into lower altitudes, became more continuous and formed significant barriers to gene flow for taxa living in open dry habitats [67, 71, 72, 98]. The bottom of GRV was filled by the palaeolakes, which prevented gene flow of savanna-dwelling species, leading to diversification, or even speciation [73]. Because of wide confidence intervals of our estimates of divergence times, it is not possible to link particular splits to specific climatic events. However, it is highly probable that a majority of current genetic diversity in *spinosissimus*, *wilsoni* and *cahirinus* groups is a result of repeated fragmentation of savannas in Plio-Pleistocene, caused by climatic changes. This is further supported by parapatric distribution of lineages within major clades, where the distribution borders often correspond to predicted barriers of the gene flow (i.e. too arid or forested areas, and palaeolakes).

Species richness of *Acomys* – the need of further integrative taxonomic studies

The number of species crucially depends on adopted species concept. Rapidly increasing amount of genetic data now allows to apply the so-called integrative taxonomic approach, which usually complements the widely used typological or biological species concepts by genetic [99] and/or phylogenetic [100] species delimitations. The taxonomy of *Acomys* has been unresolved since the second half of the twentieth century. In Additional file 1 we listed several taxonomic alternatives, based mostly on morphological traits, used recently for the genus *Acomys*. The most comprehensive list was provided by Ellerman [34] with 25 species and 17 subspecies. On the other extreme, Setzer [38] recognized only five species that partly correspond to major genetic clades recovered in our study: *A. cahirinus* (7 subspecies), *A. dimidiatus* (12 subspecies), *A. russatus* (one subspecies), *A. spinosissimus* (one subspecies) and *A. subspinosus* (9 subspecies, including *A. wilsoni*). Widely accepted list of Musser and Carleton [37] contained 18 species. Monadjem et al. [36] lists 15 species in sub-Saharan Africa, including three newly delimited species in the *spinosissimus* group [45]. The most recent and comprehensive Handbook of the Mammals of the World [16] listed 21 species and 12 subspecies.

We used several genetic species delimitation methods and their estimates of spiny mice species richness differ significantly from 57 species (mPTP) to 26 species (STACEY as well as our prior delimitation based on geographical distribution of genetic diversity). The species delimitation based only on mitochondrial markers (ABGD and mPTP) have the tendency to overestimate the number of revealed species and they often identify as separate species also genetic lineages that are traditionally considered as intraspecific phylogeographic structure. For example ABGD revealed four species within *Ngu* lineage, while Petruželka et al. [44] recently showed on multi-locus dataset that they represent only phylogeographic structure of *A. ngurui*. Below we use the most conservative estimate (26 MOTUs, here considered as “genetic lineages” and named according Fig. 1a) and compare these species delimitations with previous taxonomic work. Multi-species coalescent approaches to species delimitation (like STACEY) in fact diagnose the genetic structure, with no distinction between structure due to populations or due to species [49]. Therefore, the aim of the following part is not to perform a formal taxonomic revision, but to show the genetic clades and geographic regions where further integrative taxonomic analyses (employing combination of genetic, morphological, ecological and other data) could lead either to new descriptions or synonymization of *Acomys* taxa.

The *subspinosus* group

This group is monotypic and contains a single lineage *Sub. (1) Sub*

Distribution: South Africa (Cape Province).

Available name: *Acomys subspinosus* (Waterhouse, 1838).

Type locality: Western Cape Province, Cape of Good Hope, South Africa.

Karyotype: $2n = 64$, $NF = 70$ [101, 102].

Additional information: Based on its unique dental and skull morphology, *A. subspinosus* has been placed in its own subgenus *Subacomys* with an “ancestral” karyotype ($2n = 64$, $NF = 70$; [51, 101–104]). Its separation from other *Acomys* was also indicated by phylogenetic analyses of *CYTB* [31, 32, 45]. Using for the first time the combination of mitochondrial and nuclear markers, we unequivocally showed its sister relationship with *spinosissimus* group, i.e. it does not represent the first cladogenetic split of *Acomys*. As a consequence, the validity of the subgenus *Subacomys* (mentioned erroneously as *Preacomys* in Denys et al. [16]) is questionable.

The *spinosissimus* group

This strongly supported monophyletic group has been revised repeatedly [41, 44, 45] and four genetic lineages were distinguished and named. However, the genetic data from the southern part of its distribution are still very limited and especially the taxonomy and distribution of *A. selousi* and *A. spinosissimus* should be further explored.

(2) *Ngu*

Distribution: Lineage *Ngu* is distributed in three well supported parapatric sublineages from Tanzania (East of EAM) to central Mozambique (north of the Zambezi River; [44]).

Available name: *Acomys ngurui* Verheyen et al., 2011.

Type locality: Nguru Ya Ndege, Tanzania.

Karyotype: $2n = 60$, $NFa = 68$ [45].

Additional information: This species is very similar to *A. muzei* from which it differs by relatively shorter tail and non-overlapping distribution [44, 45]. Barome et al. [41] reported it as *A. cf. selousi* from Berega. Three genetically distinct sublineages probably represent intraspecific variation [44].

(3) *Sel*

Distribution: Northern part of South Africa, only one genetically confirmed locality from the Kruger National Park [45]. Northern limits of its distribution are not fully resolved.

Available name: *Acomys selousi* De Winton, 1896.

Type locality: Essex Farm, Zimbabwe.

Karyotype: $2n = 58–62$, $NFa = 68$ [101, 102].

Additional information: According some authors [35, 37, 38]; *A. transvaalensis* and *A. selousi* are synonyms of *A. spinosissimus*. Here we follow the view of Verheyen et al. [45] and Monadjem et al. [36] and consider this

lineage as a separate species, but further taxonomic investigation of the *spinossissimus* group in South African region is required.

(4) *Spi*

Distribution: Mozambique, Zimbabwe, southern Malawi.

Available name: *Acomys spinossissimus* Peters, 1852.

Type locality: Tette and Buio, Mozambique.

Karyotype: $2n = 60$, $NFa = 68$ [101].

Additional information: We follow the view of Verheyen et al. [45] and Monadjem et al. [36] and include only populations from central Mozambique and southern Malawi into this species. Petružela et al. [44] recently showed that its distribution north of the Zambezi River is much more restricted compared to maps in Monadjem et al. [36] and the two well distinct genetic sublineages of this species seem to be separated by the Zambezi river.

(5) *Muz*

Distribution: Central and western Tanzania, Malawi (west of the Lake Malawi), Zambia.

Available name: *Acomys muzei* Verheyen et al., 2011.

Type locality: Muze, Tanzania.

Karyotype: $2n = 58-62$, $NFa = 68$ [45].

Additional information: Recent analyses showed that the populations in Malawi and Zambia (reported as *A. spinossissimus* in Monadjem et al. [36]) belong to this species [44]. Further, the highest genetic diversity of the species was recorded west of the Lake Malawi, while Tanzanian populations represent only relatively recent colonization event [44].

The *russatus* group

The *russatus* group is monotypic with only one lineage *Rus*. The phylogenetic relationships with its sister groups *cahirinus* and *wilsoni* are not fully resolved (Fig. 1).

(6) *Rus*

Distribution: Egypt (separate subspecies *aegyptiacus* was described in Eastern Desert), Sinai, Jordan, Israel, Saudi Arabia, Yemen and Oman. Genotyped material in this study originates only from Jordan and Israel.

Available name: *Acomys russatus* (Wagner, 1840).

Type locality: Sinai, Egypt.

Karyotype: $2n = 66$, $NF \geq 66$ [40].

Additional information: Denys et al. [51] referred that *A. russatus* is very distinctive in its molar morphology and chromosomal traits and previous *CYTB* [31, 41, 45] as well as our multilocus genetic analyses showed that it is not closely associated with any other *Acomys* taxon. *Acomys russatus* and *A. dimidiatus* (= *Cah5* in this study) can live in sympatry, and their differences in ecology, physiology, and activity patterns (especially in Israel) have been extensively documented (see references in [105]).

The *wilsoni* group

This group is well supported in all phylogenetic analyses (Fig. 1 in this study, [31, 41]), but its relationships with *russatus* and *cahirinus* groups are not completely resolved. Distribution of the *wilsoni* group is limited to the Somali region [sensu 3]. Based on multilocus species delimitation we recognize four genetic lineages, but the species limits must be further investigated.

(7) *Wil1*

Distribution: South Ethiopia, Kenya (along GRV).

Available name: *Acomys percivali* Dollman, 1911.

Type locality: Chanler Fall, Nyiro, Kenya.

Karyotype: $2n = 36$ and $NF = 68$ [40].

Additional information: Phylogenetically the most distinct MOTU within the *wilsoni* group (Fig. 1). It was not included in previous phylogenetic studies. Distribution of *A. percivali* reported by Monadjem et al. [36] and Denys et al. [16] is very similar to that of *Wil1*. Janeczek et al. [103] regarded *A. percivali* as the species genetically most closely related to *A. wilsoni* (= *Wil4*). Both clades were found sympatric at several localities in southern Ethiopia, where they can be distinguished by external morphology (our unpublished data) and karyotypes [16].

(8) *Wil2*

Distribution: Southern Kenya.

Available name: None. Based on Barome et al. [31], we use in Fig. 1 the name *A. sp.* 'Magadi'.

Type locality: Not relevant.

Karyotype: Not known.

Additional information: Known only from three localities from southern Kenya, all of them reported by Barome et al. [31]. They mentioned this MOTU as two different species, *A. sp.* 'Magadi' and *A. wilsoni*, and this structure was reflected also by mPTP and ABGD analyses in our study. The conspecificity with *Wil3* and/or *Wil4* are plausible hypotheses and should be tested.

(9) *Wil3*

Distribution: NW Tanzania, southern Kenya, most localities in the bottom of GRV.

Available name: None. Based on Mgode [42], we use in Fig. 1 the name *Acomys* aff. *percivali*.

Type locality: Not relevant.

Karyotype: $2n = 58$ [42].

Additional information: Mgode [42] named 13 spiny mice from northern Tanzania (localities Tingatinga, Longido, Mt. Gelai-Olikisima and Kilimamoja-Karatu) belonging to this lineage as *Acomys* cf. *percivali*. They differ from *Wil4* (= *A. wilsoni*) in skull morphology and karyotype. Because the type locality of *A. percivali* (Mt. Nyiro, Kenya) is very far from the distribution of *Wil3*, the name *A. percivali* more probably belongs to *Wil1*,

while *Wil3* likely deserves a formal description as a new species.

(10) *Wil4*

Distribution: Southern Ethiopia (Somali region), Kenya (east of GRV), north-eastern Tanzania.

Available name: *A. wilsoni* Thomas, 1892.

Type locality: Mombasa, Kenya.

Karyotype: 2n = 62 and NF = 76 [42, 106].

Additional information: Verheyen et al. [45] suggested that *A. wilsoni* (meaning *Wil2* and *Wil4* included in their study of mitochondrial variation) is probably a species complex, which is confirmed by our data. The conspecificity of *Wil4* with *Wil2* and/or *Wil3* should be further investigated as they might represent intraspecific phylogeographic structure (see similar patterns in savanna-dwelling rodent species in southern part of Somali region, e.g. in *Gerbilliscus vicinus*; [72] or *Saccostomus umbriventer*; [71]). If *Wil2* and *Wil4* are different species, the analysis of the type material of *A. wilsoni* will be required to decide, which of them is true *A. wilsoni* (both are distributed around the type locality of *wilsoni*).

The *cahirinus* group

This is the most diversified *Acomys* group comprising of three main clades, “Djibuti” (one MOTU *Dji*), “*ignitus*” (four MOTUs *Ign1* – *Ign4*) and “*cahirinus-dimidiatus*” (11 MOTUs *Cah1* – *Cah11*), with unresolved mutual relationships.

(11) *Dji*

Distribution: Djibuti, Afar province in Ethiopia, probably also Somalia (from where no genetic data are available).

Available name: *Acomys louisae* Thomas, 1896.

Type locality: 65 km S of Berbera, Somalia.

Karyotype: 2n = 68 and NF = 68 ([107]; our unpubl. data).

Additional information: *Acomys louisae* was placed in a separate subgenus *Peracomys* based on dental characters [51]. We have not checked the skull morphology of our material from *Dji*, but the recently collected animals from eastern Ethiopia (Dire Dawa region) likely assigned to *A. louisae* by morphological characters (by C. Denys, unpubl. data) clustered with *Dji* at *CYTB* (not included in this study). According to Petter [108], *A. louisae* cannot be distinguished from the “*cahirinus-dimidiatus* complex” (sensu [109, 110]) on the basis of skull or external characteristics. This species may co-occur with *A. mullah* (= *Cah6*) in the Afar triangle (larger, HB > 100 mm with grey or greyish-brown dorsal pelage). *A. louisae* should have a bright rufous or brown dorsal pelage and relatively very long tail (> 100% of HB; [36]). In our pilot analysis we were not able to find significant external size differences between individuals from lineages

Dji and *Cah6* (considered as *A. mullah*, see below), but more detailed morphological investigation is needed.

(12) *Ign1*

Distribution: Eastern part of Ethiopia (Babile).

Available name: None. Based on Lavrenchenko et al. [111], we use the name *Acomys* sp. C in Fig. 1.

Type locality: Not relevant.

Karyotype: 2n = 44, NF = 68 [111, 112].

Additional information: This presumably new species was mentioned for the first time as genetically and cytogenetically very divergent lineage (*Acomys* sp. C) by Lavrenchenko et al. [111] from the Babile Elephant Sanctuary in Eastern Ethiopia. This lineage is only known from the Babile Elephant Sanctuary, where it is very common and abundant species. It can have wider distribution in poorly sampled regions of southeastern Ethiopia and Somalia (see similar pattern in gerbils, [72]). The conspecificity with other lineages of the *ignitus* clade should be further tested. The comparison with the type material of *A. mullah*, described from nearby town Harar, is necessary (see also below).

(13) *Ign2*

Distribution: South-eastern Ethiopia.

Available name: None.

Type locality: Not relevant.

Karyotype: Not known.

Additional information: This lineage is reported here for the first time. It is known only from two localities in the south-eastern slope of Ethiopian Highlands (Sof Omar caves and Imi; each locality has very distinct mitochondrial haplotypes). It might be more widespread in poorly sampled Somali region of Ethiopia, and in Somalia. Its sister lineage *Ign3* (= *A. ignitus*) is geographically distant, but the conspecificity with other lineages of the *ignitus* clade are worth of further taxonomic work.

(14) *Ign3*

Distribution: Southern Kenya, northernmost Tanzania.

Available name: *Acomys ignitus* Dollman, 1919. In Alhajeri et al. [46] was this species incorrectly mentioned as *A. percivali*.

Type locality: Voi, Kenya.

Karyotype: 2n = 50, NF = 66–68 [102].

Additional information: *A. ignitus* has been recorded in and around Tsavo National Park [36], which corresponds to the distribution of this MOTU. Whether or not other lineages of the *ignitus* clade (especially *Ign2*) are conspecific with *A. ignitus* must be investigated by integrative taxonomy approach.

(15) *Ign4*

Distribution: Kenya and southernmost Ethiopia (east of GRV).

Available name: *Acomys kemp* Dollman, 1911.

Type locality: Chanler Falls, N Guaso Nyiro, Kenya.

Karyotype: Not known.

Additional information: This species was previously listed as subspecies of *A. ignitus* (Hollister, 1919) [113] or *A. cahirinus* (Setzer, 1975) [38], but rehabilitated as clearly distinct species by Janecek [103]. *Acomys kemp* was found sympatric with *A. percivali* (= *Willi*) at several localities in Kenya and southern Ethiopia and these two taxa can be easily distinguished, e.g. by coat coloration (the latter being usually greyish and darker). There is no evidence of distributional overlap with *Ign3* (*A. ignitus*), but further sampling in southern Kenya would be desirable.

(16) *Cah1*

Distribution: North-west Kenya (the only region from where the genetic data is available), Sudan, South Sudan. Very probably also in Uganda.

Available name: *Acomys cineraceus* Heuglin, 1877.

Type locality: Doka, Sudan.

Karyotype: $2n = 48-50$ [114].

Additional information: Formerly included in *A. cahirinus* [38, 108], but Dieterlen (in litt.) noted that *A. cineraceus* is a distinct species [37]. Separation of *A. cineraceus* from *A. cahirinus* is supported by chromosomal data ($2n = 48$ or 50 for *A. cineraceus*, $2n = 36$ for *A. cahirinus*; [37, 114]). Limits of the geographic range of *A. cineraceus* are unresolved, especially its western part [37]. Compared to the distribution maps in Denys et al. [16] and Happold [115], we were not able to confirm its occurrence in western Ethiopia.

(17) *Cah2*

Distribution: Burkina Faso, Mali.

Available name: None. Based on Barome et al. [30], we call this MOTU as *Acomys* sp. 2 in Fig. 1.

Type locality: Not relevant.

Karyotype: $2n = 66-68$, $NF = 66-72$ [116].

Additional information: Barome et al. [30] called this MOTU as *Acomys* sp. 2, Granjon and Duplantier [116] included it in *A. johannis* species complex. The distribution of *Cah2* in West Africa is overlapping with *Cah3* and *Cah4*, and its specific status should be investigated by integrative taxonomy approach using larger material and multi-locus genetic analysis.

(18) *Cah3*

Distribution: Burkina Faso.

Available name: None. Based on Barome et al. [30], we call this MOTU as *Acomys* sp. 1 in Fig. 1.

Type locality: Not relevant.

Karyotype: $2n = 66-68$, $NF = 66-72$ [116].

Additional information: Barome et al. [30] called this MOTU as *Acomys* sp. 1, Granjon and Duplantier [116]

included it in *A. johannis* species complex. The distribution of *Cah3* in West Africa is overlapping with *Cah2*, *Cah4* and *Cah11*. The West African *A. johannis* species complex (paraphyletic in our study, grouping *Cah2*, *Cah3* and *Cah4*; see also [116]) requires taxonomic revision.

(19) *Cah4*

Distribution: Chad, Cameroon, Niger, Nigeria, Benin.

Available name: *A. johannis* Thomas, 1912.

Type locality: Bauchi Plateau Kabwir, North Nigeria.

Karyotype: $2n = 66-68$, $NF = 66-72$ [116].

Additional information: This MOTU was included in previous phylogenetic studies as *A. johannis* [30, 31, 37, 43, 46]. Formerly it was included in *A. cahirinus* [38] or *A. cineraceus* [104]. Sicard and Tranier [63] provided a detailed report on the geographic distribution of three pelage colour phenotypes of *Acomys* occurring in Burkina Faso [117], assigned them to *A. johannis*, and contrasted their external, cranial, and dental morphology with *A. chudeaui* (= *Cah11*). Using *CYTB* sequences, Barome et al. [31] reported the specimens from Burkina Faso and Mali as *Acomys* sp. 1 (= *Cah3*) and *Acomys* sp. 2 (= *Cah2*) and specimens from Niger, Benin, Cameroon, and Niger as *A. johannis* (= *Cah4*), but their conspecificity has never been tested by the combination of multi-locus genetic and phenotypic data.

(20) *Cah5*

Distribution: Egypt (Sinai only), Arabian Peninsula, South Iran.

Available name: *Acomys dimidiatus* Cretzschmar, 1826.

Type locality: Sinai, Egypt.

Karyotype: $2n = 36-38$, $NF = 68-70$ [110].

Additional information: *Acomys dimidiatus* is nearly indistinguishable from *A. cahirinus* with regard to external morphology, which resulted in great confusions relative to its classification [16]. With few exceptions (e.g., [34, 37, 38, 46, 118, 119]) *A. dimidiatus* usually has been listed in the synonymy of *A. cahirinus* [36, 37, 82, 120]. A morphological and cytogenetical review of *Acomys* species made by Denys et al. [51], who provided the external, skull and dental characteristics of all the type specimens available, validated *A. cahirinus* as distinct from *A. dimidiatus*. Frynta et al. [33] referred two major lineages in northern Africa and Middle East, which should represent *A. cahirinus* and *A. dimidiatus*, respectively. The type localities of these two species are very close each other (Cairo and Sinai) but their distributions seem to be separated by the Isthmus of Suez.

(21) *Cah6*

Distribution: Horn of Africa (S Eritrea, Djibouti, E Ethiopia and N Somalia).

Available name: *Acomys mullah* Thomas, 1904.

Type locality: Harar, Ethiopia.

Karyotype: Not known.

Additional information: Awaiting more detailed taxonomic revision, we assigned the name *A. mullah* to the lineage *Cah6* distributed in the margins of the Afar triangle in Ethiopia. This species is considered a member of the “*cahirinus-dimidiatus*” complex [16, 121], which is confirmed by our phylogenetic study. This species may co-occur with *A. louisae* (= *Dji*). It should be also taken in consideration that the type locality of *A. mullah* (Harar) is very close to the only known locality of *Ign1* and is separated from the Afar lowland by the Chercher Mts. Comparison of *Ign1* and *Cah6* with the type material of *A. mullah* (and *A. brockmani* considered as its synonym) is necessary.

(22) *Cah7*

Distribution: North-west Ethiopia (Mai Temen and Alatish NP).

Available name: None. Based on Lavrenchenko et al. [112], we use the name *Acomys* sp. B in Fig. 1.

Type locality: Not relevant.

Karyotype: 2n = 40, NF = 68 [112].

Additional information: Ivlev et al. [25] and Lavrenchenko et al. [112] called this lineage *Acomys* sp. B. It is sympatric with *Cah8*, but the two lineages significantly differ by karyotypes, physiological and behavioural traits [112] and thus might represent different biological species.

(23) *Cah8*

Distribution: Alatish NP, Ethiopia.

Available name: None. Based on Lavrenchenko et al. [112], we use the name *Acomys* sp. A in Fig. 1.

Type locality: Not relevant.

Karyotype: 2n = 52, NF = 68 [112].

Additional information: Ivlev et al. [25] and Lavrenchenko et al. [112] called this lineage *Acomys* sp. A. It is also very abundant in the neighbouring Dinder NP in Sudan (J. Bryja et al., unpublished data). The taxonomic revision of *Cah7*, *Cah8*, *Cah9* and *Cah10* is necessary and more intensive sampling in Sudan and northern Ethiopia would be very helpful.

(24) *Cah9*

Distribution: Egypt, Greece (Crete), Cyprus, Turkey, Libya, northern Chad.

Available name: *Acomys cahirinus* (É. Geoffroy Saint-Hilaire, 1803).

Type locality: Cairo, Egypt.

Karyotype: 2n = 36–42, NF = 68 [54, 110].

Additional information: The species was described from Cairo (Egypt). It seems very likely that it colonized eastern Mediterranean area during Antiquity. Weak genetic differences revealed also by our multilocus analysis support the view that *A. cahirinus* should be synonymized

with *A. minous* Bate, 1906 from Crete, *A. cilicicus* Spitzenberg, 1978 from Turkey and *A. nesiotetes* Bate, 1903 from Cyprus (see [16, 54] and references there). The relationships with *A. seurati* (a distinct taxon from rocky areas in southern Algeria, differing by karyotype and dental morphology; [51]) should be investigated by using genetic data from the Algerian material.

(25) *Cah10*

Distribution: Sheraro, Ethiopia.

Available name: None.

Type locality: Not relevant.

Karyotype: Not known.

Additional information: *Cah10* is known only from one locality in North Ethiopia. It is a sister MOTU either to *Cah9* or *Cah11* and its conspecificity with *A. cahirinus* and/or *A. chudeaui* should be tested.

(26) *Cah11*

Distribution: Niger, Mauritania, Mali, Chad.

Available name: *Acomys chudeaui* Kollman, 1911.

Type locality: Atar, SW of Biskra, Mauritania.

Karyotype: 2n = 40–46, NFa = 66 [116].

Additional information: This taxon has been previously listed as a synonym of *A. cahirinus*, but most recent works consider it as a distinct species [36, 46, 116]. Nicolas et al. [43] synonymized *A. airensis* and *A. chudeaui* and provided a detailed phylogeographic analysis of this taxon.

Conclusions

Using multilocus genetic data, comprehensive geographic sampling and multiple phylogenetic approaches, we revealed that the spiny mice (*Acomys*) are composed of five main species groups: *subspinosus*, *spinosissimus*, *russatus*, *wilsoni* and *cahirinus*. Three of them (*spinosissimus*, *wilsoni* and *cahirinus*) clearly represent species complexes. We delimited 26 genetic lineages as potential *Acomys* species, and their taxonomic status should now be assessed by multidisciplinary investigations. The origin of the genus is dated to the late Miocene in savannas of eastern Africa, when the first vicariance between “southern” and “northern” groups was probably caused by the development of the coast-to-coast forest belt. The evolutionary history of the genus in Plio-Pleistocene was influenced by global climatic transitions as well as by local geomorphological features (e.g. deserts, mountain blocks and/or large water bodies) and is characterized by repeated cycles of diversifications, especially in eastern Africa, and repeated dispersal events mainly to the North and West. The spiny mice can be thus used as very suitable model for testing specific hypotheses of the role of historical factors on the formation of current biodiversity of seasonally dry environments of Afro-Arabia.

Methods

Sampling

The genetic dataset is based on 700 individuals of spiny mice. We produced original genetic data from 421 individuals collected at more than one hundred localities, and complemented them by 279 georeferenced mitochondrial sequences from GenBank. This material covers large part of the distribution of the genus as predicted by the IUCN [122] (see Fig. 2a). All individuals were DNA-barcoded at mitochondrial markers to get as precise distributional maps of genetic clades as possible, but part of sequences was removed as redundant from subsequent phylogenetic analyses (see Additional file 5). All fieldwork performed in the frame of this study complied with legal regulations in particular countries and sampling was in accordance with local legislation (see more details in Ethics approval section). Rodents were trapped in Sherman live traps (H.B. Sherman Traps Inc., Tallahassee, USA) and snap traps baited with a mixture of peanut butter, maize flour and dried fish. Mice caught in live traps were euthanized by cervical dislocation or an overdose of Isoflurane prior to dissection (Directive 2010/63/EU). When present, the spiny mice are generally the most abundant component of the small mammal communities and are not listed as endangered. Each individual was identified to the genus by the external features and the tissue sample (tail, toe, spleen, etc.) was stored in 96% ethanol until DNA extraction. GPS coordinates of each locality were recorded. For more details on particular specimens, localities and collectors, see Additional file 5.

DNA extraction, amplification and sequencing

DNA from 96% ethanol-preserved tissue samples was extracted using a DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. For phylogenetic analysis we selected four genetic markers; two mitochondrial fragments, cytochrome *b* (*CYTB*) and control region (*D-loop*) and two nuclear exons, Interphotoreceptor Binding Protein gene (*IRBP*) and Recombination activating gene 1 (*RAG1*). Individual markers were amplified by the polymerase chain reaction (PCR) using following combination of primers: L14723 and H15915 [123] for *CYTB*; 'primers 1–4' [124] for *D-loop*; IRBP217 and IRBP1531 [125] for *IRBP* and RAG1F1705 and RAG1R2951 [126] for *RAG1*. Each locus was amplified using a final concentration of 3 mM of MgCl₂ (for *IRBP* only 2 mM), 0.2 mM of each dNTP, 0.4 μM of each primer, 1 x Taq buffer (Thermo Fisher Scientific, Waltham, USA), 0.2 μl of Taq polymerase (5 U/μl, Thermo Fisher Scientific), 30 ng/μl of genomic DNA, and ddH₂O to a total volume of 15 μl. PCR products were purified with Calf Intestine Alkaline Phosphatase and Exonuclease I (New England Biolabs, Ipswich,

USA), and Sanger-sequenced in both directions using the BigDye® Terminator chemistry (Thermo Fisher Scientific) either at the Institute of Vertebrate Biology CAS on an 'Applied Biosystems® 3130xl Genetic Analyzer' or commercially through the GATC Biotech company (Konstanz, Germany). All corresponding sequences were deposited in GenBank under accession numbers MH044731-MH045045 (see Additional file 5).

Phylogenetic analysis

The final dataset for phylogenetic analyses consisted of 373 unique sequences of *CYTB*, 71 sequences of *IRBP*, 59 sequences of *RAG1* and 96 sequences of *D-loop*. The remaining mitochondrial sequences (usually identical and/or shorter sequences and/or from the same or neighbouring localities) were unambiguously assigned to particular mtDNA lineages by preliminary phylogenetic analysis and they were removed as redundant (see Additional file 5). These data were used only to increase the precision by which we mapped the geographical distribution of phylogenetic clades. Nuclear exons were sequenced only in the representative subset of 102 individuals, covering the geographic distribution and mitochondrial diversity as much as possible (see Additional files 5 and 6). As outgroups we used four taxa from subfamily Deomyinae, to which *Acomys* belong (*Deomys ferrugineus*, *Lophuromys flavopunctatus*, *Lophuromys sikapusi* and *Uranomys ruddi*; see Additional file 5). Sequences were aligned in MUSCLE [127] and the concatenated dataset with total length 4005 bp was created in Mesquite. For all three protein-coding genes (*CYTB*, *IRBP*, *RAG1*), we used Mesquite 3.03 [128] to check the coding frame for possible errors or stop codons.

Phylogenetic reconstructions were conducted using maximum likelihood (ML) and Bayesian inference (BI). For both phylogenetic approaches were carried out partitioned analyses to improve phylogenetic accuracy [129]. The molecular dataset was divided into ten partitions: we used three partitions for each of the protein-coding genes, and one partition for the control region (*D-loop*). The best partitioning scheme and substitution models were determined with PartitionFinder v1 [130] using a greedy heuristic algorithm with 'linked branch lengths' option. The Bayesian information criterion (BIC) was used to compare partitioning schemes and substitution models following the recommendation of Ripplinger and Sullivan ([131]; Table 2).

Maximum likelihood analyses were performed using RAxML v8.2.8 [132] for separate gene trees (*CYTB* and *D-loop* not shown same topology as Fig. 1, *IRBP* Additional file 2, *RAG1* Additional file 3) as well as for concatenate matrix. Based on the BIC results in PartitionFinder we used seven partitions for concatenate matrix, five partitions with GTR + I + G model and two

Table 2 The substitution models used in particular phylogenetic analyses. They were selected by PartitionFinder using BIC model selection, greedy search, linked branch length

Partitions	RAxML	MrBayes	BEAST
1 <i>CYTB_pos1</i>	GTR+I+G	GTR+I+G	GTR+I+G
2 <i>CYTB_pos2</i>	GTR+I+G	HKY+I+G	HKY+I+G
3 <i>CYTB_pos3</i>	GTR+I+G	GTR+I+G	GTR+I+G
4 <i>IRBP_pos1, RAG1_pos2</i>	GTR+G	HKY+I	TrN+I
5 <i>IRBP_pos2, RAG1_pos3</i>	GTR+G	HKY+G	TrN+G
6 <i>IRBP_pos3, RAG1_pos1</i>	GTR+I+G	HKY+I+G	HKY+I+G
7 <i>D-loop</i>	GTR+I+G	GTR+I+G	GTR+I+G

with GTR + G substitution model (Table 2). The ML tree was obtained using heuristic searches with 100 random addition replicates and the clade support was then assessed using a non-parametric bootstrap procedure with 1000 replicates. Following Hillis and Bull [133], nodes supported by bootstrap values (BP) ≥ 70 were considered strongly supported.

Bayesian inference analyses were carried out using MrBayes v3.2.6 [134] with seven partitions (Table 2). Two independent runs with four MCMC (one cold and three incrementally heated) were conducted: they ran for 50 million generations, with trees sampled every 1000 generations. A conservative 25% burn-in was applied after checking for stability on the log-likelihood curves and the split-frequencies of the runs. Support of nodes for MrBayes analyses was provided by clade posterior probabilities (PP) as directly estimated from the majority-rule consensus topology. Following Erixon et al. [135], nodes supported by PP ≥ 0.95 were considered strongly supported.

Estimates of species richness

For estimation of *Acomys* species richness we applied multiple species delimitation methods (as suggested by Carstens et al. [136]): (1) “by-eye” analysis of genetic structure (based primarily on *CYTB* barcodes) and geographical distribution of genetic lineages; (2) species discovery approach to assign individuals to putative groups based on the variability of *CYTB* sequences [137, 138]; (3) species delimitation based on multi-locus data and multispecies coalescent methods [139].

In the first simplest approach, we produced ML tree based only on *CYTB* sequences. We then compared the revealed clades with the species names used in previous studies (e.g. [31, 32, 41, 43, 45]), and the distribution of particular clades with positions of type localities of nominal species. By using this approach we newly identified several highly supported phylogenetic clades with parapatric distribution to previously analysed and named species, which might represent new species and are worth of further taxonomic studies. Genetic distances

(within species and to the genetically nearest lineage) from BI tree were additionally also computed using the species delimitation package [140] implemented in Genious v9.1.8 [141].

Second, we performed two analyses of species discovery based on diversity of *CYTB* marker. The Automatic Barcode Gap Discovery approach (ABGD; [137]) was used to identify barcode gap between intraspecific and interspecific genetic distances. An alternative Poisson Tree process (PTP) approach models intra- and inter-species processes by directly using the number of substitutions [138]. We used a recently improved algorithm based on PTP, the so-called multi-rate PTP (mPTP; [142]), which works better for phylogenies that have different rates of speciation-coalescence and allows to account for the different rates of branching events within each delimited species [142]. Both analyses (ABGD and mPTP) were performed using the ultrametric *CYTB* phylogeny produced by Bayesian method with strict clock in BEAST v2.4.7 [143].

The last used species delimitation approach, Species Tree And Classification Estimation, Yarely (STACEY; [139]), is a Bayesian method based on the multispecies coalescent model and estimates the probability of distinct species delimitation hypotheses given multilocus data. By utilizing multi-species coalescent theory and phylogenetic inference under a full probability Bayesian network, STACEY simultaneously estimates gene trees, the species tree and species delimitations under the assumption that all individuals that are affected by the same coalescent process, also belong to the same species/clade. We assumed conspecificity of individuals bearing mtDNA of the same lineage, identified by the first arbitrary approach described above. All but one lineages were represented at least by two individuals genotyped minimally at 3–4 markers (see Additional file 5). To relax the prior assumptions about species delimitation, we estimated a species tree using the birth-death-collapse model [144] as implemented in STACEY for BEAST 2 [145]. STACEY does not require guide tree, therefore errors resulting from a priori phylogenetic assumptions are avoided. Parameters and priors for the analysis were set according to the recommendations of STACEY manual [139]. Sequence alignments were imported into BEAUTI where they were assigned separate and unlinked substitution, clock and tree models. For mitochondrial markers (*CYTB* + *D-loop*) the ploidy was set of 0.5, for nuclear genes (*IRBP*, *RAG1*) 2.0. For the species tree prior the collapse height was set to 10^{-3} . Three independent MCMC chains were run for 10^{-7} generations and log every 5000 generations. The burn-in was 25%, and the outputs from three runs were combined in LogCombiner 2.4.7 [146]. The similarity matrix was created using SpeciesDelimitationAnalyser version 1.8.9 [147] with 25% burn-in and

collapse height of 10^{-3} . Species tree was visualised as a cloudogram using DensiTree [148].

Divergence dating

To calibrate a molecular clock, we compiled the set of usable fossils for the genus *Acomys* and its ancestors: (1) an extinct genus †*Preacomys* with three described species: †*P. griffini* Mein et al., 2004 and †*P. karsticus* Mein et al., 2004 from Harasib, Namibia (9 Ma; [83, 149]) and †*P. kikiae* Geraads, 2001 from Chorora, Ethiopia (8.5 Ma; [150, 151]). Because the position of these three fossils on phylogenetic tree is not unequivocally clear, we used a minimum age 8.5 Ma as a root for the genus *Acomys*. (2) *Acomys* from Lemudong'o locality in Kenya (6.08–6.12 Ma; [152, 153]) is the oldest *Acomys* and we considered it as the most recent common ancestor (MRCA) for taxa living currently in the northern part of eastern Africa (with the centre of their distribution in Somali-Masai savanna) and in Arabia, i.e. the clade encompassing *cahirinus* + *wilsoni* + *russatus* groups. (3) The oldest fossil of the *spinosissimus* group (sensu Verheyen et al. [45]) was discovered in Transvaal, South Africa (3 Ma; [154]), and we used it as MRCA for this group. Bayesian analyses of divergence dating were conducted on a species tree in *BEAST v2.4.7 [143]. The species were defined based on STACEY results. The mitochondrial (*CYTB* + *D-loop*) and nuclear genes (*IRBP*, *RAG1*) were imported in BEAUTI where they were assigned separate and unlinked substitution, clock and tree models. Bayesian analysis run with uncorrelated lognormal relaxed clocks [155], birth-death tree prior [156] and selected fossil constraints were defined by using lognormal statistical distributions (see Table 3 for more details). Two independent runs were carried out for 10^7 generations with sampling every 1000 generations in BEAST. We discarded first 25% as burn-in and the resulting parameter and tree files were examined for convergence and effective sample sizes (> 200) in Tracer 1.6 [157]. The two runs were combined in LogCombiner and the species tree was visualized in TreeAnnotator.

Biogeographical reconstructions

The BioGeoBEARS approach [158] was used to reconstruct the ancestral distributions and diversification patterns. Six major biogeographic regions with *Acomys*

occurrence were defined on the basis of Holt et al. [159] and Linder et al. [3]: South Africa region (S – South Africa), Zambebian region (Z – Zambebian region), Somali region (E – East Africa), Sudanian region (W – West Africa), Sahara region (N – North Africa) and the Arabian region (A – Arabian region). Dispersal rate between adjacent areas (S-Z, Z-E, E-W, E-N, N-A) was fixed to 1, whereas the dispersal of 0.5 (S-E, Z-W, Z-N, E-A) was defined for long distance dispersal (i.e. biogeographical areas separated by another region) or whenever a geographical barrier had to be crossed (e.g. multiple water bodies). Dispersal was disallowed between geographical areas separated by two or more areas (S-W, S-A, S-N, Z-N, Z-A, W-A). Biogeographic reconstruction relied on the Dispersal-Extinction Cladogenesis model (DEC) of range evolution [160]. DEC model estimates geographical range evolution using a phylogenetic tree with branch lengths scaled to time, geographical (habitat) areas for all tips, and an adjacent matrix of plausibly connected areas [85]. Because of concerns with its statistical validity [161] we did not use the +J model of Matzke [158] in our analyses.

Species distribution modelling

Assuming phylogenetic niche conservatism [162] and generally similar ecological requirements for all taxa of spiny mice, we modelled the present and past distribution of suitable climatic conditions for the genus *Acomys* by the maximum entropy approach [163]. We used 282 presence records (unique localities, Additional file 5, Fig. 2) as the input data to train the model. We modelled the suitable conditions in the recent, but we also produced paleoclimatic projections for the last glacial maximum (21 ka; MIROC resolution 2.5 min [164]) and for the last interglacial (120–140 ka; resolution 30 s [165]) using 19 bioclimatic variables from the WorldClim database [166]. The background was restricted to whole Africa and Arabia (Figs. 2 and 3). The species distribution modelling (SDM) analysis was performed using MaxEnt v3.3.3 k [167]. We used 10 replicates and the importance of environmental variables was tested using jackknife option, and for the regularization multiplier we used the default value of 1. The SDM results were converted in a map using QGIS with a maximized sum threshold [168, 169].

Table 3 List of fossils associated with the genus *Acomys* used in the divergence dating. The offset and mean represent the specification of lognormal priors used for the calibration of molecular clock. All fossil constraints were used as a crown

Fossil	MRCA	Locality	Author	Age (Ma)	Offset	Mean
† <i>Preacomys kikiae</i>	<i>Acomys</i>	Chorora, Ethiopia	Geraads (2001, 2002); Suwa et al., (2015)	8.5	8.5	1.0
† <i>Acomys</i> l. Geoffroy	<i>cahirinus</i> + <i>wilsoni</i> + <i>russatus</i>	Lemudong'o, Kenya	Manthi (2007); Manthi and Ambrose (2007)	6.08–6.12	6.08	1.0
† <i>Acomys spinosissimus</i> Peters	<i>spinosissimus</i>	Transvaal, South Africa	Denys (1999)	3	3	1.0

Additional files

Additional file 1: Taxonomic classifications of the genus *Acomys*. Bold italic names represent species reported in particular lists, standard italics represent subspecies (in Ellerman [34]; Setzer [38]; Denys et al. [16]). (XLSX 41 kb)

Additional file 2: ML phylogenetic tree based on *IRBP* sequences (nexus file). (NEXUS 6 kb)

Additional file 3: ML phylogenetic tree based on *RAG1* sequences (nexus file). (NEXUS 5 kb)

Additional file 4: Maximum clade credibility tree from STACEY with PP support (nexus file). (NEXUS 24 kb)

Additional file 5: Complete list of individuals used in this study, with details on localities and genetic data. (XLSX 97 kb)

Additional file 6: Alignment of 369 ingroup and 4 outgroup concatenated sequences of *CYTB*, *IRBP*, *RAG1* and *D-loop*. (NEXUS 1470 kb)

Abbreviations

2n: Diploid number of chromosomes; a.s.l.: Above sea level; ABGD: Automatic Barcode Gap Discovery; BI: Bayesian Inference; BIC: Bayesian information criterion; bp: Base pairs; BP: Bootstrap value; *CYTB*: Cytochrome b gene; DEC: Dispersal-Extinction Cladogenesis model; *D-loop*: Control region; EAM: Eastern Arc Mountains; EARS: The East African Rift System; GRV: The Great Rift Valley; HPD: Highest posterior density; iNHG: The intensification of Northern Hemisphere glaciation; *IRBP*: Interphotoreceptor Binding Protein gene; ka: Thousand years; LGM: Last Glacial Maximum; LIG: Last Interglacial; Ma: Million of years ago; MaxEnt: Maximum entropy; MCMC: Markov chain Monte Carlo; ML: Maximum likelihood; MOTU(s): Molecular operational taxonomic unit(s); mPTP: Multi-rate Poisson Tree process; MRCA: The most recent common ancestor; MSC: Messinian salinity crisis; Mt. (s): Mountain(s); mtDNA: Mitochondrial DNA; NF: Fundamental number of chromosome arms; NFa: Fundamental number of autosomal chromosome arms; NP: National park; PCR: The polymerase chain reaction; PP: Posterior probability; PTP: Poisson Tree process; *RAG1*: Recombination activating gene 1; SDM: Species distribution modelling; SRM: Southern Rift Mountains; STACEY: Species Tree And Classification Estimation, Yarely

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Availability of data and materials

All corresponding sequences were deposited in GenBank under accession numbers MH044731-MH045045 (see Additional file 5). The final alignment of concatenated sequences used in phylogenetic analyses is in Additional file 6.

Authors' contributions

DF, RS and JB conceived and designed the study; TA, DF, RS, LL, YM, JS, JV, JM, DM, JB collected important part of samples; TA and KP performed laboratory analysis; TA analysed data; TA, JB, KP drafted the first version of the manuscript. All authors made substantial contribution on acquisition of data, revised the draft, gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- Mayaux P, Bartholomé E, Fritz S, Belward A. A new land-cover map of Africa for the year 2000. *J Biogeogr.* 2004;31:861–77.
- Sayre R, Comer P, Hak J, Josse C, Bow J, Warner H, Larwanou M, Kelbessa E, Bekele T, Kehl H, Amena R, Andriamasimanana R, Ba T, Benson L, Boucher T, Brown M, Cress J, Dasserling O, Friesen B, Gachathi F, Houcine S, Keita M, Khamala E, Marangu D, Mokuia F, Morou B, Mucina L, Mugisha S, Mwavu E, Rutherford M, Sanou P, Syampungani S, Tomor B, Vall A, Weghe JV, Wangui E, Waruingi L. A new map of standardized terrestrial ecosystems of Africa. Washington DC: Association of American Geographers; 2013.

3. Linder HP, de Klerk HM, Born J, Burgess ND, Fjeldså J, Rahbek C. The partitioning of Africa: statistically defined biogeographical regions in sub-Saharan Africa. *J Biogeogr.* 2012;39:1189–205.
4. Maslin MA, Trauth MH. Plio-Pleistocene east African pulsed climate variability and its influence on early human evolution. In: Grine FE, Leakey RE, Fleagle JG, editors. *The first humans - origins of the genus homo*. New York: Springer Verlag; 2009. p. 151–8.
5. Kingston JD, Deino AL, Edgar RK, Hill A. Astronomically forced climate change in the Kenyan Rift Valley 2.7–2.55 Ma: implications for the evolution of early hominin ecosystems. *J Hum Evol.* 2007;53:487–503.
6. Maslin MA, Christensen B. Tectonics, orbital forcing, global climate change, and human evolution in Africa: introduction to the African paleoclimate special volume. *J Hum Evol.* 2007;53:443–64.
7. Pickford M. Uplift of the roof of Africa and its bearing on the evolution of mankind. *Hum Evol.* 1990;5:1–20.
8. Prömmel K, Cubasch U, Kaspar F. A regional climate model study of the impact of tectonic and orbital forcing on African precipitation and vegetation. *Palaeogeogr Palaeoclimatol Palaeoecol.* 2013;369:154–62.
9. Cerling TE, Harris JM, Macfadden BJ, Leakey MG, Quadek J, Eisenmann V, Ehleringer JR. Global vegetation change through the Miocene/Pliocene boundary. *Nature.* 1997;389:153–8.
10. Cerling TE, Ehleringer JR, Harris JM. Carbon dioxide starvation, the development of C4 ecosystems, and mammalian evolution. *Philos Trans R Soc B Biol Sci.* 1998;353:159–71.
11. Huang W, Takebayashi N, Qi Y, Hickerson MJ. MTML-msBayes: approximate Bayesian comparative phylogeographic inference from multiple taxa and multiple loci with rate heterogeneity. *BMC Bioinf.* 2011;12:1.
12. Le Houérou HN. Outline of the biological history of the Sahara. *J Arid Environ.* 1992;22:3–30.
13. Swezey CS. Cenozoic stratigraphy of the Sahara. *Northern Africa J African Earth Sci.* 2009;53:891–21.
14. Brito JC, Godinho R, Martínez-Freiria F, Pleguezuelos JM, Rebelo H, Santos X, Vale CG, Velo-Antón G, Boratyński Z, Carvalho SB, Ferreira S, Gonçalves DV, Silva TL, Tarroso P, Campos JC, Leite JV, Nogueira J, Alvares F, Sillero N, Sosa AS, Fahd S, Crochet P-A, Carranza S. Unravelling biodiversity, evolution and threats to conservation in the Sahara-Sahel. *Biol Rev Camb Philos Soc.* 2014;89:215–31.
15. Gonçalves DV, Martínez-Freiria F, Crochet PA, Geniez P, Carranza S, Brito JC. The role of climatic cycles and trans-Saharan migration corridors in species diversification: biogeography of *Psammophis schokari* group in North Africa. *Mol Phylogenet Evol.* 2018;118:64–74.
16. Denys C, Taylor PJ, Aplin KP. Family Muridae. In: Wilson DE, Lacher Jr TE, Mittermeier RA, editors. *Handbook of the mammals of the world, volume 7: rodents II*. Barcelona: Lynx Edicions; 2017.
17. Carere C, Casetti R, De Acetis L, Perretta G, Cirulli F, Alleva E. Behavioural and nociceptive response in male and female spiny mice (*Acomys cahirinus*) upon exposure to snake odour. *Behav Process.* 1999;47:1–10.
18. Frynta D, Fraňková M, Čížková B, Skarlandtová H, Galeštoková K, Průšová K, Šmilauer P, Šumbera R. Social and life history correlates of litter size in captive colonies of precocial spiny mice (*Acomys*). *Acta Theriol (Warsz)*. 2011;56:289–95.
19. Gutman R, Dayan T. Temporal partitioning: an experiment with two species of spiny mice. *Ecol.* 2005;86:164–73.
20. Haim A, Yedidia I, Haim D, Zisapel N. Photoperiodicity in daily rhythms of body temperature, food and energy intake of the golden spiny mouse (*Acomys russatus*). *Isr J Zool.* 1994;40:145–50.
21. Kronfeld-Schor N, Shargal E, Haim A, Dayan T, Zisapel N, Heldmaier G. Temporal partitioning among diurnally and nocturnally active desert spiny mice: energy and water turnover costs. *J Therm Biol.* 2001;26:139–42.
22. Seifert AW, Kiama SG, Seifert MG, Goheen JR, Palmer TM, Maden M. Skin shedding and tissue regeneration in African spiny mice (*Acomys*). *Nature.* 2012;489:561–5.
23. Ehrhardt N, Heldmaier G, Exner C. Adaptive mechanisms during food restriction in *Acomys russatus*: the use of torpor for desert survival. *J Comp Physiol B.* 2005;175:193–200.
24. Horowitz M, Borut A. The spiny mouse (*Acomys cahirinus*) - a rodent prototype for studying plasma volume regulation during thermal dehydration. *Isr J Zool.* 1994;40:117–25.
25. Ivlev YF, Lavrenchenko LA, OF Chernova, Bekele A. Response to overheating in spiny mice (the genus *Acomys*) from arid regions in northwestern Ethiopia. *Dokl Biol Sci.* 2011;440:335–9.
26. Weissenberg S, Shkolnik A. Metabolic rate and water economy in the desert and Mediterranean populations of the common spiny mouse (*Acomys cahirinus*) in Israel. *Isr J Zool.* 1994;40:135–43.
27. Dieterlen F. Vergleichende Untersuchungen zur Ontogenese von Stachelmaus (*Acomys*) und Wanderratte (*Rattus norvegicus*). Beiträge zum Nesthocker-Nestflüchter-Problem bei Negetieren. *Z Säugetierkd.* 1963;28:193–227.
28. Hadid Y, Pavlicek T, Beiles A, Ianovici R, Raz S, Nevo E. Sympatric incipient speciation of spiny mice *Acomys* at "Evolution Canyon," Israel. *Proc Natl Acad Sci.* 2014;111:1043–8.
29. Tučková V, Šumbera R, Čížková B. Alloparental behaviour in Sinai spiny mice *Acomys dimidiatus*: a case of misdirected parental care? *Behav Ecol Sociobiol.* 2016;70:437–47.
30. Barome PO, Monnerot M, Gautun JC. Intrageneric phylogeny of *Acomys* (Rodentia, Muridae) using mitochondrial gene cytochrome b. *Mol Phylogenet Evol.* 1998;9:560–6.
31. Barome PO, Monnerot M, Gautun JC. Phylogeny of the genus *Acomys* (Rodentia, Muridae) based on the cytochrome b mitochondrial gene: implications on taxonomy and phylogeography. *Mammalia.* 2000;64:423–38.
32. Barome PO, Lymberakis P, Monnerot M, Gautun JC. Cytochrome b sequences reveal *Acomys minous* (Rodentia, Muridae) paraphyly and answer the question about the ancestral karyotype of *Acomys dimidiatus*. *Mol Phylogenet Evol.* 2001a;18:37–46.
33. Frynta D, Palupčíková K, Bellinvia E, Benda P, Skarlandtová H, Schwarzová L, Modrý D. Phylogenetic relationships within the *cahirinus-dimidiatus* group of the genus *Acomys* (Rodentia : Muridae): new mitochondrial lineages from Sahara, Iran and the Arabian Peninsula. *Zootaxa.* 2010;2660:46–56.
34. Ellerman JR. The Families and Genera of Living Rodents. In: *Family muridae, volume II*. London: British Museum (Natural History); 1941.
35. Honacki JH, Kinman KE, Koepl JW. *Mammal species of the world: a taxonomic and geographic reference*. Kansas: Allen Press Inc. and The Association of Systematics Collections; 1982.
36. Monadjem A, Taylor P, Denys C, Cotterill FPD. *Rodents of sub-Saharan Africa. A biogeographic and taxonomic synthesis*. Berlin, Boston: De Gruyter; 2015.
37. Musser GG, Carleton MD. Superfamily Muroidea. In: Wilson DE, DAM R, editors. *Mammal Species of the World. A Taxonomic and Geographic Reference*. Baltimore: The Johns Hopkins University Press; 2005. p. 894–1531.
38. Setzer HW. Genus *Acomys*. In: Meester J, Setzer HW, editors. *The mammals of Africa: an identification manual*. Washington: Smithsonian Institution Press; 1975. p. 1–2.
39. Chevret P, Denys C, Jaeger JJ, Michaux J, Catzeflis FM. Molecular evidence that the spiny mouse (*Acomys*) is more closely related to gerbils (Gerbillinae) than to true mice (Murinae). *Proc Natl Acad Sci U S A.* 1993;90:3433–6.
40. Matthey R. Cytogenetique et taxonomie du genre *Acomys A. percivali* Dollman et *A. wilsoni* Thomas, especes D'Abyssinie. *Mammalia.* 1968;32:621–7.
41. Barome PO, Volobouev V, Monnerot M, Mfune JK, Chitaukali W, Gautun JC, Denys C. Phylogeny of *Acomys spinosissimus* (Rodentia, Muridae) from North Malawi and Tanzania: evidence from morphological and molecular analysis. *Biol J Linn Soc.* 2001b;73:321–40.
42. Mgode GF. Application of a multidisciplinary approach to the systematics of *Acomys* (Rodentia: Muridae) from northern Tanzania. PhD thesis: University of Pretoria, Zoology and Entomology; 2006. <http://www.suaire.sua.ac.za:8080/xmlui/bitstream/handle/123456789/1433/Mgode18.pdf?sequence=1&isAllowed=y>. Accessed 8 Feb 2019.
43. Nicolas V, Granjon L, Duplantier JM, Cruaud C, Dobigny G. Phylogeography of spiny mice (genus *Acomys*, Rodentia: Muridae) from the south-western margin of the Sahara with taxonomic implications. *Biol J Linn Soc.* 2009;98:29–46.
44. Petružela J, Šumbera R, Aghová T, Bryjová A, Katakweba AS, Sabuni CA, Chitaukali WN, Bryja J. Spiny mice of the Zambezian bioregion—phylogeny, biogeography and ecological differentiation within the *Acomys spinosissimus* complex. *Mamm Biol.* 2018;91:79–90.
45. Verheyen W, Hulselmans J, Wendelen W, Leirs H, Corti M, Backeljau T, Verheyen E. Contribution to the systematics and zoogeography of the east-African *Acomys spinosissimus* Peters 1852 species complex and the description of two new species (Rodentia: Muridae). *Zootaxa.* 2011;3059:1–35.
46. Alhajari BH, Hunt OJ, Steppan SJ. Molecular systematics of gerbils and deomyines (Rodentia: Gerbillinae, Deomyiinae) and a test of desert adaptation in the tympanic bulla. *J Zool Syst Evol Res.* 2015;53:312–30.

47. Bryja J, Kostin D, Meheretu Y, Šumbera R, Bryjová A, Kasso M, Mikula O, Lavrenchenko LA. Reticulate Pleistocene evolution of Ethiopian rodent genus along remarkable altitudinal gradient. *Mol Phylogenet Evol.* 2018;118:75–87.
48. Correa C, Vásquez D, Castro-Carrasco C, Zúñiga-Reinoso Á, Ortiz JC, Palma RE. Species delimitation in frogs from south American temperate forests: the case of *Eupsophus*, a taxonomically complex genus with high phenotypic variation. *PLoS One.* 2017;12:e0181026.
49. Sukumaran J, Knowles LL. Multispecies coalescent delimits structure, not species. *Proc Natl Acad Sci U S A.* 2017;114:1607–12.
50. Houghton CL, Gawriluk TR, Seifert AW. The biology and husbandry of the African spiny mouse (*Acomys cahirinus*) and the research uses of a laboratory Colony. *J Am Assoc Lab Anim Sci.* 2016;55:9–17.
51. Denys C, Gautun J-C, Tranier M, Volobouev V. Evolution of the genus *Acomys* (Rodentia, Muridae) from dental and chromosomal patterns. *Isr J Zool.* 1994;40:215–46.
52. Bray TC, Bennett NC, Mohammed OB, Alagaili AN. On the genetic diversity of spiny mice (genus *Acomys*) and gerbils (genus *Gerbillus*) in the Arabian peninsula. *Zool Middle East.* 2013;59:283–8.
53. Fernandes CA, Rohling EJ, Siddall M. Absence of post-Miocene Red Sea land bridges: biogeographic implications. *J Biogeogr.* 2006;33:961–6.
54. Giagia-Athanasopoulou EB, Rovatos MTH, Mitsainas GP, Martimianakis S, Lymberakis P, Angelou LXD, Marchal JA, Sánchez A. New data on the evolution of the Cretan spiny mouse, *Acomys minous* (Rodentia: Murinae), shed light on the phylogenetic relationships in the cahirinus group. *Biol J Linn Soc.* 2011;102:498–509.
55. Brouat C, Tatar d C, Bâ K, Cosson J-F, Dobigny G, Fichet-Calvet E, Granjon L, Lecompte E, Loiseau A, Mouline K, Piry S, Duplantier J-M. Phylogeography of the Guinea multimammate mouse (*Mastomys erythroleucus*): a case study for Sahelian species in West Africa. *J Biogeogr.* 2009;36:2237–50.
56. Dobigny G, Tatar d C, Gauthier P, Ba K, Duplantier J, Granjon L, Kergoat GJ. Mitochondrial and Nuclear Genes-Based Phylogeography of *Avicanthis niloticus* (Murinae) and Sub-Saharan Open Habitats Pleistocene History. *PLoS One.* 2013; doi.org/10.1371/journal.pone.0077815.
57. Granjon L, Colangelo P, Tatar d C, Colyn M, Dobigny G, Nicolas V. Intrageneric relationships within *Gerbilliscus* (Rodentia, Muridae, Gerbillinae), with characterization of an additional west African species. *Zootaxa.* 2012; 25:1–25.
58. Kingdon J. The Kingdon field guide to African mammals. 2nd ed. London: Bloomsbury Publishing Plc; 2015.
59. Douady CJ, Catzeflis F, Raman J, Springer MS, Stanhope MJ. The Sahara as a vicariant agent, and the role of Miocene climatic events, in the diversification of the mammalian order Macroscelidea (elephant shrews). *Proc Natl Acad Sci U S A.* 2003;100:8325–30.
60. Franck P, Garnery L, Loiseau A, Oldroyd BP, Hepburn HR, Solignac M, Cornuet JM. Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. *Heredity.* 2001;86:420–30.
61. Guillaumet A, Crochet PA, Pons JM. Climate-driven diversification in two widespread *Galerida* larks. *BMC Evol Biol.* 2008;8:32.
62. Rosevear D. The rodents of West Africa. London: Trustees of the British Museum Natural History; 1969.
63. Sicard B, Tranier M. Caractères et répartition de trois phénotypes d'*Acomys* (Rodentia, Muridae) au Burkina Faso. *Mammalia.* 1996;60:53–68.
64. Diamond JM, Hamilton AC. The distribution of forest passerine birds and quaternary climatic change in tropical Africa. *J Zool.* 1980;191:379–402.
65. Kingdon J. Where have the colonists come from? A zoogeographical examination of some mammalian isolates in eastern Africa. *Afr J Ecol.* 1981; 19:115–24.
66. Jesus FF, Wilkins JF, Solferini VN, Wakeley J. Expected coalescence times and segregating sites in a model of glacial cycles. *Genet Mol Res.* 2006;5:466–74.
67. Colangelo P, Verheyen E, Leirs H, Tatar d C, Denys C, Dobigny G, Duplantier JM, Brouat C, Granjon L, Lecompte E. A mitochondrial phylogeographic scenario for the most widespread African rodent, *Mastomys natalensis*. *Biol J Linn Soc.* 2013;108:901–16.
68. Bryja J, Mikula O, Šumbera R, Meheretu Y, Aghová T, Lavrenchenko LA, Mazoch V, Ogue N, Mbau JS, Welegerima K, Amundala N, Colyn M, Leirs H, Verheyen E. Pan-African phylogeny of *Mus* (subgenus *Nannomys*) reveals one of the most successful mammal radiations in Africa. *BMC Evol Biol.* 2014;14:256–76.
69. Bryja J, Konvičková H, Bryjová A, Mikula O, Makundi R, Chitaukali WN, Šumbera R. Differentiation underground: range-wide multilocus genetic structure of the silvery mole-rat does not support current taxonomy based on mitochondrial sequences. *Mamm Biol.* 2018;93:82–92.
70. Mazoch V, Mikula O, Bryja J, Konvičková H, Russo IR, Verheyen E, Šumbera R. Phylogeography of a widespread sub-Saharan murid rodent *Aethomys chrysophilus*: the role of geographic barriers and paleoclimate in the Zambezi bioregion. *Mammalia.* 2018;82:373–87.
71. Mikula O, Šumbera R, Aghová T, Mbau JS, Katakweba AS, Sabuni CA, Bryja J. Evolutionary history and species diversity of African pouched mice (Rodentia: Nesomyidae: *Saccostomus*). *Zool Scr.* 2016;45:595–617.
72. Aghová T, Šumbera R, Piálek L, Mikula O, McDonough MM, Lavrenchenko LA, Meheretu Y, Mbau JS, Bryja J. Multilocus phylogeny of east African gerbils (Rodentia, *Gerbilliscus*) illuminates the history of the Somali-Masai savanna. *J Biogeogr.* 2017;44:2295–307.
73. Trauth MH, Maslin MA, Deino AL, Junginger A, Lesolyoia M, Odada EO, Olago DO, Olaka LA, Strecker MR, Tiedemann R. Human evolution in a variable environment: the amplifier lakes of eastern Africa. *Quat Sci Rev.* 2010;29:2981–8.
74. Bartáková V, Reichard M, Blažek R, Poláček M, Bryja J. Terrestrial fishes: rivers are barriers to gene flow in annual fishes from the African savanna. *J Biogeogr.* 2015;42:1832–44.
75. McDonough MM, Šumbera R, Mazoch V, Ferguson AW, Phillips CD, Bryja J. Multilocus phylogeography of a widespread savanna-woodland-adapted rodent reveals the influence of Pleistocene geomorphology and climate change in Africa's Zambezi region. *Mol Ecol.* 2015;24:5248–66.
76. Keller C, Roos C, Groeneveld LF, Fischer J, Zinner D. Introgressive hybridization in southern African baboons shapes patterns of mtDNA variation. *Am J Phys Anthropol.* 2010;142:125–36.
77. Cotterill FPD. A biogeographic review of tsessebe antelopes *Damaliscus lunatus* (Bovidae: *Alcelaphini*) in south-Central Africa. *Durban Mus Novit.* 2003;28:45–55.
78. Bryja J, Granjon L, Dobigny G, Patzenhauerová H, Konečný A, Duplantier J-M, Gauthier P, Colyn M, Durnez L, Lalis A, Nicolas V. Plio-Pleistocene history of west African Sudanian savanna and the phylogeography of the *Praomys daltoni* complex (Rodentia): the environment/geography/genetic interplay. *Mol Ecol.* 2010;19:4783–99.
79. Jacquet F, Denys C, Verheyen E, Bryja J, Hutterer R, Peterhans JCK, Stanley WT, Goodman SM, Couloux A, Colyn M, Nicolas V. Phylogeography and evolutionary history of the *Crocidura olivieri* complex (Mammalia, Soricomorpha): from a forest origin to broad ecological expansion across Africa. *BMC Evol Biol.* 2015;15:1–15.
80. Aghová T, Kimura Y, Bryja J, Dobigny G, Granjon L, Kergoat GJ. Fossils know it best: using a new set of fossil calibrations to improve the temporal phylogenetic framework of murid rodents (Rodentia: Muridae). *Mol Phylogenet Evol.* 2018;128:98–111.
81. Steppan SJ, Schenk JJ. Muroid rodent phylogenetics: 900-species tree reveals increasing diversification rates. *PLoS One.* 2017;12:e0183070. <https://doi.org/10.1371/journal.pone.0183070>.
82. Ellerman JR, TCS M-S. A Checklist of Palaearctic and Indian Mammals, 1758–1946. London: Trustees of the British museum (natural history); 1951.
83. Pickford M. Age of supergene ore bodies at berg Aukas and Harasib 3a, Namibia. *Commun Geol Surv Namibia.* 1992;8:147–50.
84. Geraads D. Rongeurs du Miocene superieur de Chorora (Ethiopie): Dendromuridae, Muridae et conclusions. *Palaeovertebrata.* 2001;30:89–109.
85. Bryja J, Šumbera R, Peterhans K, Julian C, Aghová T, Bryjová A, Mikula O, Nicolas V, Denys C, Verheyen E. Evolutionary history of the thicket rats (genus *Grammomys*) mirrors the evolution of African forests since late Miocene. *J Biogeogr.* 2017;44:182–94.
86. Couvreur TL, Chatrou LW, Sosef MS, Richardson JE. Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biol.* 2008;6:54.
87. Lorenzen ED, Heller R, Siegmund HR. Comparative phylogeography of African savannah ungulates. *Mol Ecol.* 2012;21:3656–70.
88. Randi E, D'Huart JP, Lucchini V, Aman R. Evidence of two genetically deeply divergent species of warthog, *Phacochoerus africanus* and *P. aethiopicus* (Artiodactyla: Suiformes) in eastern Africa. *Mamm Biol.* 2002;67:91–6.
89. Brown DM, Brenneman RA, Koepfli K-P, Pollinger JP, Milá B, Georgiadis NJ, Louis EE, Grether GF, Jacobs DK, Wayne RK. Extensive population genetic structure in the giraffe. *BMC Biol.* 2007;5:57.
90. Fennessy J, Bidon T, Reuss F, Kumar V, Elkan P, Nilsson MA, Vamberger M, Fritz U, Janke A. Multi-locus analyses reveal four giraffe species instead of one. *Curr Biol.* 2016;26:1–7.

91. Hsü KJ, Giovanoli F. Messinian event in the black sea. *Palaeogeogr Palaeoclimatol Palaeoecol*. 1979;29:75–93.
92. Hodell DA, Curtis JH, Siero FJ, Raymo ME. Correlation of Late Miocene to early Pliocene sequences between the Mediterranean and North Atlantic. *Paleoceanography*. 2001;16:164–78.
93. Maslin MA, Haug GH, Sarnthein M, Tiedemann R, Erlenkeuser H, Stax R. 21. Northwest Pacific site 882: The initiation of Northern Hemisphere Glaciation. In: Rea DK, Basov IA, Scholl DW, Allan JF, editors. *Proceedings of the Ocean Drilling Program Vol 145. Scientific Results*; 1995. p. 179–94.
94. Maslin MA, Li XS, Loutre MF, Berger A. The contribution of orbital forcing to the progressive intensification of northern hemisphere glaciation. *Quat Sci Rev*. 1998;17:411–26.
95. Ravelo AC, Andreasen DH, Lyle M, Lyle AO, Wara MW. Regional climate shifts caused by gradual global cooling in the Pliocene epoch. *Nature*. 2004;429:263–70.
96. Berger WH, Jansen E. Mid-Pleistocene climate shift: the Nansen connection. *Geophys Monogr Ser*. 1994;85:295–311.
97. Potts R. Hominin evolution in settings of strong environmental variability. *Quat Sci Rev*. 2013;73:1–13.
98. Kebede F, Rosenbom S, Khalatbari L, Moehlman PD, Beja-Pereira A, Bekele A. Genetic diversity of the Ethiopian Grevy's zebra (*Equus grevyi*) populations that includes a unique population of the Alleddeghi plain. *Mitochondrial DNA A DNA Mapp Seq Anal*. 2016;27:397–400.
99. Baker RJ, Bradley RD. Speciation in mammals and the genetic species concept. *J Mammal*. 2006;87:643–62.
100. Groves CP. The nature of species: a rejoinder to Zachos et al. *Mamm Biol*. 2013;78:7–9.
101. Dippenaar NJ, Rautenbach IL. Morphometrics and karyology of the southern African species of the genus *Acomys* I. Geoffroy saint-Hilaire, 1838 (Rodentia: Muridae). *Ann Transvaal Museum*. 1986;34:129–83.
102. Matthey R. Etudes de cytogenetique Sur des Murinae Africains appartenant aux genres *Arvicanthis*, *Acomys* et *Mastomys* (Rodentia). *Mammalia*. 1965;29:228–49.
103. Janecek LL, Schlitter DA, Rautenbach IL. A genetic comparison of spiny mice, genus *Acomys*. *Am Soc Mammal*. 1991;72:542–52.
104. Musser G, Carleton M. Family Muridae. In: Wilson DE, Reeder DM, editors. *Mammal species of the world*. Washington DC: Smithsonian Institution Press; 1993.
105. Nevo E. Natural selection of body size differentiation in spiny mice, *Acomys*. *Z Säugetierkd*. 1989;54:81–99.
106. Corti M, Castiglia R, Colangelo P, Capanna E, Beolchini F, Bekele A, Oguge N, Makundi RH, Sichilima AM, Leirs H, Verheyen W, Vergagen R. Cytotaxonomy of rodent species from Ethiopia, Kenya, Tanzania and Zambia. *Belgian J Zool*. 2005;135:197–216.
107. Sokolov VE, Orlov VN, Baskevich MI, Bekele A, Mebrate A. A karyological study of the spiny mouse *Acomys* Geoffroy 1838 (Rodentia, Muridae) along the Ethiopian Rift Valley. *Trop Zool*. 1993;6:227–35.
108. Petter F. Eléments d'une révision des *Acomys* africains. Un sous-genre nouveau, *Peracomys* Petter and Roche, 1981 (Rongeurs, Muridés). *Ann Mus R Afr Cent Sci Zool*. 1983;237:109–19.
109. Volobouev VT, Gautun JC, Tranier M. Chromosome evolution in the genus *Acomys* (Rodentia, Muridae): chromosome banding analysis of *Acomys cahirinus*. *Mammalia*. 1996;60:217–22.
110. Volobouev V, Auffray JC, Debat V, Denys C, Gautun JC. Species delimitation in the *Acomys cahirinus* – *dimidiatus* complex (Rodentia, Muridae) inferred from chromosomal. *Biol J Linn Soc*. 2007;91:203–14.
111. Lavrenchenko LA, Kruskop SV, Bekele A, Belay G, Morozov PN, Mlev YF, Warshavsky AA. Mammals of the Babille elephant sanctuary (eastern Ethiopia). *Russ J Theriol*. 2010;9:47–60.
112. Lavrenchenko LA, Nadjafova RS, Bulatova NS. Three new karyotypes extend a Robertsonian fan in Ethiopian spiny mice of the genus *Acomys* I. Geoffroy, 1838 (Mammalia, Rodentia). *Comp Cytogenet*. 2011;5:423–31.
113. Hollister N. East African mammals in the United States National Museum. II. Rodentia, Lagomorpha and Tubulidentata. *Bull U S Natn Mus*. 1919;99:1–184.
114. Kunze B, Dieterlen F, Traut W, Winking H. Karyotype relationship among four species of spiny mice (*Acomys*, Rodentia). *Z Säugetierkd*. 1999;64:220–9.
115. Happold DCD, editor. Volume 3: Rodents, Hares and Rabbits. *Mammals of Africa*. London: Bloomsbury Publishing; 2013. p. 789.
116. Granjon L, Duplantier JM. Les Rongeurs de l'Afrique sahélo-soudanienne. Marseille: Editions de l'IRD (Collection Faune et Flore tropicales); 2009.
117. Gautun JC, Sankhon I, Tranier M. Nouvelle contribution à la connaissance des rongeurs du massif guinéen des monts Nimba (Afrique occidentale). *Systématique et aperçu quantitatif*. *Mammalia*. 1986;50:205–17.
118. Morrison-Scott TCS. The identity of the *Acomys megalotis* (Lichtenstein), described from Arabia. *Ann Mag Nat Hist*. 1939;11:238–40.
119. Setzer HW. The spiny mice (*Acomys*) of Egypt. *J Egypt Public Health Assoc*. 1959;34:93–101.
120. Ellerman JR, Morrison-Scott TCS, Hayman RW. *Southern African mammals*. London: British Museum; 1953.
121. Yalden DW, Largen MJ, Kock D. Catalogue of the mammals of Ethiopia. *Ital J Zool*. 1976;1:1–118.
122. IUCN The IUCN red list of threatened species. Version 2017–3. 2017. <http://www.iucnredlist.org>. Accessed 17 Dec 2017.
123. Ducroz JF, Volobouev V, Granjon L. A molecular perspective on the systematics and evolution of the genus *Arvicanthis* (Rodentia, Muridae): inferences from complete cytochrome *b* gene sequences. *Mol Phylogenet Evol*. 1998;10:104–17.
124. Bellinva E. A phylogenetic study of the genus *Apodemus* by sequencing the mitochondrial DNA control region. *J Zool Syst Evol Res*. 2004;42:289–97.
125. Stanhope MJ, Czelusniak J, Si JS, Nickerson J, Goodman M. A molecular perspective on mammalian evolution from the gene encoding interphotoreceptor retinoid binding protein, with convincing evidence for bat monophyly. *Mol Phylogenet Evol*. 1992;1:148–60.
126. Teeling EC, Scally M, Kao DJ, Romagnoli ML, Springer MS, Stanhope MJ. Molecular evidence regarding the origin of echolocation and flight in bats. *Nature*. 2000;403:188–92.
127. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 2004;32:1792–7.
128. Maddison WP, Maddison DR. Mesquite: a modular system for evolutionary analysis. Version 3.31. 2017. <http://mesquiteproject.org>.
129. Nylander JAA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey JL. Bayesian phylogenetic analysis of combined data. *Syst Biol*. 2004;53:47–67.
130. Lanfear R, Calcott B, Ho SY, Guindon S. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol*. 2012;29:1695–701.
131. Ripplinger J, Sullivan J. Does choice in model selection affect maximum likelihood analysis? *Syst Biol*. 2008;57:76–85.
132. Stamatakis A. RAXML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30:1312–3.
133. Hillis DM, Bull JJ. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol*. 1993;42:182–92.
134. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. 2012;61:539–42.
135. Erixon P, Suvnblad B, Britton T, Oxelman B. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Syst Biol*. 2003;52:665–73.
136. Carstens BC, Pelletier TA, Reid NM, Satler JD. How to fail at species delimitation. *Mol Ecol*. 2013;22:4369–83.
137. Puillandre N, Lambert A, Brouillet S, Achaz G. ABGD, automatic barcode gap discovery for primary species delimitation. *Mol Ecol*. 2012;21:1864–77.
138. Zhang J, Kapli P, Pavlidis P, Stamatakis A. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*. 2013;29:2869–76.
139. Jones G. Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *J Mat Biol*. 2017;74:447–67.
140. Masters BC, Fan V, Ross HA. Species delimitation – a geneious plugin for the exploration of species boundaries. *Mol Ecol Resources*. 2011;11:154–7.
141. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 2012;28:1647–9.
142. Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, Flouri T. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics*. 2017;33:1630–8.
143. Drummond AJ, Bouckaert RR. *Bayesian evolutionary analysis with BEAST*. Cambridge: Cambridge University Press; 2015.
144. Jones G, Aydin Z, Oxelman B. DISSECT: an assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. *Bioinformatics*. 2015;31:991–8.

145. Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput Biol*. 2014;10:e1003537.
146. Rambaut A, Drummond AJ. LogCombiner v2.4.7; 2017.
147. Jones G. Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. *J Math Biol*. 2017;74:447–67.
148. Bouckaert RR. DensiTree: making sense of sets of phylogenetic trees. *Bioinformatics*. 2010;26:1372–3.
149. Senut B, Pickford M, Mein P, Conroy G, Van Couvering J. Discovery of 12 new Late Cretaceous fossiliferous sites in palaeokarsts of the Otavi Mountains, Namibia. *Comptes rendus de l'Académie des sciences. Série 2, Mécanique, Physique, Chimie, Sciences de l'univers. Sci Terre*. 1992;314:727–33.
150. Geraads D. Plio-pleistocene mammalian biostratigraphy of Atlantic Morocco. *Quaternaire*. 2002;13:43–53.
151. Suwa G, Beyene Y, Nakaya H, Bernor RL, Boisserie JR, Bibi F, Ambrose SH, Sano K, Katoh S, Asfaw B. Newly discovered cercopithecoid, equid and other mammalian fossils from the Chorora formation, Ethiopia. *Anthropol Sci*. 2015;123:19–39.
152. Manthi FK. A preliminary review of the rodent fauna from Lemudong'o, southwestern Kenya, and its implication to the late Miocene paleoenvironments. *Kirtlandia*. 2007;56:92–105.
153. Deino AL, Ambrose SH. ⁴⁰Ar/³⁹Ar dating of the Lemudong'o late Miocene fossil assemblages, southern Kenya rift. *Kirtlandia*. 2007;56:65–71.
154. Denys C. Of mice and men. Evolution in East and South Africa during Plio-Pleistocene times. In: Bromage GT, Schrenk F, editors. *African Biogeography, Climate Change and Human Evolution*. New York: Oxford University Press; 1999. p. 226–52.
155. Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. Relaxed phylogenetics and dating with confidence. *PLoS Biol*. 2006;4:e88.
156. Gernhard T. The conditioned reconstructed process. *J Theor Biol*. 2008;253:769–78.
157. Rambaut A, Suchard MA, Xie D, Drummond AJ. Tracer v1.6; 2014.
158. Matzke NJ. Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Syst Biol*. 2014;63:951–70.
159. Holt BG, Lessard JP, Borregaard MK, Fritz SA, Araújo MB, Dimitrov D, Fabre PH, Graham CH, Graves GR, Jønsson KA, Nogués-Bravo D, Wang Z, Whittaker RJ, Fjeldså J, Rahbek C. An update of Wallace's zoogeographic regions of the world. *Science*. 2013;339:74–8.
160. Ree RH, Smith SA. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst Biol*. 2008;57:4–14.
161. Ree RH, Sanmartín I. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *J Biogeogr*. 2018;45:741–9.
162. Wiens JJ, Donoghue MJ. Historical biogeography, ecology, and species richness. *Trends Ecol Evol*. 2004;19:639–44.
163. Phillips SJ, Anderson RP, Schapire RE. Maximum entropy modeling of species geographic distributions. *Ecol Model*. 2006;190:231–59.
164. Braconnot P, Otto-Bliesner B, Harrison S, Joussaume S, Peterchmitt JY, Abe-Ouchi A, Crucifix M, Driesschaert E, Fichefet T, Hewitt CD, Kageyama M, Kitoh A, Laîné A, Loutre MF, Marti O, Merkel U, Ramstein G, Valdes P, Weber SL, Yu Y, Zhao Y. Results of PMIP2 coupled simulations of the mid-Holocene and last glacial maximum - part 1: experiments and large-scale features. *Clim Past*. 2007;3:261–77.
165. Otto-Bliesner BL. Simulating arctic climate warmth and icefield retreat in the last interglaciation. *Science*. 2006;311:1751–3.
166. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol*. 2005;25:1965–78.
167. Phillips SJ, Dudík M, Schapire RE. A maximum entropy approach to species distribution modeling. *Proceedings of the 21st International Conference on Machine Learning*. Canada: Banff; 2004. p. 655–62.
168. Jiménez-Valverde A, Lobo JM. Threshold criteria for conversion of probability of species presence to either-or presence-absence. *Acta Oecol*. 2007;31:361–9.
169. Manel S, Williams HC, Ormerod SJ. Evaluating presence absence models in ecology; the need to count for prevalence. *J Appl Ecol*. 2001;38:921–31.

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