

Phylogeny and conservation priorities of afrotherian mammals (Afrotheria, Mammalia)

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Submitted: 29 November 2009

Accepted: 5 September 2010

doi: 10.1111/j.1463-6409.2010.00452.x

Kuntner, M., May-Collado, L. J. & Agnarsson, I. (2011). Phylogeny and conservation priorities of afrotherian mammals (Afrotheria, Mammalia). — *Zoologica Scripta*, 40, 1–15.

Phylogenies play an increasingly important role in conservation biology providing a species-specific measure of biodiversity – evolutionary distinctiveness (ED) or phylogenetic diversity (PD) – that can help prioritize conservation effort. Currently, there are many available methods to integrate phylogeny and extinction risk, with an ongoing debate on which may be best. However, the main constraint on employing any of these methods to establish conservation priorities is the lack of detailed species-level phylogenies. Afrotheria is a recently recognized clade grouping anatomically and biologically diverse placental mammals: elephants and mammoths, dugong and manatees, hyraxes, tenrecs, golden moles, elephant shrews and aardvark. To date, phylogenetic studies have focused on understanding higher level relationships among the major groups within Afrotheria. Here, we provide a species-level phylogeny of Afrotheria based on nine molecular loci, placing nearly 70% of the extant afrotherian species (50) and five extinct species. We then use this phylogeny to assess conservation priorities focusing on the widely used evolutionary distinctiveness and global endangeredness (EDGE) method and how that compares to the more recently developed PD framework. Our results support the monophyly of Afrotheria and its sister relationship to Xenarthra. Within Afrotheria, the basal division into Afroinsectiphilia (aardvark, tenrecs, golden moles and elephant shrews) and Paenungulata (hyraxes, dugongs, manatees and elephants) is supported, as is the monophyly of all afrotherian families: Elephantidae, Procaviidae, Macroscelididae, Chrysochloridae, Tenrecidae, Trichechidae and Dugongidae. Within Afroinsectiphilia, we recover the most commonly proposed topology (Tubulidentata sister to Afroscorica plus Macroscelidea). Within Paenungulata, Sirenia is sister to Hyracoidea plus Proboscidea, a controversial relationship supported by morphology. Within Proboscidea, the mastodon is sister to the remaining elephants and the woolly mammoth sister to the Asian elephant, while both living elephant genera, *Loxodonta* and *Elephas* are paraphyletic. Top ranking evolutionarily unique species always included the aardvark, followed by several species of elephant shrews and tenrecs. For conservation priorities top ranking species always included the semi-aquatic Nimba otter shrew, some poorly known species, such as the Northern shrew tenrec, web-footed tenrec, giant otter shrew and Giant golden mole, as well as high profile conservation icons like Asian elephant, dugong and the three species of manatee. Conservation priority analyses were broadly congruent between the EDGE and PD methodologies. However, for certain species EDGE overestimates conservation urgency as it, unlike PD, fails to account for the status of closely related, but less threatened, species. Therefore, PD offers a better guide to conservation decisions.

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Introduction

Phylogenies have transformed comparative biology (Felsenstein 1985; Harvey & Pagel 1991) and have begun to play an increasingly important role in conservation biology (Faith 1992, 2007, 2008; Redding & Mooers 2006; Helmus *et al.* 2007; Isaac *et al.* 2007; Steel *et al.* 2007; Graham & Fine 2008; Mooers *et al.* 2008). Phylogenies provide information on shared and taxon-specific evolutionary history. Hence, they offer species-specific measures of biodiversity such as evolutionary distinctiveness (ED) and a family of measures based upon phylogenetic diversity (PD; Faith 1992; Faith *et al.* 2004). Both can indicate the amount of unique evolutionary history that a taxon represents. This information, in turn, can be utilized along with species imperilment estimates [global endangeredness (GE)], such as those provided by the IUCN (<http://www.iucnredlist.org/>) to help prioritize conservation effort [e.g. evolutionary distinctiveness and global endangeredness (EDGE), see Isaac *et al.* 2007; expected PD, Witting & Loeschcke 1995; Faith 2008]. Currently, one of the main constraints on integrating phylogeny and extinction risk to establish conservation priorities for mammals is the lack of detailed species-level phylogenies, other than summary supertrees (e.g. Jones *et al.* 2002; Cardillo *et al.* 2004; Bininda-Emonds 2005; Price *et al.* 2005). Afrotheria is a particularly interesting clade for the analyses of evolutionary uniqueness, because it is an old group containing a third of mammalian orders, yet it is relatively species poor. Within Afrotheria then, relatively few species represent a large amount of unique evolutionary history. This group as a whole, therefore, merits special conservation attention.

Typical higher level clades within placental mammals that were proposed based exclusively on morphology, such as Altungulata, Anagalida, Archonta, Artiodactyla, Lipotyphla, Ungulata and Volitantiata have not survived recent molecular analyses, with a few exceptions such as Xenarthra (Springer *et al.* 2007). Recent molecular and combined studies have largely established the division of placental mammals into four higher level groups: Afrotheria, Xenarthra, Laurasiatheria and Euarchontoglires, but their interrelationships have not been fully resolved and continue to be debated (Madsen *et al.* 2001; Delsuc *et al.* 2002; Hallstrom *et al.* 2007; Tabuce *et al.* 2008). Here, we focus on Afrotheria, a clade which groups elephants and their extinct relatives, mammoths, with anatomically and biologically very dissimilar relatives, such as dugong and manatees, elephant shrews, hyraxes, tenrecs, golden moles and aardvark (Fig. 1). The extraordinary common descent of the largest living land animals (elephants) with a group of marine mammals and many smaller enigmatic African placental mammals, is now broadly accepted and Afrotheria is considered as one of four mammalian superorders (Hedges

2001; Nishihara *et al.* 2005; Wildman *et al.* 2007; Arnason *et al.* 2008; Tabuce *et al.* 2008).

Afrotheria as a phylogenetic hypothesis was unexpected given classical morphological data and early morphological evidence, such as similarities in the male reproductive tract, were discarded as convergence (Glover 1973; Porter *et al.* 1996). Afrotheria was first proposed based on molecular data (for review, see Tabuce *et al.* 2008; Asher *et al.* 2009) in the 1980s (Dejong *et al.* 1981; Goodman 1982). The understanding of afrotherian relationships developed with additional molecular data (Springer *et al.* 1997, 1999; Stanhope *et al.* 1998) demonstrating that Insectivora was not monophyletic and did not contain golden moles. These were instead hypothesized to belong to a clade that contained hyraxes, manatees, elephants, elephant shrews and aardvark, a group of African origin (but, see Zack *et al.* 2005), hence the name Afrotheria. It is only recently that researchers have started to look for, and identify, cytogenetic (Robinson *et al.* 2004, 2008; Kellogg *et al.* 2007), genomic (Redi *et al.* 2007; Ruiz-Herrera & Robinson 2007) and morphological synapomorphies in support of Afrotheria. Detailed look at morphology has revealed putative synapomorphies such as late eruption of permanent teeth, vertebral anomalies, testicondy and others (Asher *et al.* 2003; Carter *et al.* 2004; Cox 2006; Sanchez-Villagra *et al.* 2007; Seiffert 2007; Asher & Lehmann 2008).

As it was proposed, Afrotheria (or Afroplacentalia) has been repeatedly supported by numerous molecular studies (Liu & Miyamoto 1999; Springer *et al.* 1999; van Dijk *et al.* 2001; Madsen *et al.* 2001; Murphy *et al.* 2001a,b, 2007; Malia *et al.* 2002; Amrine-Madsen *et al.* 2003; Douady *et al.* 2003; Nishihara *et al.* 2005; Matthee *et al.* 2007; Arnason *et al.* 2008). However, the exact placement of Afrotheria within mammals has not been well resolved. Arnason *et al.* (2008) and Amrine-Madsen *et al.* (2003) recovered Afrotheria as sister to the remaining placental mammals, but most recent analyses place it as sister to Xenarthra (anteaters, sloths and relatives), and this clade, in turn, sister to the remaining placental mammals (Wildman *et al.* 2007; Arnason *et al.* 2008). The interrelationships within Afrotheria, likewise, differ among studies, but generally consist of the major groups Hyracoidea (hyraxes or rock daisies), Proboscidea (elephants, including the extinct mammoth and mastodon), Sirenia (manatees and dugong), Tenrecidea (tenrecs), Tubulidentata (aardvark), Macroscelidea (elephant shrews) and Chrysochloridea (golden moles) (Murata *et al.* 2003; Arnason *et al.* 2008; Tabuce *et al.* 2008).

In addition to its conservation importance, Afrotheria is also a promising group for comparative evolutionary studies. The research into body size evolution alone contrasts

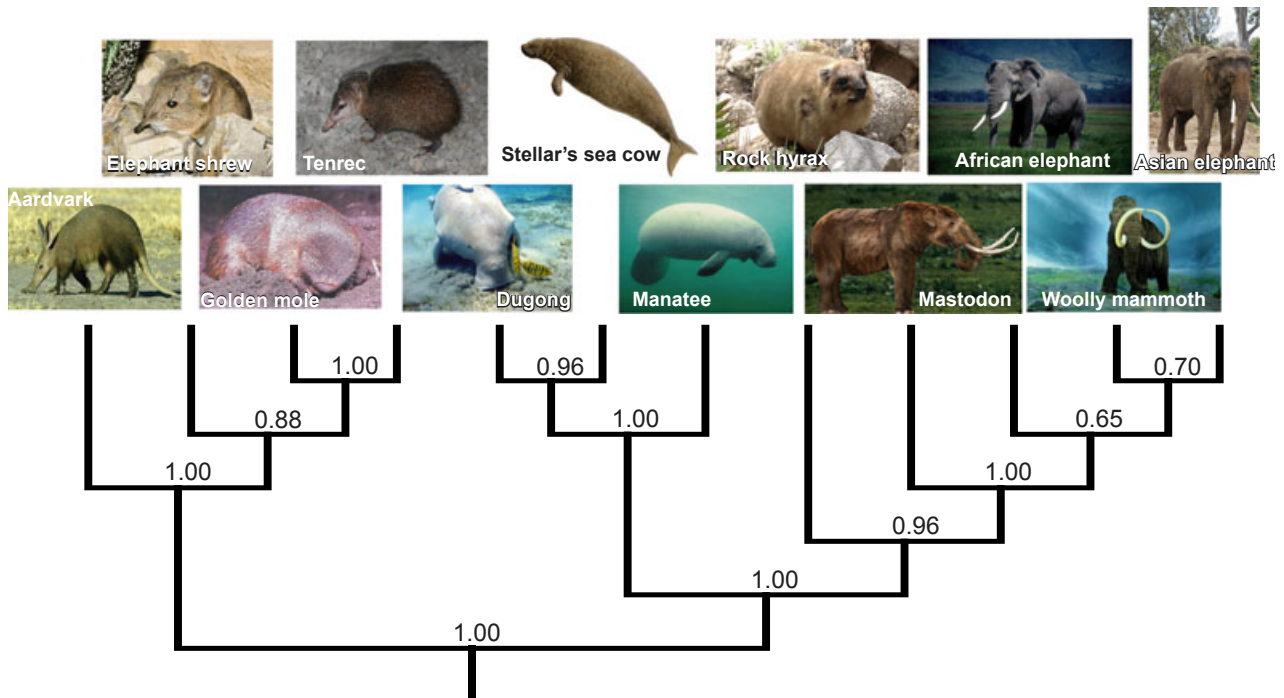


Fig. 1 Selected members of Afrotheria representing major clades, with relationships among them and clade support. The results support the division of Afrotheria into Afroinsectiphilia (aardvark, tenrecs, golden moles and elephant shrews) and Paenungulata (hyraxes, dugongs, manatees and elephants). Within Afroinsectiphilia, Tubulidentata is sister to Afroscoricida (tenrecs and golden moles) plus Macroscelidea (elephant shrews). Within Paenungulata, Sirenia is sister to Hyracoidea plus Proboscidea. Images from Wikipedia Commons (license under: http://commons.wikimedia.org/wiki/Commons:GNU_Free_Documentation_License_version_1.2): Asian elephant – Fir0002/Flagstaffotos; Elephant shrew – O. Lellinger; Golden mole – Killer18; Dugong – J. Willem; Manatee – US Geological Survey; Mastodon modified from – Dantheman9758; Woolly mammoth – Tracy O. Other images by B. Joubert, National Geographic Society (Aardvark), T. Kuntner (Rock hyrax), J.B. Hlíðberg (Stellar sea cow), M. Kuntner (Tenrec) and I. Agnarsson (African elephant).

two extremes of a range, the largest living terrestrial animals, elephants, and very small representatives such as tenrecs. Furthermore, afrotherian diversity encompasses interesting adaptations, which are convergent with those in other mammal groups. Afrotheria as well as Laurasiatheria both contain unrelated aquatic, ungulate and insectivore-like forms (Madsen *et al.* 2001), such as aquatically adapted fusiform habitus of sirenians and cetaceans, and the general appearance and habits of Malagassy tenrecs resembling those of hedgehog. Furthermore, most of afrotherian taxa are marked by various modifications of the proboscis, which sets them apart from most other mammal groups. Thus, a better understanding of phylogenetic relationships among and within Afrotheria can help reveal evolutionary patterns in this unusual group, yet, available phylogenies only contain a small portion of the total diversity of this clade.

In this study, we provide a species-level phylogenetic hypothesis of Afrotheria based on molecular data, thereby providing a tool for comparative analyses and conservation

biology. We test the monophyly and placement of Afrotheria, its composition and the species-level relationships. We base our analysis on mitochondrial cytochrome *b* (*cytb*) data, which has proven to be an exceptionally reliable marker in mammal phylogenetics (May-Collado & Agnarsson 2006; Agnarsson & May-Collado 2008; Agnarsson *et al.* 2010), and a set of three additional mitochondrial markers (12S, 16S and NADH2) and five nuclear markers [adrenergic alpha-2B-receptor (ADRA2B), androgen receptor (AR), growth hormone receptor (GHR), von Willebrand factor (vWF) and interphotoreceptor retinoid-binding protein (IRBP)]. We evaluate our Bayesian phylogenetic results by comparison to the previously proposed topologies. In particular, we discuss the classification consequences of the newly recovered topology within Proboscidea, as elephant interrelationships have not been resolved and it is still unclear how many species the African and Asian lineages represent (Debruyne *et al.* 2003; Debruyne 2005; Roca & O'Brien 2005; Johnson *et al.* 2007). Finally, we discuss the conservation implications of our findings, through

comparison of the EDGE of the species and phylogenetic diversity (PD, represented by HEDGE) metrics.

Methods

Taxon sample

We datamined GenBank for nine molecular markers that are available for a significant portion of afrotherian taxa. These include four mitochondrial loci, the protein coding genes *cytb* and NHDH dehydrogenase subunit 2 (ND2), and the ribosomal RNA 12S and 16S, and five nuclear protein coding genes: ADRA2B, AR, GHR, vWF and IRBP. We compiled matrices for each of these loci for all available afrotherian species, and 14 outgroups representing the remaining placental mammal diversity, and using two marsupial mammals as primary outgroups (Supporting information, Table S1). Combining these loci, we can represent about 70% of afrotherian species diversity in the matrix. The majority of taxa have *cytb* (68 taxa, 54 afrotherians, all outgroups), whereas other loci are available for about half or less of the taxa (22–41 taxa in total per locus). Where available, we used several terminals of widespread species/subspecies maximizing their geographic ranges. For example, the African and Asian elephants were represented by 6 and 13 terminals, respectively (Table S1), but exclusively by *cytb* sequences. In addition to extant taxa, we used the available mitogenomic data for several fossil elephantids (e.g. Gheerbrant 2009): a mastodon (*Mammuth americanum*), a woolly mammoth (*Mammuthus primigenius*) and three extinct *Elephas* taxa, the Syrian elephant (*Elephas maximus asurus*), and two pigmy elephants from the Greek island Tilos sometimes identified as *Palaoloxodon antiquus falconeri* (Table S1). Hyraxes (family Procaviidae) were represented by eight terminals of three species (out of about five). Elephant shrews (family Macroscelididae) were well represented although with several unidentified *Elephantulus* haplotypes (Table S1). We included all sirenid species: the dugong (*Dugong dugon*) and the extinct Steller's sea cow (*Hydrodamalis gigas*) of the family Dugongidae, and the three manatee species (family Trichechidae; Table S1). Finally, we included the armadillo (*Oryzomys afer*), as the single representative of the family Oryzomysidae. As the outgroup to Afrotheria is disputed, our taxon sample included representatives of Perisodactyla, Cetartiodactyla, Carnivora, Rodentia, Primates, Xenarthra, Pholidota and marsupial mammals as primary outgroups (Table S1).

Matrix composition and outgroup chimera

Composing the matrix via combination of mitochondrial and nuclear data involved creating some taxon chimeras (Poux et al. 2008). Within outgroups, our *Equus caballus* is a chimera of *E. caballus* (eight genes) and *Equus asinus* (one

gene). Our Primates is a chimera of *Homo sapiens* (eight genes) and *Pan troglodytes* (vVF).

Phylogenetic analysis

Bayesian analyses are increasingly becoming the standard for mammal molecular phylogenetic reconstruction (Kelly 2005; May-Collado & Agnarsson 2006; Agnarsson & May-Collado 2008; Agnarsson et al. 2010; but, see Waddell et al. 2001). We used Mesquite v. 2.71 (Maddison & Maddison 2009) to fetch sequence data from GenBank, and to store and manipulate them. Sequences were aligned using the default settings of the OPAL module (Wheeler & Kececioglu 2007) of Mesquite. Alignments were checked by eye, and minor modifications made to fix conspicuously misaligned blocks in 12S, 16S and AR. The aligned matrices are available from the authors. Modeltest (Posada & Crandall 1998) was used for model selection using the AIC criterion (Posada & Buckley 2004) with a parsimony tree chosen as the basis for Modeltest. We found that GTR+ Γ +I was always the best fitting model, although slightly simpler related models were not significantly worse (and thus favored by Modeltest). Hence, as GTR+ Γ +I represents a good fit for all partitions, for simplicity, we here use it for all partitions. Bayesian analysis was performed using MRBAYES V3.1.2 (Huelsenbeck & Ronquist 2001) with the settings following our previous phylogenetic studies (May-Collado & Agnarsson 2006; Agnarsson & May-Collado 2008; Agnarsson et al. 2010). Each analysis ran the Markov chain Monte Carlo search with four chains for 10 000 000 generations (repeated twice), sampling the Markov chain every 1000 generations, and the sample points of the first 5 000 000 generations were discarded as 'burnin'.

Analyses of conservation priorities

We used the Tuatara module 1.01 (Maddison & Mooers 2007) in the evolutionary analysis packet Mesquite (Maddison & Maddison 2009) to assess conservation priorities for afrotherian species. To estimate evolutionary uniqueness of species, we used all the methods implemented in Tuatara (Supporting information, Table S2), but focus on two of the methods: EDGE and the PD-type method HEDGE. The well-known ED is a measure of a species terminal branch length and its share in ancestral branches (Isaac et al. 2007), while the expected terminal branch length (HED) in addition considers extinction probabilities of species to calculate the expected terminal branch length of the focal taxon (Steel et al. 2007). The latter, as is HEDGE (see below), is a special case of the expected phylogenetic diversity (PD) framework promoted by Faith (2008) and going back to Witting & Loeschcke (1995). Other methods implemented in Tuatara (see Supporting

information, Tables S2 and S3, and for details see Maddison & Mooers 2007 and software documentation) are Evolutionary Distinctiveness Clades (EDc), Expected Evolutionary Distinctiveness Species (Exp.ED), Expected evolutionary Distinctiveness Clades (Exp.EDc), May's Distinctiveness (May 1990), Terminal Branch Length (Term.Br.Lng) and the method of Vane-Wright *et al.* (1991). We then weighted ED and HED by 'GE' producing EDGE, and HEDGE estimates (Steel *et al.* 2007). We calculate extinction risk by translating IUCN threat categories to extinction probabilities, needed as input values in Tuatara, using the 'pessimistic' approach (Mooers *et al.* 2008), the 'Isaac' method (Isaac *et al.* 2007), as well as the IUCN50 method (for review of methods, see Mooers *et al.* 2008). The 'pessimistic' transformation gives relatively great weight to phylogenetic distinctiveness of taxa in the lower threat categories, as even these are considered at some considerable risk of extinction. The IUCN50 method, in contrast, considers low risk taxa as essentially safe, thus all 'least concern' taxa score a value near zero under the EDGE and HEDGE methods. The Isaac method gives results that are somewhat intermediate between pessimistic and IUCN50. To allow the inclusion of 'data deficient' species we, arbitrarily, but probably conservatively, estimated their extinction risk as in between the 'least concern' and 'near threatened' categories (see Agnarsson *et al.* 2010 for justification). We limited our analyses to pessimistic option for all the additional methods explored (Mooers *et al.* 2008). We then compared the results of the widely used EDGE and the HEDGE method, the latter of which, like the expected PD method (Faith 2008), considers the extinction probabilities of relatives, when estimating the contribution of a given species to evolutionary diversity (Tables S2 and S3).

Results

Phylogenetic results

Our results (Figs 1–3) unequivocally support the monophyly of Afrotheria and its subdivision into Afroinsectiphilia (aardvark, tenrec, golden moles and elephant shrews) and Paenungulata (hyraxes, sirenians and proboscideans). Similar to previous works (Waddell *et al.* 2001; Murphy *et al.* 2007; Wildman *et al.* 2007; Arnason *et al.* 2008; Prasad *et al.*, 2008), we recover a monophyletic Notoplacentalia (Afrotheria plus Xenarthra, Fig. 3) sister to the remaining placental mammals (Euarchontoglires plus Laurasiatheria) (Arnason *et al.* 2008; Hallstrom & Janke 2008). Within Afroinsectiphilia (Fig. 2), we recover the 'traditional' relationships with aardvark sister to the remaining taxa, and tenrecs grouping with golden moles in the order Afrosoricida, this sister to elephant shrews (Stanhope *et al.* 1998). Within Paenungulata, Sirenia (dug-

ongs and manatees) is sister to a clade containing Hyracoidea (hyraxes) plus Proboscidea (elephants and mammoths) (Fig. 2), thus supporting neither Tethytheria [Sirenia plus Proboscidea (Arnason *et al.* 2008)] nor the commonly recovered clade Sirenia plus Hyracoidea (Malia *et al.* 2002; Douady *et al.* 2003; Poux *et al.* 2008).

Our results support the monophyly of all afrotherian families (Elephantidae, Procaviidae, Macroscelididae, Chrysochloridae, Tenrecidae, Trichechidae and Dugongidae; Fig. 2). The family Orycteropodidae and the order Tubulidentata are monotypic with the aardvark as the only living representative.

A long and well supported branch corroborates the monophyly of elephants and mammoths (Elephantidae; Fig. 2). The American mastodon (*M. americanum*) is sister to all remaining elephants (Fig. 2). The African elephant (*Loxodonta*) is not monophyletic and the recovered phylogenetic structure is incongruent with the grouping of the African elephant populations into two distinct species, the eastern savannah elephant (*Loxodonta africana*) and the western forest elephant (*Loxodonta cyclotis*). Sister to the woolly mammoth (*M. primigenius*) is a clade of 'Asian elephants', which contains the living Asian elephant populations, usually named *Elephas maximus maximus* (from Sri Lanka) and *Elephas maximus indicus* (from India and Southeast Asia). However, these populations do not form a monophyletic group, as two clades of extinct small elephants (*E. m. asurus* from Syria and a clade of small elephants from Greek islands sometimes named *PalaeoLoxodon* spp.) nest in-between the clusters of Asian elephants.

Within hyraxes, *Heterohyrax brucei* forms two distinct clades with two additional genera nesting within (Fig. 2). Within elephant shrews, *Elephantulus* is not monophyletic due to the placement of *Elephantulus rozeti* with *Macroscelides* and *Petrodromus* as previously reported by Douady *et al.* (2003). Within tenrecs, *Microgale* is paraphyletic, containing *Limnogale*, and within golden moles, *Amblysomus* is also paraphyletic (Fig. 2).

Establishing conservation priorities

We summarize first the ED and EDGE/HEDGE analyses, where we examined the effect of different criteria used to transform IUCN categories to extinction risk. We then discuss and compare the results of the two focal methods (EDGE/HEDGE) to integrate phylogeny and extinction risk; for this latter comparison we restricted our analyses to the 'pessimistic' transformation.

The ED analysis ranked the aardvark (*O. afer*) as the most evolutionarily unique afrotherian species, followed by a number of elephant shrews and tenrecs (Fig. 4A; Tables S2 and S3). Results of the EDGE and HEDGE analyses were somewhat sensitive to the method used to

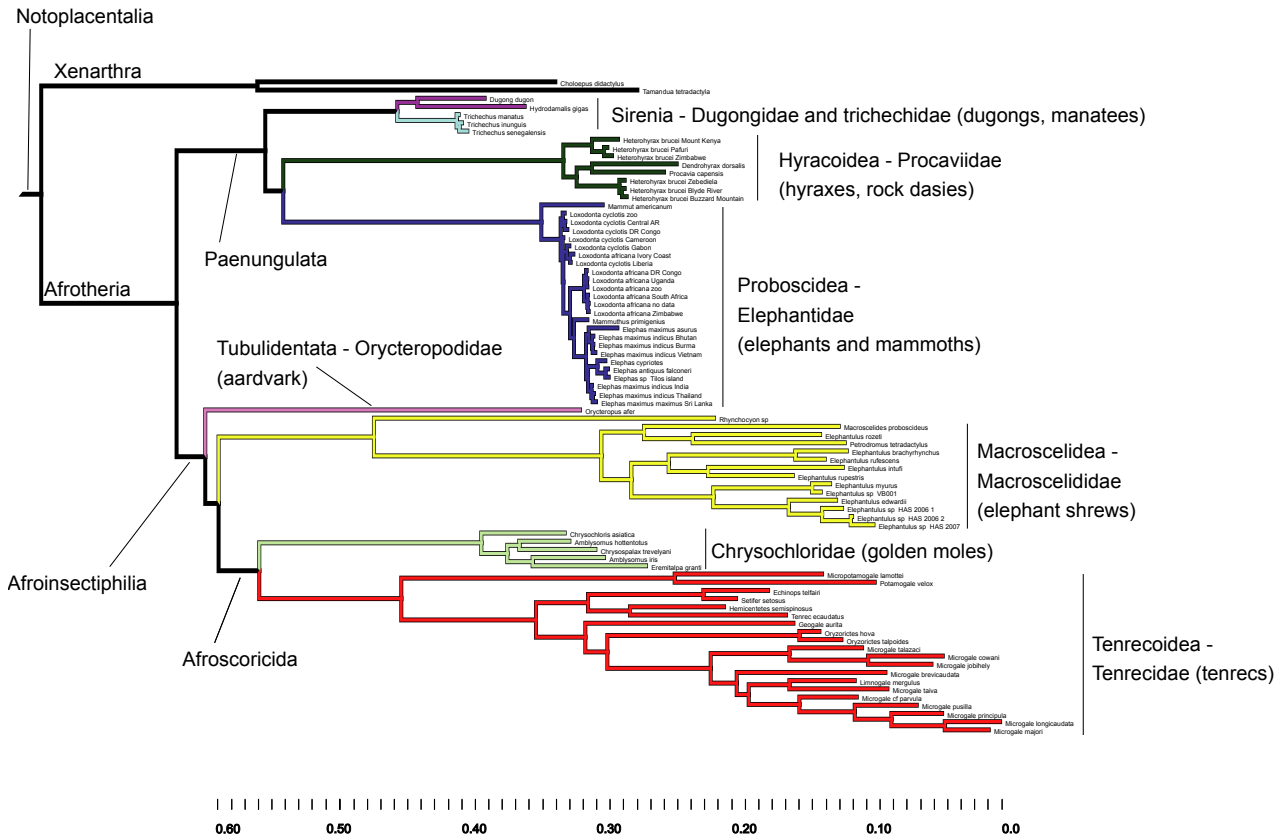


Fig. 2 Results of the Bayesian analysis with branch lengths and major clade names, showing only Afrotheria and its sister group Xenarthra.

translate IUCN categories to % extinction risk (see Mooers *et al.* 2008). Nevertheless, the results from different transformation methods broadly agree on many of the high priority taxa (Tables S2 and S3). For example, under all transformations the Nimba otter shrew (*Micropotamogale lamottei*) ranks as the highest priority species for conservation (Fig. 4B; Table S2). Other high priority taxa include Northern shrew tenrec (*Microgale jobihelyi*), giant golden mole (*Chrysofalax trevelyani*), Asian elephant (*E. maximus*), web-footed tenrec (*Limnogale mergulus*), dugong (*D. dugon*), aardvark (*O. afer*) and the three manatee species (*Trichechus* spp.).

Comparing EDGE and HEDGE, the exact ranking of taxa may differ somewhat, but in general the results are similar regardless of method. For example, EDGE lists the second to tenth critical species as *M. jobihelyi*, *Rhynchocyon* sp., *C. trevelyani*, *E. m. indicus*, *D. dugon*, *L. mergulus*, *O. afer*, *Trichechus senegalensis* and *Potamogale velox*. Under HEDGE, this list is only slightly different: *D. dugon*, *Rhynchocyon* sp., *T. senegalensis*, *P. velox*, *Trichechus inunguis*, *Trichechus manatus*, *O. afer*, *M. jobihelyi* and *C. trevelyani*,

while *E. m. indicus* and *L. mergulus*, rank twelfth and thirteenth (Table S2). There are differences in ED results among methods when comparing those that do consider extinction risk (Exp. ED) to those that do not (e.g. ED). For example, the aardvark is the top ED species, but ranks 14th under Exp. ED, and the African elephant (*L. africana*) ranks 35th under ED, but 9th under Exp. ED (Table S2).

However, the more relevant comparison is between the conservation priorities suggested by these methods. Of the 20 species that emerge as top conservation priorities under EDGE, 18 are also among the top 20 under HEDGE. The ranks of species given by EDGE and HEDGE are highly correlated ($R^2 = 0.89$, $P < 0.001$; Fig. 5). Results from other methods are also similar. However, certain taxa do differ in their rankings sufficiently to potentially impact conservation decisions. For example, *M. jobihelyi* ranks second under EDGE but ninth under HEDGE. Likewise, the ranking of the three *Trichechus* species differs substantially. In those cases, the results of PD methods such as HEDGE may be a better guide for conservation decisions (Faith 2008; see Discussion).

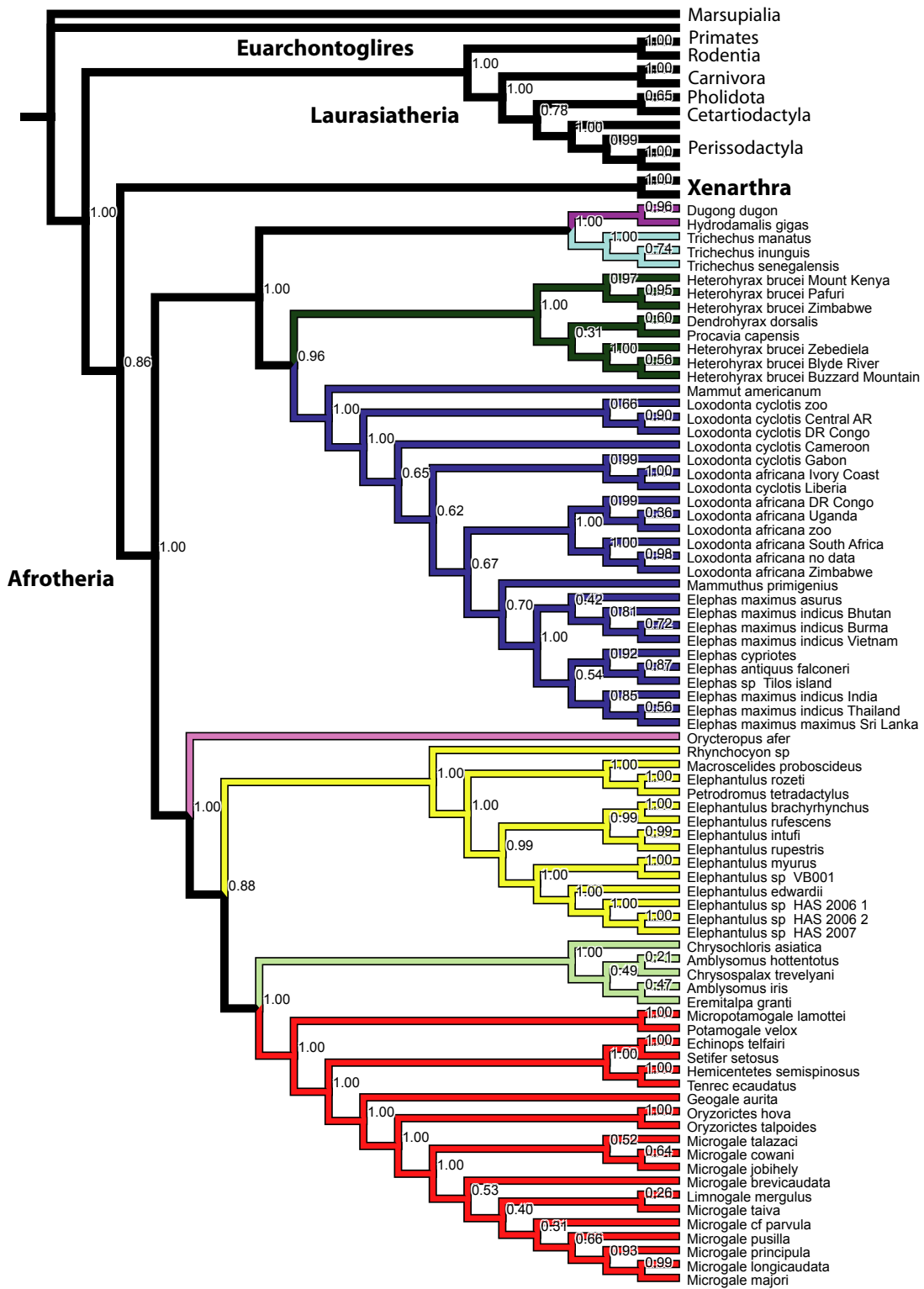


Fig. 3 Species-level relationships among Afrotheria and outgroups, with posterior probabilities. For clarity, branch length information is omitted.

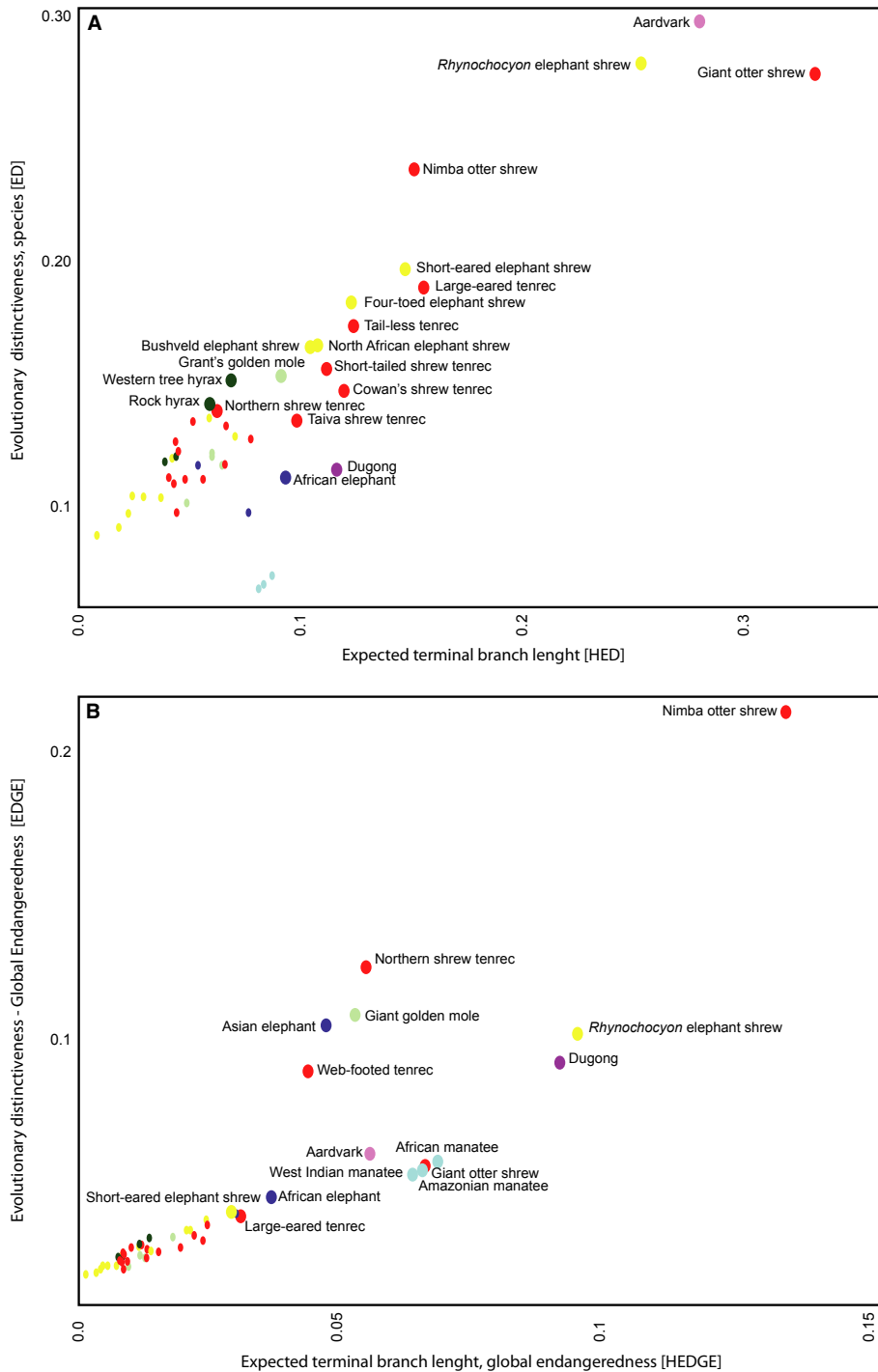


Fig. 4 Scattergram summarizing ED/HED (A) and EDGE/HEDGE (B) analyses under the ‘pessimistic’ approach (see text). Top ED/HED and top priority taxa for conservation are highlighted, names of lower ranked taxa have been omitted for clarity (see Table S2 for details). Species dots are coloured by families as in Fig. 2.

Discussion

We present the first species-level phylogeny for Afrotheria, which provides a much needed tool for comparative

biological studies and for conservation priorities of African placental mammals. The general shape of the tree with long but species poor branches (Fig. 2) implies that certain

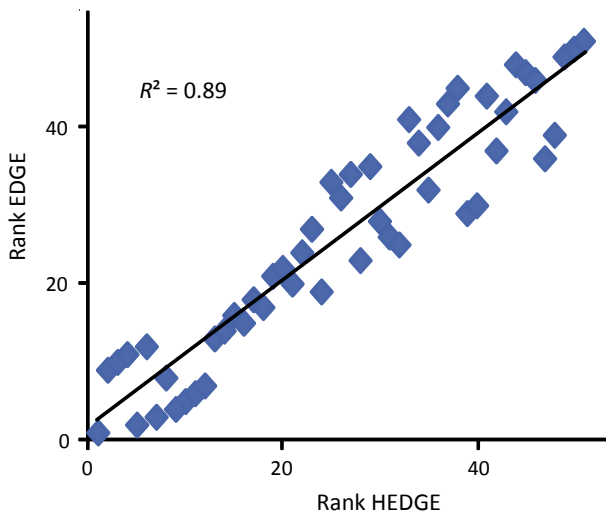


Fig. 5 Comparison of conservation priority ranks of species given by the two target methods for establishing conservation priorities (EDGE and HEDGE, see Results). The two metrics correspond closely ($R^2 = 0.89$, $P < 0.001$), however, the subtle differences may have important conservation implications (see Discussion).

groups and species of Afrotheria contain inordinate amount of unique evolutionary history, which has implications for phylogenetically informed conservation assessments (Fig. 4; Tables S2 and S3).

To resolve afrotherian species-level relationships, we performed a Bayesian analysis of nine molecular markers for 50 extant and 5 extinct afrotherians and representatives of all other placental and marsupial mammal superorders. Our results support the sister relationship of Afrotheria and Xenarthra (Fig. 2) as in several prior studies (Waddell *et al.* 2001; Murphy *et al.* 2007; Wildman *et al.* 2007; Arnason *et al.* 2008; Prasad *et al.*, 2008), refuting the basal Afrotheria (Murphy *et al.* 2001b; Amrine-Madsen *et al.* 2003; Nishihara *et al.* 2007), or the basal Xenarthra hypotheses (Delsuc *et al.* 2002).

Afrotheria monophyly

Although Afrotheria as a group is not disputed by recent molecular phylogenetic studies (Stanhope *et al.* 1998; Madsen *et al.* 2001; Murphy *et al.* 2001a,b; Malia *et al.* 2002; Nishihara *et al.* 2005, 2006, 2007; Kriegs *et al.* 2006; Arnason *et al.* 2008), morphological character support for it has been limited. However, in recent studies strong anatomical synapomorphies for Afrotheria have been established (Carter *et al.* 2006; Mess & Carter 2006), in particular skeletal and dental synapomorphies (Sanchez-Villagra *et al.* 2007; Tabuce *et al.* 2007). Seiffert (2007) reported no less than nine unambiguous synapomorphies for Afrotheria regardless of topological instabilities within, based on simul-

aneous genomic and morphological (including fossil) data. In addition, members of Afrotheria have been shown to exhibit exceptionally large genome sizes, among the highest in placental mammals (Redi *et al.* 2005, 2007). All evidence combined provides a very compelling evidence for Afrotheria. Our results, likewise, strongly support the monophyly of Afrotheria, clearly a clade that is becoming a benchmark in mammalian phylogenetics.

Relationships within Afrotheria

Our results support the division of Afrotheria into Afroinsectiphilia and Paenungulata and all families within Afrotheria (Fig. 2). Our dataset is in agreement with the majority of other studies recovering Tubulidentata as sister to the remaining afroinsectiphilians (Tabuce *et al.* 2008), and the group Afrosoricida, uniting tenrecs and golden moles (Cox 2006; Nishihara *et al.* 2006).

Our results strongly support Paenungulata, containing hyraxes, sirenians and proboscideans, which has been a benchmark clade in previous molecular studies (Stanhope *et al.* 1998; Springer *et al.* 1999; Nishihara *et al.* 2006; Arnason *et al.* 2008). We find no support for Hyracoidea sister to Tethytheria (Sirenia plus Proboscidea), contradicting many prior studies (Murata *et al.* 2003; Nishihara *et al.* 2005; Kjer & Honeycutt 2007; Seiffert 2007; Arnason *et al.* 2008), or for Sirenia plus Hyracoidea contradicting others (Liu & Miyamoto 1999; Nishihara *et al.* 2006). Instead, our results support the clade elephants plus hyraxes (to the exclusion of sirenians), as suggested to date only by Cox's (2006) analysis of orbital skeletal features. Given the support for different relationships within Paenungulata, further research is needed to resolve these ambiguities.

Species-level relationships

Tenrecs. Not surprisingly, our results regarding the relationships within tenrecs closely resemble those of Poux *et al.* (2008), although we here included many more taxa. We recovered paraphyletic *Microgale* including *Limnogale* (Poux *et al.* 2008) and a monophyletic *Oryzorictes*. However, all other terminals represented unique genera, and thus their monophyly could not be tested.

Golden moles. Golden mole monophyly is strongly supported but the relationships within are practically unresolved due to low clade support (Fig. 3). Hence, the implied paraphyly of *Amblysomus* requires further testing.

Elephant shrews. As found by Douady *et al.* (2003), *Elephantulus* is not monophyletic due to the placement of *E. rozeti* in a clade with *Petrodomus* and *Macroscelides*, a

clade that is sister to the remaining *Elephantulus* (Fig. 2). *Elephantulus* minus *E. rozeti* is apparently a more speciose genus than hitherto appreciated. Our results suggest that several of the terminals may represent new species: E sp. VB001 appears to be a new species, and deep divergences imply further new species for haplotypes HAS 2006-1, 2 and 2007. Future work on this group should aim to broadly sample geographic ‘populations’ of *Elephantulus* species, which may reveal as yet unappreciated species diversity.

The deep divergence between *Rhynchocyon* sp. and the clade containing all other elephant shrews mirrors the results of Douady *et al.* (2003), who contend that rhynchocyonines are typical living fossils, being an ancient group (43 MYA), with little morphological deviation from the ancestral groundplan, and low taxonomic diversity.

Hyraxes. *Heterohyrax brucei* forms two strongly supported clades that are incongruent with geography (Fig. 3). These groups are not sister clades, which implies that *H. brucei* as currently delimited contains much genetic structure, and suggests the possibility that these populations may be taxonomically distinct. The monophyly of *Dendrohyrax* and *Procavia* could not be tested in the current taxon sample.

Sirenians. Our analysis recovered the monophyly of Dugongidae and Trichechidae (Fig. 2). The relationships in this group reflect the biogeographical separation between the two main families. Manatees are thought to have become restricted to coastal areas in South America where there was a great source of aquatic macrophytes, and true grasses, while dugongids inhabited West Atlantic and Caribbean marine waters where they exploited seagrass meadows (Domning 1982). Manatees are thought to have outcompeted dugongids in sea grass meadow habitats resulting in the extinction of dugongids in the West Indies (Domning 1982).

Contrasting Vianna *et al.* (2006) our results do not support a sister relationship between freshwater Amazonian (*T. inunguis*) and the Antillean (*T. manatus*) manatee, but rather supported a sister relationship of the Amazonian and West African manatee (*T. senegalensis*).

Proboscideans. Our results corroborate the generic relationships of recent studies utilizing the newly available mitogenomic mammoth data (Rogaev *et al.* 2006; Debruyne *et al.*, 2008; Roca 2008) where American mastodon was used to root the elephant tree, and mammoth and Asian elephant grouped to the exclusion of the African elephant (Fig. 2). In our study, the data for most elephant individuals comes only from the *cytb* gene and these results should

thus be interpreted with care, however, as in prior studies, our results are incongruent with the current taxonomic status of both African living elephants. While the savannah elephants (see exception below) form a monophylum (*L. africana*), this is sister to a clade containing *Elephas* s.l. and *Mammuthus*, but the remaining *Loxodonta* terminals (labelled as *L. cyclotis*) are scattered at the base of the elephant (minus *Mammuth*) clade (Fig. 2). This would render the genus *Loxodonta* and the species *L. cyclotis* paraphyletic. Johnson *et al.* (2007) found that African elephant mitogenomic diversity is complicated and could be interpreted as evidence of more than two species, however, both Debruyne (2005) and Johnson *et al.* (2007) made the point of retaining the taxonomy of a single African species of elephant. Considering the important conservation implications of elephant taxonomy, it is an urgent priority to fully resolve the African and Asian elephant relationships and diversity.

Our results suggest, as have prior studies, that the fossil pygmy elephants (*E. m. asurus*, *Elephas cypriotes*, *Elephas antiquus falconeri*), were all small representatives of the common Asian elephant, *E. maximus*.

Establishing conservation priorities

We focus our discussion on results obtained using the ED/HED and EDGE/HEDGE analyses (Fig. 4; Tables S1–S3). Faith (2008) reviewed the methods for assessing taxonomic ED, including those that take into account extinction risk, such as the expected phylogenetic diversity measure (Faith 2008) and those that do not, such as ED. Faith (2008) argues that expected PD is superior to ED weighted by extinction risk (EDGE) as it better captures the idea of shared responsibility for the persistence of evolutionary history, by taking into account the status of close relatives through their extinction probabilities. For phylogenetic analyses of conservation priorities, the ED/EDGE method is probably the most commonly used among those available (e.g. the EDGE of existence program), a popularity that may partially be explained by the method being fully implemented in Tuatara. However, if Faith’s (2008) argument holds, then it will be important to understand how often and why results from EDGE and PD type analyses differ. Further research on the sensitivity of PD type analyses will also be necessary. For example, it will be important to analyse the sensitivity of different methods to uncertainty in extinction probabilities (Mooers *et al.*, 2008). It is obviously extremely difficult to estimate with any accuracy the per cent extinction risk of any given species (Mooers *et al.* 2008), thus these estimates are far from being ‘hard numbers’. Our results here suggest that in this test case EDGE methodology is quite sensitive to how IUCN cat-

egories are transformed to % extinction risk, but we are not aware of similar sensitivity analyses for the PD methodology. Several authors have shown that using different methods results in differing ranking of species and therefore can impact conservation decisions (Moore *et al.*, 2007; Faith 2008). Our approach here is to contrast the most widely used method (EDGE) with the arguably superior PD method framework (Faith 2008) as exemplified by HEDGE. We found relatively few differences in conservation priority assessments among different methods used (Tables S2 and S3). The top 20 lists of species for conservation priorities based on EDGE and HEDGE, for example, share 18 species, and the rankings suggested by these two methods are highly correlated (Fig. 5).

Nevertheless, the relative ranking of some individual species differs somewhat among methods, which could have consequences for species conservation (Faith 2008). An example that highlights the differences in approaches is the Northern shrew tenrec (*M. jobibely*), which ranked second under EDGE but only ninth under HEDGE. Here, EDGE fails to take into account the fact that its sister species are not listed as vulnerable. Another case of inconsistent results is in the ranking of the three manatee (*Trichechus*) species. A PD approach (HEDGE) ranks them all high because closely related species are all listed as vulnerable, whereas EDGE simply ranks each species separately and fails to highlight the need to protect deeper branches (Faith 2008). Furthermore, EDGE overestimated the conservation importance of *L. mergulus* and *E. maximus*; in both cases HEDGE recognizes that their closest relatives are not equally vulnerable, and hence these species should not rank as high as indicated by EDGE. As these results show, although the methods give overall similar results, the PD approach as implemented by HEDGE more appropriately accounts for extinction risk over a broader phylogenetic context. Therefore, we agree with Faith (2008) that conservation priorities are better based on the PD approach. Furthermore, optimization strategies have been developed for the PD framework that seek the best set of species for conservation rather than simply relying on the rank of individual species (for further discussion, see Faith 2008).

The most evolutionary distinct afrotherian species according to our results is the aardvark (Fig. 4A). The uniqueness of the aardvark was early on recognized due to its unusual morphological characteristics, as a somewhat pig-like animal with sparse hair, four fingers, five toes, long and slender snout, long naked ears and unique type of teeth (Lehmann 2009). The aardvark is the only living representative of Tubulidentata and thus alone preserves the unique morphological and molecular evolutionary history of the lineage. Currently, the aardvark is listed under

the IUCN Red List 2009 as a least concern species due to the lack of indications of population reduction across its wide distribution. There are no major threats reported for the species and most populations appear to inhabit protected areas across its distribution (<http://www.iucnredlist.org/>). The relatively high ranking of this species for conservation priorities shows the relevance of phylogeny in making conservation decisions. Even though relatively safe at the moment, the aardvark merits attention due to its uniqueness. Apart from the aardvark, the majority of the most ED species are elephant shrews and tenrecs (Fig. 4A). This reflects the general phylogenetic structure within these groups, marked by long, species poor branches (Fig. 2).

The Nimba otter shrew (*M. lamottei*) ranks highest for conservation priorities among afrotherians (Fig. 4B), with the Northern shrew tenrec (*M. jobibely*), giant golden mole (*Eremitalpa granti*), and Asian elephant (*E. maximus*) being other top priority conservation taxa independent of methods used (Tables S2 and S3). The Nimba otter shrew, Asian elephant and giant golden mole are all high ranking taxa in the EDGE of Existence programme (<http://www.edgeofexistence.org/>) and active conservation effort exists to secure the future of these species (<http://www.iucnredlist.org/>). However, the Northern shrew tenrec is not identified in the EDGE of Existence program as a high priority taxon, presumably because it was recently discovered and its phylogenetic history has just begun to be revealed (Goodman *et al.* 2006). Our analysis suggests that the Northern shrew tenrec should receive immediate conservation attention. Like the Nimba otter shrew, this species has a very restricted distribution in the Tsaratanana Massif in northern Madagascar, and none of the records of the species have come from currently protected areas (Goodman *et al.* 2006).

Especially for taxa in the 'least concern' IUCN category, species ranking is somewhat method-dependent (Tables S2 and S3). Under IUCN50 transformation of threat category to % extinction risk, species in lower risk categories are considered essentially safe, and therefore not in need of urgent conservation planning. However, given the rapid ongoing global change and unforeseeable changes in populations of species, other methods that consider even 'least concern' species to be at some significant risk are probably more realistic. Under the 'pessimistic' approach, further high priority species include: web-footed tenrec (*L. mergulus*), dugong (*D. dugon*), giant otter shrew (*P. velox*), aardvark (*O. afer*), the three species of manatee (*Trichechus* spp.) and African elephant (*L. africana*). Many of these species are also ranked highly in the EDGE of Existence program and for these species extensive conservation efforts exist (<http://www.iucnredlist.org/>). However,

others like web-footed tenrec and giant otter shrew are very poorly known and have received relatively little conservation attention to date.

Conclusions

The monophyly of Afrotheria, a recently discovered but already undisputed mammal clade, is confirmed. The placement of Afrotheria as sister to Xenarthra is supported, these being sister to all other placental mammals (Laurasiatheria + Euarchontoglires). The basal division of Afrotheria into Afroinsectiphilia (with aardvark, tenrecs, golden moles and elephant shrews) and Paenungulata (hyraxes, dugongs, manatees and elephants) is supported, and all familial groups are monophyletic. However, within Paenungulata the sister relationship of elephants and hyraxes dispute most recent studies, thus reflecting the state of the art in afrotherian phylogenetic research. These results point to urgent priorities in resolving afrotherian phylogenetic relationships. Similarly, the taxonomic status of the African and Asian elephants will likely need to be redefined.

Afrotheria, although relatively species poor, represents an inordinate amount of unique evolutionary diversity (see e.g. Afrotheria Specialist Group at <http://www.afrotheria.net/ASG.html>). This is evidenced by the shape of the tree (Fig. 2), marked by long branches, but relatively low diversity per branch. Our conservation priority analyses support the importance of ongoing conservation effort for several afrotherian species, but also highlight some poorly known species, such as the Northern shrew tenrec (*M. jobibely*), that urgently need conservation attention. We find overall high correspondence between the EDGE and PD methodology, but conclude that the small differences can have important conservation implications and that in such cases PD offers conservation priorities that more appropriately account for 'shared responsibility for the persistence of evolutionary history' (Faith 2008: 1461).

In sum, our results are broadly congruent with recent research on Afrotheria interrelationships, but for the first time the majority of afrotherian species are placed phylogenetically. This phylogeny thus provides the most detailed tool currently available for comparative species-level biological studies within Afrotheria, and for establishing conservation priorities.

Acknowledgements

This research was supported in part by the Slovenian Research Agency (research grant to IA, program financing support for MK), the Scientific Research Centre of the Slovenian Academy of Sciences and Arts (international travel grant to MK) and the Department of Biology, University of Puerto Rico (IA, LMC). We thank D. Faith for a most constructive review.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Terminals used for phylogenetic analyses with GenBank accession numbers ('see text' refers to '*Matrix composition and taxon chimera*' under Methods).

Table S2. Results of ED, EDGE and HEDGE analyses using the 'pessimistic', Isaac, and IUCN50 translations of IUCN categories to % extinction risk. Table shows absolute values and ranks, with the 10 top priority species for conservation in each analysis highlighted.

Table S3. Results of all conservation priority analyses implemented in Tuatara (see Methods), species ranked by ED from the most to the least evolutionarily distinct.

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