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Letter to the Editor

Phylogenetics of the Tenrecidae (Mammalia): a response to Douady et al., 2002

The mammalian family Tenrecidae is comprised of 10 extant genera and at least three fossil forms. Eight of the living taxa are known from Madagascar; two living and three extinct genera comprise the African record. Using a morphological dataset sampled for tenrecs and other living and extinct mammals, I have previously (Asher, 1999, 2000) described support for the paraphyly of Malagasy tenrecs. Recently, Douady et al. (2002) discussed the implications of new sequence data for this issue. Their analysis provides yet more molecular support for paraphyly of the Lipotyphla and for an endemic clade of African mammals. However, it misrepresents the conclusions of Asher (1999) and contains methodological oversights that I would like to briefly address in this letter.

Douady et al. (2002) correctly recognized that the morphological data discussed by Asher (1999) did not support an endemic clade of African mammals and were ambiguous regarding the monophyly of the Lipotyphla. However, their characterization of "Asher's paraphyletic (Tenrecinae + Chrysochloridae) hypothesis" (Douady et al., 2002, p. 361), i.e., that African golden moles nest within the Tenrecidae as sister taxa to spiny tenrecs in the subfamily Tenrecinae, is inaccurate. I found no consistent pattern of chrysochlorid affinities across the analyses discussed in Asher (1999), and summarized my findings as follows: "...the position of the Chrysochloridae varies from nesting within the Tenrecidae to comprising the sister taxon to either the Tenrecidae or the Talpidae. Without a clear rationale for preferring some... assumption sets over others, precise statements on... chrysochlorid affinities do not seem justified at present" (Asher, 1999, p. 238). Stated with regard to tenrecid monophyly, I did not seriously question the integrity of this taxon and should not be cited for "Asher's hypothesis of a paraphyletic Tenrecidae" (Douady et al., 2002, p. 361).

Douady et al. (2002) also misrepresented my hypothesis of Malagasy tenrec paraphyly. The only well-supported clade discussed by Asher (1999) in favor of paraphyly was one comprised of Malagasy *Limnogale* and African potamogalines. This clade appeared throughout the eight analyses presented by Asher (1999) with support indices comparable to those of the Soricidae (used in that paper as a standard of good support). In contrast, the Malagasy *Geogale*, *Microgale*, and *Oryzorictes* were variably unresolved (set 6 of Asher, 1999), grouped with other tenrecids and golden moles to the exclusion of *Limnogale* and potamogalines (sets 7 and 8), or were attached to the base of a *Limnogale*—potamogaline clade (sets 1–5). Because of this ambiguity, I depicted the relationships of *Geogale*, *Microgale*, and *Oryzorictes* as unresolved (Asher, 1999, Fig. 3) and stated that "the exact positions of *Geogale*, *Oryzorictes*, and *Microgale* are unclear based on the present data set" (Asher, 1999, p. 239). Hence, because the only tenrecid data sampled by Douady et al. (2002) come from two tenrecines, one African otter shrew, plus 12S rRNA from *Oryzorictes*, and because they lack data for *Limnogale*, their analysis is a weak test of Malagasy tenrec monophyly and has little bearing on the results from Asher (1999).

Douady et al. (2002) do not address in their analysis such issues as vagaries in sequence alignment, exclusion of "alignment ambiguous" regions, and the treatment of gaps as information during phylogenetic reconstruction, despite a growing literature on the importance of each issue (see, for example, Giribet and Wheeler, 1999; Lee, 2001; Lutzoni et al., 2000; Morrison and Ellis, 1997). Thus, all of the tests presented in their tables two and three are compromised because of their peculiar homology decisions (Fig. 1), excluded data (e.g., gaps and "alignment ambiguous" sequences), and narrow choice of analysis parameters. For example, they do not address how the non-independent patterns of base substitution in rRNA genes, discussed previously by one of the coauthors (Springer and Douzery, 1996) affect their statements on topology and statistical significance, their interpretation of a "clock-like" mutation pattern in tenrecid molecules, or to what extent this nonindependence violates the IID requirement of bootstrapping (Felsenstein, 1985) and other phylogenetic tests of "significance."

A superficial inspection of their rRNA alignment revealed many differences from that used in Stanhope et al. (1998) ; see alignment "ds34832" from ftp://ftp.

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A
Amblysomus        GUG AGAAU ACCCU UU
Tenrec            GUG AAAAU GCCCU UA
Echinops          GUG AAAAU GCCCU UA
Micropotamogale GUG AGAUG CCCUU GA

B                 6         7
Amblysomus        GUG AGAAU ACCCU UU
Tenrec            GUG AAAAU GCCCU UA
Echinops          GUG AAAAU GCCCU UA
Micropotamogale GUG AGAAU GCCCU UG
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Fig. 1. Homology decisions made by Douady et al. (A) Fragment of their 12S rRNA alignment starting at their position 67. (B) Sequences submitted to GenBank (courtesy of Michael Stanhope, same accession numbers as reported by Douady et al., 2002, Table 1) fit to 12S model of Springer and Douzery (1996). Numbers indicate stems defined by Springer and Douzery (1996). Boldface "A" in *Micropotamogale* was not included in the alignment (A) of Douady et al. (2002).

embl-heidelberg.de/pub/databases/embl/align) and the secondary structure model for 12S presented by Springer and Douzery (1996), apparently used by Douady et al. (2002) to "refine" their ClustalX-hand alignment. To name a few, the alignment of Douady et al. contained no gaps. Also, their *Micropotamogale* and *Tenrec* alignments lack a nucleotide between stems 2 and 1′ (as defined by Springer and Douzery, 1996), *Micropotamogale* lacks a nucleotide between stems 6 and 7, and their *Echinops* alignment retained several bases comprising stem 8 that were missing altogether in the rRNA alignment used by Stanhope et al. (1998); based on the same *Echinops* rRNA sequence, AF069540). None of these regions was identified by Springer and Douzery (1996) as "alignment ambiguous"; and complete sequences deposited on GenBank show that missing bases do not represent deletion events. Instead, they have been omitted from the aligned datafiles.

The region identified by Springer and Douzery (1996) as the upstream half of stem 7 is composed in most taxa by the motif "GCCCU" (Springer and Douzery, 1996, p. 363). By omitting the base between stems 6 and 7, Douady et al. (2002) have, in contrast, homologized a "CCCUU" series of bases in *Micropotamogale* with "GCCCU" in most of the remaining taxa (Fig. 1). If their intent was to follow the secondary structure pattern identified by Springer and Douzery (1996), then this appears to be a mistake, as there exists about 20 bases downstream in *Micropotamogale* the motif "AGGAGC" which matches (with the second "A" comprising an unpaired "bulge," see Kjer, 1995) the complementary stem 7′ in most taxa listed in Springer and Douzery (1996), and which would have complemented the proximal stem 7 in *Micropotamogale* had Douady et al. (2002) identified it correctly. In fairness, they may not have intended this part of their 12S rRNA alignment to correspond with stem 7 of Springer and Douzery (1996), but because their alignments lack any reference to secondary structure landmarks, it is impossible to be sure.

More importantly, the fact that the rRNA alignment of Douady et al. differs from those of Springer and Douzery (1996) and Stanhope et al. (1998) underscores the subjective nature of such manual alignments. Even given constraints such as secondary structure and amino acid triplets, considerable leeway exists in assessing molecular homology (Gatesy et al., 1993; Kjer, 1995; Wheeler, 1995, 2001; Winnepenninckx and Backeljau, 1996). To make their analysis more compelling, Douady et al. should explore alternative homology assessments (a minute fraction of which has been identified above), or at least attempt to justify their apparently unique decisions on homology and exclusion of "ambiguous" data.

To their credit, Douady et al. (2002) acknowledged in their paper the proposal by Butler (1978, 1984) that the east African Miocene fossil *Parageogale* could be related to the extant Malagasy *Geogale* to the exclusion of other tenrecs, supporting paraphyly of Malagasy tenrecs. In fact, in their original description, Butler and Hopwood (1957) had placed this animal in the same genus as the extant *Geogale aurita*, naming the fossil *G. aletris*. (This taxon has since been renamed *Parageogale* by Butler, 1984; see also Poduschka and Poduschka, 1985.)

Asher (2000) tested this proposal by including *Parageogale* and another Miocene tenrec from Kenya, *Erythrozootes* (Butler, 1984) in a phylogenetic study of similar scope as that of Asher (1999). In addition to an osteological dataset, I incorporated soft tissue (see Asher, 2001) and 12S rRNA sequence data for some taxa. Unfortunately, these data are unavailable for most tenrecids (e.g., *Limnogale*) and fossils. Nevertheless, despite the large amount of missing data for *Parageogale*, it consistently appeared as the sister taxon to *Geogale*, as Butler and Hopwood (1957) originally suggested, with one exception. In this case, *Parageogale* was unresolved within a golden mole–tenrecid–*Apternodus* clade. (The latter taxon is extinct and best known from the late Eocene of western North America.) Although these morphological data are not decisive about many aspects of intra-tenrecid relationships, they do consistently support the association of tenrecid fossils summarized by Butler (1984) with one or more extant tenrecids from Madagascar. Hence, the hypothesis of Malagasy tenrec paraphyly extends to taxa known only from fossils; and studies that wish to comprehensively address this issue should incorporate these extinct taxa, in addition to the diversity of living forms.

It is entirely possible that the morphological similarities between African potamogalines and the Malagasy *Limnogale* (Asher, 1999, 2000) are homoplastic. Individual taxa within other Malagasy radiations show extraordinary anatomical similarities to mainland groups

(e.g., cranial vasculature in Malagasy cheirogaleid and Afro-Asian lorisiform primates, see Yoder, 1992) that upon closer phylogenetic examination appear to be convergent (Yoder et al., 1996). Yet molecular data are also susceptible to homoplasy (Bull et al., 1997; Cunningham et al., 1997), and definitive tests one way or the other require multiple datasets and an adequate taxon sample—fossil and living. Given their limited sample, the study of Douady et al. is not a compelling analysis of intra-tenrecid relationships and does not test any previously existing hypothesis on Malagasy tenrecid paraphyly (Asher, 1999; Butler, 1978). Future studies of tenrecid phylogeny and of mammals generally need to address these issues of sampling and homology assessment.

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