Molecular evidence for multiple origins of Insectivora and for a new order of endemic African insectivore mammals

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ABSTRACT The traditional views regarding the mammalian order Insectivora are that the group descended from a single common ancestor and that it is comprised of the following families: Soricidae (shrews), Tenrecidae (tenrecs), Solenodontidae (solenodons), Talpidae (moles), Erinaceidae (hedgehogs and gymnures), and Chrysochloridae (golden moles). Here we present a molecular analysis that includes representatives of all six families of insectivores, as well as 37 other taxa representing marsupials, monotremes, and all but two orders of placental mammals. These data come from complete sequences of the mitochondrial 12S rRNA, tRNA-Valine, and 16S rRNA genes (2.6 kb). A wide range of different methods of phylogenetic analysis groups the tenrecs and golden moles (both endemic to Africa) in an all-African superordinal clade comprised of elephants, sirenians, hyracoids, aardvark, and elephant shrews, to the exclusion of the other four remaining families of insectivores. Statistical analyses reject the idea of a monophyletic Insectivora as well as traditional concepts of the insectivore suborder Soricomorpha. These findings are supported by sequence analyses of several nuclear genes presented here: vWF, A2AB, and α-β hemoglobin. These results require that the order Insectivora be partitioned and that the two African families (golden moles and tenrecs) be placed in a new order. The African superordinal clade now includes six orders of placental mammals.

Throughout most of this century, the placental (eutherian) mammals with extant representation have been classified into 18 orders. During this period, the order Insectivora has been among the least stable higher taxa in Eutheria, both in terms of phylogenetic position and taxonomic content. Beginning with Huxley (1) and later embellished by Mathew (2), insectivores have been thought to possess features that rendered them closer to the ancestral stock of mammals. Despite this presumed central position of insectivores in the evolutionary history of mammals, the composition of the group never has been widely agreed on. The prevalent morphological view (3) suggests that the following extant families of "insectivores" descended from a single common ancestor and as such should be those groups that are regarded as the constituents of the order Insectivora (Lipotyphla): Soricidae (shrews), Tenrecidae (tenrecs), Solenodontidae (solenodons), Talpidae (moles), Erinaceidae (hedgehogs and gymnures), and Chrysochloridae (golden moles).

Butler (4) listed six morphological characteristics that, in his opinion, supported a monophyletic Insectivora including (*i*) absence of cecum; (*ii*) reduction of pubic symphysis; (*iii*) maxillary expansion within orbit, displacing palatine; (*iv*) mobile proboscis; (*v*) reduction of jugal; and (*vi*) hemochorial placenta. More recently, MacPhee and Novacek (3) have reviewed the evidence and concluded that characteristics (*i*) and (*ii*) support lipotyphlan monophyly, characteristic (*iii*) possibly does, and (*iv–vi*), as currently defined, do not, leaving two to three characteristics that, in their opinion, support the order Insectivora.

The six families of insectivores are most often grouped into two clades of subordinal rank: the Erinaceomorpha (hedgehogs) and the Soricomorpha (all other families). Within the Soricomorpha, Butler (4) suggested that the golden moles and tenrecs form a clade and that moles and shrews cluster together, followed by solenodons. MacPhee and Novacek (3), however, proposed three clades of subordinal rank: Chrysochloromorpha (Chrysochloridae), Erinaceomorpha (Erinaceidae), and Soricomorpha (Soricidae, Talpidae, Solenodontidae, and Tenrecidae). This latter organization is based on their view that the Chrysochloridae is "spectacularly autapomorphic." In their opinion, golden moles show no shared derived traits with the soricomorphs and therefore should be separated from that suborder. This recommendation echoes earlier views regarding the group that have suggested that golden moles are a separate order or suborder (5–7).

A recent molecular study of mammalian phylogeny, which included three insectivore families, demonstrated that golden moles are not part of the Insectivora but instead belong to a clade of endemic African mammals that also includes elephants, hyraxes, sea cows, aardvarks, and elephant shrews (8). Evidence for this now comes from a wide range of disparate molecular loci including the nuclear AQP2, vWF, and A2AB genes as well as the mitochondrial 12S–16S rRNA genes (8, 9). The fossil record of golden moles indicates that the geographic distribution of this group has been restricted to Africa throughout its temporal range (10, 11). The fossil record of tenrecs also suggests an African origin (10, 11). This paleontological record, along with the morphological study of Butler (4), suggests a possible common ancestry for golden moles and tenrecs. However, it also may be that the autapomorphic qualities of golden moles reflect their evolutionary history as a singular distinct lineage of African insectivores, separate from the rest of the order. At present, there is no published molecular phylogenetic perspective on the evolutionary history of the Tenrecidae, there is no molecular study that includes a representative from all families of insectivores, and there are no molecular sequence data (data banks or published accounts) for solenodons.

Here, we report a molecular phylogenetic analysis involving all families of insectivores, using complete sequences of the mitochondrial 12S rRNA, 16S rRNA, and tRNA-Valine genes. These data, along with additional sequences from four disparate nuclear

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genes, are used to examine the extent of insectivore paraphyly or polyphyly, the conflicting hypotheses regarding the origin of the family Tenrecidae, and the possibility of an African clade of insectivores.

MATERIALS AND METHODS

The 12S rRNA, tRNA-Valine, and 16S rRNA genes were amplified and sequenced [as described elsewhere refs. 8 and 12] from the following taxa: tenrec (*Echinops telfairi*; Insectivora; AF069540), hairy armadillo (*Chaetophractus villosus*; Xenarthra; U61080, AF069534), three-toed sloth (*Bradypus tridactylus*; Xenarthra; AF038022, AF069535), tube-nosed fruit bat (*Nyctimene robinsoni*; Chiroptera; U93061, AF069536), flying fox (*Pteropus hypomelanus*; Chiroptera; U93073, AF069537), false vampire bat (*Megaderma lyra*; Chiroptera; AF069538), mole (*Scalopus aquaticus*; Insectivora; AF069539), solenodon (*Solenodon paradoxus*; Insectivora; AF076646) and capybara (*Hydrochaeris hydrochaeris*; Rodentia; U61081, AF069533). These were combined with sequences already available in GenBank. Accession numbers for many of these sequences are tabulated elsewhere (9); here we list only those accession numbers that did not appear in that recent tabulation. In addition to the above species, the mitochondrial data set included the following taxa: aardvark (*Orycteropus afer*; Tubulidentata), long-nosed armadillo (*Dasypus novemcinctus*; Xenarthra; Y11832), elephant shrew (*Elephantulus rufescens*; Macroscelidea), golden mole (*Amblysomus hottentotus*; Insectivora), African elephant (*Loxodonta africana*; Proboscidea), manatee (*Trichechus manatus*; Sirenia), hyrax (*Procavia capensis*; Hyracoidea), Indian rhino (*Rhinoceros unicornis*; Perissodactyla; X97336), white rhino (*Ceratotherium simum*; Perissodactyla; Y07726), horse (*Equus caballus*; Perissodactyla), harbor seal (*Phoca vitulina*; Carnivora), gray seal (*Halichoerus grypus*; Carnivora), cat (*Felis catus*; Carnivora), fin whale (*Balaenoptera physalus*; Cetacea), blue whale (*Balaenoptera musculus*; Cetacea), cow (*Bos taurus*; Artiodactyla), deer (*Odocoileus virginianus*; Artiodactyla; M35874), mouse deer (*Tragulus napu*, Artiodactyla, M55539), pangolin (*Manis* sp.; Pholidota), shrew (*Sorex palustris*; Insectivora), human (*Homo sapiens*; Primates), gorilla (*Gorilla gorilla*; Primates), orangutan (*Pongo pygmaeus*; Primates), gibbon (*Hylobates lar*; Primates; U99256), rabbit (*Oryctolagus cuniculus*; Lagomorpha; AJ001588), hedgehog (*Erinaceus europaeus*; Insectivora), mouse (*Mus domesticus*; Rodentia), rat (*Rattus norvegicus*; Rodentia), guinea pig (*Cavia porcellus*; Rodentia), monito del monte (*Dromiciops gliroides*; Marsupialia), wallaroo (*Macropus robustus*; Marsupialia; Y10524), bandicoot (*Echymipera kalubu*; Marsupialia), opossum (*Didelphis virginiana*; Marsupialia), and platypus (*Ornithorhynchus anatinus*; Monotremata), for a total of 43 species encompassing 16 placental orders (all eutherians except Dermoptera and Scandentia), 4 marsupial orders, one monotreme, and all insectivore families.

Initial sequence alignments were constructed using CLUSTAL W and then were refined by hand following secondary structure models (13). Regions in which alignments were ambiguous (complex indels) were eliminated from phylogenetic analyses. This resulted in 2,086 nt positions for 43 taxa. All phylogenetic trees involving this data set were rooted at platypus. Upon completion of analyses involving the total mitochondrial data set, these data were analyzed with the African taxa as ingroup, using long-nosed armadillo, cow, rat, and human as outgroups in separate analyses. Again, these alignments were constructed using CLUSTAL W and then refined by hand. Ambiguous regions again were removed; however, a consequence of eliminating 35 taxa from the sequence comparisons was that a larger number of sequence alignment positions could be considered as unambiguous and thus included in the ingroup analyses. A further consequence is a slightly different number of sequence positions represented in alignments involving each of the four outgroups (armadillo: 2,506; cow: 2,624; rat: 2,603; human: 2,560).

To provide a corroborating test of our mitochondrial results, we also obtained sequences for the representative tenrec from

two nuclear genes: exon 28 of the gene for von Willebrand factor (vWF; AF076478) and the α-2B adrenergic receptor gene (A2AB; Y17692). New sequence data were also obtained for vWF from mole (AF076479), and hairy armadillo (AF076480). These sequences were combined with already existing data for both of these genes arising from previous studies (8, 9, 14). For vWF, this included the following taxa: human, galago (*Galago crassicaudatus*; Primates), agouti (*Dasyprocta agouti*; Rodentia), rat, mole-rat (*Spalax polinicus*; Rodentia), African elephant, Asian elephant (*Elephas maximus*), dugong (*Dugong dugon*; Sirenia), hyrax, aardvark, elephant shrew, harbor porpoise (*Phocoena phocoena*; Cetacea), cow, pig (*Sus scrofa*; Artiodactyla), hedgehog, golden mole, white rhinoceros, horse (*Equus asinus*), rabbit, pangolin, false-vampire bat, bar-backed fruit bat (*Dobsonia moluccensis*), cat, dog (*Canis familiaris*; Carnivora), flying lemur (*Cynocephalus variegatus*; Dermoptera), tree shrew (*Tupaiglis*; Scandentia), and three-toed sloth (scientific name indicated only when it has not been listed already for the mitochondrial data set). This resulted in a 30-taxon data set, representing all 18 eutherian orders and 4 of the 6 families of insectivores. For A2AB, the tenrec was combined with the following taxa: human, guinea pig, rat, mouse, Asian elephant, dugong, hyrax, aardvark, elephant shrew (*Macroscelides proboscideus*), cow, hedgehog, golden mole, mole (*Talpa europaea*), horse, and rabbit (scientific name listed only when that particular species is unique to the A2AB data set). The resulting A2AB alignment included 16 taxa encompassing 11 placental orders and the same 4 families of insectivores. Accession numbers for taxa involving both these genes can be found tabulated elsewhere (9). PCR and sequencing for these two loci were as described elsewhere (8, 14). Phylogenetic trees involving both of these genes were rooted with rodents; alternative rootings produced very similar results.

All of the foregoing sequence alignments are available from the European Molecular Biology Laboratory file server under the following accession numbers: vWF: DS34810; A2AB: DS34808; 12S–16S, 43 taxa: DS34832; 12S–16S, armadillo: DS34831; 12S–16S, cow: DS34811; 12S–16S, rat: DS34812; 12S–16S, human: DS34809.

Using de Queiroz's bootstrap criterion (15) as a measure of data set incongruence, the mitochondrial and the nuclear genes were judged not to be heterogenous (i.e., no conflicting nodes receiving >95% bootstrap support), and thus various data set combinations also were analyzed. These included the following: A2AB + vWF (15 taxa: human, cow, horse, hedgehog, mole, golden mole, tenrec, elephant, hyrax, dugong, aardvark, elephant shrew, rat, caviomorph rodent–guinea pig/agouti, and rabbit), vWF + mtDNA (23 taxa: human, cow, horse, hedgehog, mole, golden mole, tenrec, elephant, hyrax, dugong, aardvark, elephant shrew, rat, guinea pig/agouti, rabbit, false vampire bat, hybrid fruit bat–*Dobsonia*/*Pteropus*, white rhino, hairy armadillo, sloth, cat, pangolin, and hybrid cetacean–porpoise/fin whale), and vWF + A2AB + mtDNA (same 15 taxa as vWF + A2AB). In combined nuclear and mitochondrial data sets, the mitochondrial sequence positions represented were the same as those in the 43-taxon data set. This set of combined data sets allows maximum taxa overlap while also providing alternative perspectives on insectivore ancestries by using different numbers and combinations of taxa. All analyses involving these data set combinations were rooted with rodents.

An examination of the molecular sequence databases reveals that the only other available molecular data for the family Tenrecidae are α and β hemoglobin amino acid sequences from tenrec (*Echinops telfairi*). A variety of other mammal sequences are available for theses two peptides, many of them the same species included in this study. A tandem alignment of both of these peptides was constructed for 14 taxa including the following: human (Swiss Prot accession numbers P01922; P02023), cow (P01966; P02070), horse (P01958; P02062), rabbit (P01948; P02057), African elephant (P01955; P02085), manatee (P07414; P07415), hyrax (P01957; P02086), *Antrozous pallidus* (microbat,

P14387; P14388), rat (P01946; P02091), guinea pig (P01947; P02095), tenrec (P24291; P24292), shrew (P01950; P02060), mole (P01951; P02061), and hedgehog (P01949; P02059). Combined amino acid data sets involving A2AB, vWF, and the two hemoglobins also were constructed. In this case, the alignment consisted of the following 15 taxa: human, rat, guinea pig, elephant, dugong, hyrax, aardvark, golden mole, tenrec, elephant shrew, cow, horse, rabbit, mole, and hedgehog. In this instance, aardvark, golden mole, and elephant shrew were coded as missing data for the hemoglobins because sequences for these taxa are not available in the databases. All trees were rooted with rodents.

Maximum likelihood, minimum evolution, neighbor joining, and parsimony (all data unweighted and transversions only) were used to construct phylogenetic trees. PAUP 4.0 version d54–63 (written by D.L. Swofford) and PHYLIP (15) were used in analysis, with the exception of maximum likelihood analyses of amino acids as well as the vWF + mtDNA data set, which used PUZZLE (16). Bootstrapping was used as a measure of clade strength. All neighbor joining, parsimony, and minimum evolution analyses involved 500 replicates of the data; DNAML (15) maximum likelihood analyses involved 100 replicates. In several instances, statistical tests were conducted to assess the likelihood of constrained topologies relative to the most parsimonious trees. These included winning sites (18), Templeton (19), and Kishino–Hasegawa (20) tests and were implemented by using PAUP. Kishino–Hasegawa tests also were used to evaluate the likelihood of constrained topologies relative to the maximum likelihood trees. Maximum likelihood analyses (DNAML) used empirical base frequencies and a transition:transversion ratio of 2:1, assumed equal rates between sites, and used the global branch swapping option. Neighbor joining trees were estimated using maximum likelihood, logdet and Tamura-Nei–transversion distances. For the parsimony analyses, we used full heuristic searches, with 50 random input orders.

RESULTS

Mitochondrial Genes. All of the different types of phylogenetic analyses involving the mitochondrial data supported a clade of African-origin mammals inclusive of elephant, manatee, hyrax [these three orders generally are termed the Paenungulata; however, see comments in *Discussion* as well as in McKenna and Bell (21) for additional clarification and history of the concept], aardvark, elephant shrew, golden mole, and tenrec (Fig. 1; Table 1). Bootstrap support for this clade ranged from 56% for the very conservative transversion parsimony to 84% for neighbor joining with maximum likelihood distances. The remaining insectivores, hedgehog, solenodon, mole, and shrew were excluded from this clade in all cases. Three of these non-African families, represented by mole, shrew, and solenodon, formed a clade in the majority of tests but generally not with convincing bootstrap support. The hedgehog was in all cases on its own, ambiguously placed in various positions in the tree, depending on method of analysis. Among the non-African insectivores, the mole and shrew invariably were associated together, with moderate bootstrap support. The best tree that supported the monophyly of these non-African families added 12 substitutions to the most parsimonious tree, and this topology was judged not to be significantly different than that MP tree (Templeton: *P* = 0.377; Kishino–Hasegawa: *P* = 0.324; winning sites: *P* = 0.419). Various other nodes in the resulting trees were supported strongly and agreed with now well accepted phylogenetic hypotheses, including for example, a monophyletic Chiroptera, a Paenungulata, and an association of Artiodactyla and Cetacea (see Fig. 1 for example topology).

Within the African clade, all analyses supported a grouping of tenrec and golden mole. In the 43-taxon data set, this support ranged from 39 to 90%. However, with just the Africans as ingroup, when we used four different outgroups, support for this association was generally at our near 100% (Table 1; Fig. 3). The reason for this difference between the ingroup analyses and the
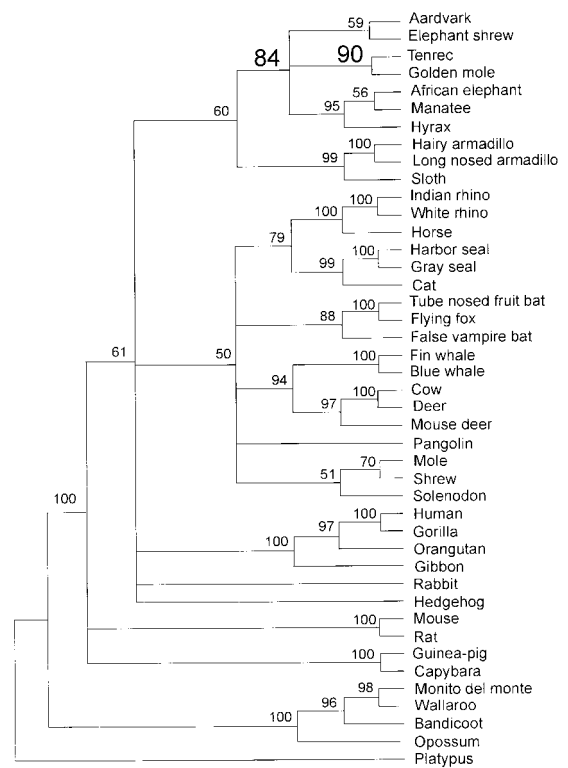

FIG. 1. Majority-rule neighbor joining bootstrap tree based on maximum likelihood distances of the 12S rRNA, tRNA-Valine, and 16S rRNA mitochondrial DNA sequences.

complete data set is that, with the inclusion of only eight taxa, there were fewer ambiguous regions in the alignment, and thus more sequence positions could be included. This, in turn, was a consequence of having only one divergent taxon to align against the Africans and, more importantly, because the Africans clearly exhibited sequence similarity in the most variable regions of the alignment. The similarity of the golden mole and tenrec sequences is further evident in alignments that exclude the outgroup. Although it is uncertain which taxon or taxa represent the most ancient branch of the African clade, the bulk of our analyses suggest that it is the elephant shrew (9). Analyses involving just the Africans, without a non-African outgroup, but instead with trees rooted at elephant shrew, resulted in golden mole/tenrec support of 100% for all analyses. In fact, all possible rootings other than the two African insectivores resulted in 100% support for golden mole/tenrec.

In addition to this strong bootstrap support for the association of golden moles and tenrecs with an all-African clade, statistical tests also support their distinction from the rest of Insectivora. Indeed, all three parsimony tests reject insectivore monophyly at *P* < 0.017 (Table 2). Similarly, the Kishino–Hasegawa test judged insectivore monophyly to be significantly worse than the highest likelihood tree (highest likelihood tree: −lnL = 31,072.10; best tree supporting insectivore monophyly: −lnL = 31,187.73; *P* = 0.0091). In addition to this ordinal distinction, the morphologically based concepts of the suborder Soricomorpha also were not supported. Butler's (4) concept has mole and shrew forming a clade, joined next by solenodon, and lastly by golden mole/tenrec. This topology costs an additional 38 substitutions and was judged significantly worse than the MP tree using all three tests (Table 2; concept of Soricomorpha was not evaluated statistically using Kishino–Hasegawa maximum likelihood test). MacPhee and Novacek (3), on the other hand, suggest Soricomorpha is comprised of tenrecs, solenodons, shrews, and moles, relative relationships undefined. This arrangement, distinct from Butler's in that golden moles are excluded, costs an additional 52 substitu-

Table 1. Bootstrap support for Afrotheria and Afrosoricida monophyly

	Level of support for monophyly of Afrotheria	Level of support for monophyly of Afrosoricida
Mitochondrial DNA (43 taxa)		
Parsimony	74	39 (98, 96, 75, 95)
Transversion parsimony	56	79 (92, 96, 55, 100)
Neighbor joining		
ML	84	90 (99, 98, 99, 100)
Logdet	82	87 (99, 97, 97, 100)
Tamura-Nei (TV)	69	75 (86, 96, 90, 94)
Maximum likelihood	77*	68* (97, 94, 98, 99)
A2AB (16 taxa)		
Parsimony	100	27
Transversion parsimony	99	36
Neighbor joining		
ML	100	25
Logdet	100	23
Tamura-Nei (TV)	93	29
Maximum likelihood	100	43
vWF (30 taxa)		
Parsimony	78	45
Transversion parsimony	80	58
Neighbor joining		
ML	77	6
Logdet	82	7
Tamura-Nei (TV)	67	26
Maximum likelihood	81†	30†
A2AB + vWF (15 taxa)		
Parsimony	100	55
Transversion parsimony	99	57
Neighbor joining		
ML	100	27
Logdet	100	28
Tamura-Nei (TV)	100	35
Maximum likelihood	100	60
Neighbor joining, amino acids	100	84
Amino acid parsimony	100	61
Amino acid maximum likelihood	96	74
A2AB + mtDNA (15 taxa)		
Parsimony	100	65
Transversion parsimony	100	68
Neighbor joining		
ML	100	89
Logdet	100	83
Tamura-Nei (TV)	100	82
Maximum likelihood	100	89
vWF + mtDNA (23 taxa)		
Parsimony	99	70
Transversion parsimony	99	94
Neighbor joining		
ML	100	63
Logdet	99	69
Tamura-Nei (TV)	99	86
Maximum likelihood	89	83
mtDNA + vWF + A2AB (15 taxa)		
Parsimony	100	76
Transversion parsimony	100	90
Neighbor joining		
ML	100	65
Logdet	100	71
Tamura-Nei (TV)	100	82
Minimum evolution − ML	100	83
Maximum likelihood	100	84
A2AB + vWF + α-β hemoglobin (15 taxa)		
Parsimony	98	87
Neighbor joining	99	88
Maximum likelihood	97	72

Numbers in parentheses indicate the results of analyses with Africans as ingroup, using armadillo, cow, rat, and human, respectively, as outgroups. Number of taxa were reduced to 24 for analysis indicated with *; this set of 24 included all of the insectivores, all of Afrotheria, in addition to the following: mouse, rat, rabbit, human, pangolin, cat, gray seal, horse, cow, fin whale, false vampire bat, long nosed armadillo, and opossum. Number of taxa was reduced to 21 for analysis indicated with † including human, rabbit, elephant shrew, golden mole, hedgehog, tenrec, aardvark, dugong, hyrax, Asian elephant, sloth, mole, tree shrew, pangolin, cow, porpoise, horse, dog, *Dobsonia*, flying lemur, and rat.

tions and also was judged significantly different than the MP tree (Table 2).

Nuclear Genes and Combined Data Sets. Both the vWF and A2AB genes were congruent with the mitochondrial data in supporting an African clade inclusive of aardvark, elephant shrew, the paenungulates, golden mole and tenrec, at the exclusion of the other insectivores in these data sets (mole and hedgehog). Bootstrap support for this African association ranges from 93 to 100% for A2AB and 67 to 82% for vWF. Like the mitochondrial data, parsimony and maximum likelihood statistical tests rejected insectivore monophyly (Table 2; A2AB-highest likelihood tree: −lnL = 7,682.90; best tree supporting insectivore monophyly: −lnL = 7,800.74; $P < 0.0001$; vWF-highest likelihood tree: −lnL = 15,996.64; best tree supporting insectivore monophyly: −lnL = 16,122.15; $P < 0.0001$). Unlike the mtDNA data set, which did not result in a clear phylogenetic placement for the hedgehog, the nuclear genes positioned it along with the mole, the other non-African insectivore in these data sets. Joining the African insectivores with the mole, in other words the construction of a partial Soricomorpha (i.e., solenodon and shrew sequences not yet available for the nuclear genes), costs an additional 34/38 and 29/54 substitutions for A2AB and vWF, respectively, all such constrained topologies judged significantly worse than the MP trees (Table 2).

Combining these two nuclear genes in a concatenated 15-taxon alignment increased African bootstrap support to 100% (or very nearly) for all analyses and resulted in some support for a golden mole/tenrec clade (Table 1). This latter grouping exhibited minimal (nucleotide sequence level) to moderate (amino acid sequence level) bootstrap support (Table 1). The classical concepts of a monophyletic Insectivora or Soricomorpha were highly significantly rejected (Table 2; highest likelihood tree: −lnL = 17,075.46; best tree supporting insectivore monophyly: −lnL = 17,264.93; $P < 0.0001$). Combining nuclear with mitochondrial data yields 100% bootstrap support for the African superordinal clade, at the exclusion of the non-African insectivores, which form a distinct well supported clade (Fig. 2) and with the majority of tests supporting an association of golden mole and tenrec at 80–95% bootstrap. Parsimony statistical tests rejected both insectivore and soricomorph monophyly at $P < 0.0001$ (Table 2), and maximum likelihood tests similarly rejected the concept of Insectivora at $P < 0.0001$ (−lnL values for highest likelihood tree and best tree supporting insectivore monophyly, respectively: A2AB + mtDNA, 22,074.83/22,316.80; vWF + mtDNA, 33,562.89/33,823.98 and vWF + A2AB + mtDNA, 31,831.15/32,152.60).

Hemoglobin analyses also supported a distinct evolutionary history for the tenrecs and the rest of Insectivora. Amino acid sequence data for these peptides include four families of Insectivora: tenrec, shrew, mole, and hedgehog. The most parsimonious tree and the NJ tree both supported a clade of shrew, mole, and hedgehog. In all cases, the tenrec was separated from that group, joining instead one (in the case of parsimony) or all of the Paenungulates. Bootstrap support for these associations was generally low; however, combining these hemoglobin data with amino acid sequences from A2AB and vWF resulted in strong support for the monophyly of the African clade (Table 2), a golden mole/tenrec grouping inside the African clade (Table 2), and the common ancestry of mole and hedgehog (the two other insectivores in this data set combination; 100% for each of parsimony, NJ and maximum likelihood). Statistical tests for this data set combination rejected insectivore monophyly, as well as the concept of Soricomorpha (Table 2).

Table 2. Results of statistical tests regarding Insectivora and Soricomorpha monophyly

		Parsimony score of alternative hypotheses (RI)			Statistical tests of alternative hypotheses								
								Soricomorpha					
	Score of the MP tree (RI)	Insectivora	Soricomorpha		Isectivora			Butler			MacPhee–Novacek		
Phylogenetic loci			B	MN	KH	Temp.	WS	KH	Temp.	WS	KH	Temp.	WS
mtDNA	5729 (0.438)	5769 (0.432)	5767 (0.432)	5781 (0.430)	0.0168	0.0154	0.0168	0.0145	0.0252	0.0119	0.0020	0.0252	0.0119
A2AB	1274 (0.474)	1310 (0.433)	1312 (0.430)	1308 (0.435)	0.0006	0.0021	0.0006	0.0002	0.0010	0.0006	0.0002	0.0010	0.0004
vWF	3094 (0.387)	3130 (0.374)	3148 (0.368)	3123 (0.377)	0.0029	0.0068	0.0084	<0.0001	0.0001	<0.0001	0.0261	0.0507	0.0218
A2AB + vWF	3020 (0.357)	3085 (0.322)	3100 (0.314)	3078 (0.326)	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A2AB + mtDNA	3766 (0.309)	3828 (0.283)	3836 (0.280)	3874 (0.264)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
vWF + mtDNA	6225 (0.310)	6288 (0.297)	6314 (0.292)	6299 (0.295)	<0.0001	<0.0001	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
mtDNA + vWF + A2AB	5560 (0.315)	5652 (0.289)	5675 (0.282)	5663 (0.286)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A2AB + vWF + α-β hemoglobin	1540 (0.376)	1553 (0.358)	1578 (0.324)	1573 (0.331)	0.0325	0.0624	0.0485	<0.0001	<0.0001	<0.0001	0.0002	0.0011	0.0003

B, Butler's concept of Soricomorpha; MN, MacPhee and Novacek's concept of Soricomorpha; RI, retention index.

DISCUSSION

The results reported here, from several disparate molecular loci, congruently support the view that tenrecs, in addition to golden moles (8), are members of the "African clade" of mammalian orders and do not belong within the classical order Insectivora. Golden moles and tenrecs are therefore more closely related to aardvarks and elephants than they are to shrews, moles, and hedgehogs. Because this African clade is well supported both from a statistical and congruence perspective but is not currently recognized taxonomically, we propose the superordinal name Afrotheria. Furthermore, the golden moles and tenrecs form an evolutionary clade within Afrotheria, and thus it is necessary to propose a new order to accommodate those taxa. For this association of African insectivore families, we propose the name "Afrosoricida" (African shrew-like mammals). Two extinct orders of mammals, Desmostylia and Embrithopoda, are believed to be closely related to the paenungulate orders (21, 22) and therefore should be included in Afrotheria based on available phylogenetic evidence. Even so, these extinct taxa may not have originated in Africa but rather from ancestors that radiated out of Africa. The oldest embrithopods are the Paleocene genera *Phenacolophus* and *Minchenella* from Asia whereas the oldest African embrithopods are Eocene (21). The oldest desmostylian fossils are from the Oligocene, and the entire fossil record for this group (six genera) is confined to the northern Pacific Rim (21). The place and time of origin of aardvarks are also uncertain; they are presumed to be of African origin, but there are no unequivocal Paleogene fossils referable to this group.

Anatomical studies have not yet provided support for such a diverse African clade, and indeed there is not a single morphological synapomorphy that defines Afrotheria. There are, however, morphological characters that support a possible common ancestry of golden moles and tenrecs, although not to the exclusion of the other insectivores. These include zalambdodont cheekteeth (also in *Solenodon*), a basisphenoid contribution to the bulla (also in Erinaceidae), and orthomesometrial implantation of the blastocyst (3). There are also data from albumin immunodiffusion comparisons that join golden moles and tenrecs in a distinct clade, separate from all other insectivores (23). These other two sources of data are therefore at least partially congruent with our proposal for an Afrosoricida. By using the recently established linear relationships describing change in 12S rRNA Tamura Nei relative rate-adjusted transversion distances with time (24), we estimate that the ancestor to this new order resided in Africa somewhere ≈70 million years ago (mya) and that the split between tenrecs and golden moles occurred between 50 and 57 million years age. We suggest, therefore, that this proposed new order, similar to other recent estimates regarding other placental orders, may predate the Cretaceous–Tertiary boundary (8, 24–27).

In addition to the lack of support for a monophyletic Insectivora, the subordinal concepts of Butler (4) and MacPhee and Novacek (3) are also not supported by our data because of the different ancestry for the golden moles and tenrecs. Although MacPhee and Novacek's hypothesis envisions a possibly very different pattern of evolutionary history for the golden moles, and as such they distinguish them from Soricomorpha, they find no such reason to exclude tenrecs. Our data, on the other hand, reject not only golden moles but also tenrecs as having any
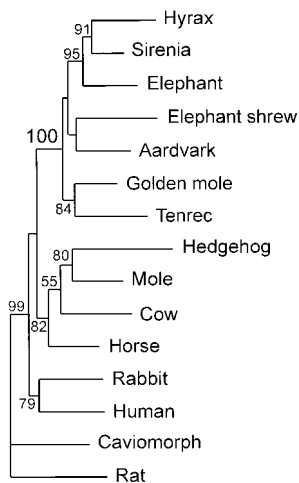

Fig. 2. Maximum likelihood tree of the combined 12S rRNA, tRNA-Valine, 16S rRNA, vWF, and A2AB data set, with branches drawn proportional to amount of sequence change, and with maximum likelihood bootstrap values added; only those bootstrap figures in excess of 50% are indicated.
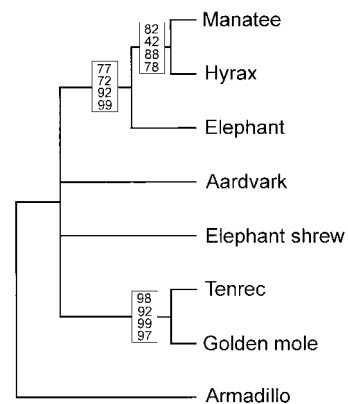

Fig. 3. Strict consensus bootstrap tree of parsimony, transversion parsimony, neighbor joining (with ML distances), and maximum likelihood analyses (bootstrap figures listed from top to bottom respectively) of the 12S rRNA, tRNA-Valine, and 16S rRNA data set, rooted with armadillo.

association with the remaining soricomorph groups, solenodon, mole, and shrew. The morphological concept of Soricomorpha in fact is based largely on the considerable differences between that suborder and hedgehogs (Erinaceomorpha). Our results, on the other hand, suggest that hedgehogs are much more closely related to other soricomorphs such as moles than are tenrecs or golden moles. Although the mitochondrial data alone do not group hedgehogs with the other non-African insectivores, the other three families do form a monophyletic group. All other data sets and combinations of data sets (including those involving mtDNA) associate hedgehog with the non-African insectivore, mole, generally with high bootstrap support (see Fig. 2 for example). These data, taken collectively, suggest that the hedgehogs, shrews, moles, and solenodons form a monophyletic group and as such should be retained in the order Insectivora.

Our demonstration of an evolutionary association of golden moles and tenrecs within an African superordinal clade has important ramifications for the evaluation of character state evolution in these and other mammals. It means, for example, that zalambdodonty evolved independently in Afrosoricida and solenodons among living insectivores. Similarly, other features currently used to define the monophyly of Insectivora, such as (*i*) hindgut simplification, with the correlated absence of cecum, (*ii*) reduction of the pubic symphysis, and (*iii*) large maxillary contribution to the orbit, probably represent convergences rather than shared-derived traits. The converse alternative [that there is molecular convergence between mitochondrial rRNA genes, as well as a number of nuclear, protein-encoding genes, of highly disparate function located on different chromosomes, including a blood clotting factor (van Willebrand factor), another involved in the neural circuitry regulating cardiovascular function (A2AB) and hemoglobins] is unparsimonious in the extreme.

These results highlight the important role of plate tectonics and biogeography in the early diversification of placental mammals. Nearly one-third of the extant orders of placental mammals belong to the superorder Afrotheria, and molecular clock estimates suggest an origin for that lineage in the mid-Cretaceous (105–90 mya) at a time when the continent was isolated (8, 24–26). When representatives of most of the extant placental orders first appear in the fossil record in the early Cenozoic (65–55 mya), many of the continents had at least periodic connection, and thus the distributions of those fossils may not accurately reflect the land areas where those groups arose (25). The challenge for paleontologists will be to locate more Cretaceous fossils of placentals to better understand the historical biogeography of mammals.

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