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Phylogenetic Relationships of the Order Insectivora Based on Complete 12S rRNA Sequences from Mitochondria

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Despite numerous studies, there is no single accepted hypothesis of eutherian ordinal relationships. Among the least understood mammalian orders is the group Insectivora. Currently, molecular and morphological data are in conflict over the possible monophyly of the living members of Insectivora (lipotyphlans), and the relationships within the group remain largely unresolved. One of the primary criticisms concerning molecular analyses is the noticeable lack of data from a well-sampled group of lipotyphlan insectivores. The mitochondrial 12S rRNA gene has been widely used to resolve interordinal and intraordinal relationships across a variety of mammalian taxa. This study compares 118 complete mammalian 12S rRNA sequences, representing all of the 18 eutherian orders and 3 metatherian orders, and includes as well taxa from each of the six families of lipotyphlan insectivores. Insectivoran lineages are thought to have diverged concurrently with the general radiation of mammalian orders. This study suggests that the 12S rRNA sequences lack the ability to resolve relationships extending into this period. This would explain the polyphyly, unusual affinities, and low support derived in this and other studies employing 12S rRNA sequences to diagnose relationships among eutherian orders. The results of these analyses suggest that even extensive taxon sampling is insufficient to provide well supported groups among eutherian orders. Additional genes and species sampling will be necessary to elucidate whether the Insectivora form a monophyletic group. © 1999 The Willi Hennig Society

INTRODUCTION

Elucidation of relationships among eutherian mammal orders has proven difficult presumably as a result of a rapid radiation at the end of the Cretaceous period. Despite numerous studies, there is no single accepted hypothesis of eutherian ordinal relationships. Among the least understood mammalian orders is the group Insectivora. These taxa are thought to have originated during the earliest radiation of placental mammals, as a group, and possess numerous primitive features.

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Therefore, resolving relationships within Insectivora may aid in the resolution of those relationships among the remaining eutherian orders. Currently, molecular and morphological data are in conflict over the possible monophyly of the living members of this group (Lipotyphla sensu Butler, 1988), and the relationships within the group remain largely unresolved (Butler, 1988; Novacek, 1986; MacPhee and Novacek, 1993; George and Sarich, 1994; Springer *et al.*, 1997). One of the primary criticisms concerning molecular analyses is the noticeable lack of data from a well-sampled group of lipotyphlan insectivores. Most studies have included only one or two insectivore taxa. If monophyly is to be properly assessed for this order, a broader range of sampling will be required.

The mitochondrial 12S rRNA gene has been widely used to resolve interordinal and intraordinal relationships across a variety of mammalian taxa (Allard *et al.*, 1992; Springer and Kirsch, 1993; Douzery and Catzeflis, 1995; Lavergne *et al.*, 1996). Indeed, a recent assessment of secondary structure and patterns of evolution among 49 complete mammalian 12S rRNA sequences concluded that the gene should provide resolution for divergence events occurring up to 100 million years ago (Springer and Douzery, 1996). In the present study, we have compiled 118 complete mammalian 12S rRNA sequences representing all of the 18 eutherian orders and 3 additional metatherian orders. In addition, each of the six families of lipotyphlan insectivores is included in an effort to assess the monophyly of Lipotyphla.

MATERIALS AND METHODS

Sequences of the mitochondrial 12S rRNA gene were obtained for 106 taxa through GenBank, one from a separate website, three from Tanhauser *et al.* (1985), and eight were produced in our lab (Table 1). The range of taxa includes at least one representative for each of the 18 orders of eutherian mammals and four members of Metatheria, which compose the outgroup. The sequences were compiled from published data and were brought together to provide a greater amount of sampling and comparable variation than previous studies of the 12S rRNA gene.

The 118 sequences were aligned by hand, with the exception of a highly variable region located near the 3′end of the gene (positions 904–1008). This region was aligned using the alignment program Clustal W 1.6 (Thompson *et al.*, 1994). The alignment DS38659 is available from EMBL upon request by electronic mail to NetServ@EBI.AC.UK. A maximum parsimony analysis was conducted by performing 100 heuristic searches utilizing the random addition sequence option and equal weighting in PAUP 3.1.1 (Swofford, 1993). This method was chosen due to the large number of taxa. Support for groups was then assessed by the parsimony jackknifing program XAC (Farris *et al.*, 1996), set for 10,000 replicates, with branch swapping of five randomly selected addition sequences per replicate (10000*/5), and a cut of 50%. The resulting tree was then reconstructed using MacClade 3.0 (Maddison and Maddison, 1992). The four metatherian taxa *Didelphis virginiana*, *Macropus giganteus*, *Macropus robustus*, and *Dromiciops gliroides* were used to root trees in analyses conducted with both PAUP 3.1.1 and XAC programs.

RESULTS

Parsimony analysis of the 12S rRNA data found 30 equally parsimonious trees. In the strict consensus of these trees, the six families of the order Insectivora are polyphyletic and scattered throughout the tree (Fig. 1). The two hedgehogs (family Erinaceidae) group together at the base of the eutherian clade; the two tenrecs (Tenrecidae) form a sister group to the golden mole (Chrysochloridae); the tenrecs and golden mole together, along with the tarsier, elephant shrew, and aardvark, fall sister to the Paenungulata clade; the shrew (Soricidae) stands alone as sister to a monophyletic Carnivora; the hairy-tailed mole (Talpidae) is sister to two megachiropteran bats; and the solenodon (Solenodontidae) is positioned outside a large group of rodents.

Although certainly intriguing, most of these relationships are not well supported by parsimony jackknifing. Only one interordinal relationship, a trichotomy composed of Proboscidea, Hyracoidea, and Sirenia (e.g., Paenungulata), is well supported by parsimony jackknifing with a group frequency of 79 (Fig. 2). Two other interordinal groups are supported less: Dermoptera

TABLE 1
List of 118 Taxa and Sources

Order	Abbreviation/species	Common name	GenBank Accession No; reference
Artiodactyla	BTA *Bos taurus*	Cow	J01394; Anderson *et al.* (1982)
Artiodactyla	BGR *Bos grunniens*	Yak	No number; Tanhauser (1985)
Artiodactyla	CHI *Capra hircus*	Goat	M55541; Kraus and Miyamoto (1991)
Artiodactyla	DDC *Damaliscus dorcas*	Bontebok	M86499; Allard *et al.* (1992)
Artiodactyla	TIM *Tragelaphus imberbis*	Lesser kudo	M86493; Allard *et al.* (1992)
Artiodactyla	MKI *Madoqua kirki*	Kirk's dikdik	M86495; Allard *et al.* (1992)
Artiodactyla	GTH *Gazella thomsoni*	Thomson's gazelle	M86501; Allard *et al.* (1992)
Artiodactyla	KEL *Kobus ellipsiprymnus*	Waterbuck	M86497; Allard *et al.* (1992)
Artiodactyla	CMA *Cephalophus maxwelli*	Maxwell's duiker	M86498; Allard *et al.* (1992)
Artiodactyla	OGA *Oryx gazella*	Gemsbok	M86500; Allard *et al.* (1992)
Artiodactyla	AME *Aepyceros melampus*	Impala	M86496; Allard *et al.* (1992)
Artiodactyla	BTR *Boselaphus tragocamelus*	Nilgai	M86494; Allard *et al.* (1992)
Artiodactyla	MRE *Muntiacus reevesi*	Chinese muntjac	M35877; Miyamoto *et al.* (1990)
Artiodactyla	CUN *Cervus unicolor*	Sambar	M35875; Miyamoto *et al.* (1990)
Artiodactyla	OVI *Odocoileus virginianus*	White-tailed deer	M35874; Miyamoto *et al.* (1990)
Artiodactyla	HIN *Hydropotes inermus*	Chinese water deer	M35876; Miyamoto *et al.* (1990)
Artiodactyla	AAM *Antilocapra americana*	Pronghorn antelope	M55540; Kraus and Miyamoto (1991)
Artiodactyla	TNA *Tragulus napu*	Mouse deer	M55539; Kraus and Miyamoto (1991)
Artiodactyla	SSC *Sus scrofa*	Pig	No number; Tanhauser (1985)
Artiodactyla	GCA *Giraffa camelopardalis*	Giraffe	No number; Tanhauser (1985)
Artiodactyla	TTA *Tayassu tajacu*	Collared peccary	X86944; Douzery and Catzeflis (1995)
Carnivora	HAU *Herpestes auropunctatus*	Small Indian mongoose	Y08506; Ledje and Arnason (1996)
Carnivora	FCO *Felis concolor*	Mountain lion	U33495; Springer *et al.* (1995)
Carnivora	FCA *Felis catus*	Domestic cat (1)	U20753; Lopez *et al.* (1996)
Carnivora	FDO *Felis domesticus*	Domestic cat (2)	Y08503; Ledje and Arnason (1996)
Carnivora	PTI *Panthera tigris*	Tiger	Y08504; Ledje and Arnason (1996)
Carnivora	PLE *Panthera leo*	Lion	Y08505; Ledje and Arnason (1996)
Carnivora	CFA *Canis familiaris*	Domestic dog	Y08507; Ledje and Arnason (1996)
Carnivora	VVU *Vulpes vulpes*	Red fox	Y08508; Ledje and Arnason (1996)
Carnivora	BGA *Bassaricyon gabbii*	Olingo	Y08509; Ledje and Arnason (1996)
Carnivora	PLO *Procyon lotor*	Raccoon	Y08510; Ledje and Arnason (1996)
Carnivora	AFU *Ailurus fulgens*	Lesser (red) panda	Y08511; Ledje and Arnason (1996)
Carnivora	ELU *Enhydra lutris*	Sea otter	Y08512; Ledje and Arnason (1996)
Carnivora	MEL *Meles meles*	European badger	Y08517; Ledje and Arnason (1996)
Carnivora	MVI *Mustela vison*	American mink	Y08514; Ledje and Arnason (1996)
Carnivora	MST *Mustela nivalis*	Least weasel	Y08515; Ledje and Arnason (1996)
Carnivora	MPU *Mustela putorius*	Domestic ferret	Y08516; Ledje and Arnason (1996)
Carnivora	MME *Mephitis mephitis*	Striped skunk	Y08517; Ledje and Arnason (1996)
Carnivora	SPU *Spilogale putorius*	Spotted skunk	Y08518; Ledje and Arnason (1996)
Carnivora	UAR *Ursus arctos*	Brown bear	Y08519; Ledje and Arnason (1996)
Carnivora	UAM *Ursus americanus*	American black bear	Y08520; Ledje and Arnason (1996)
Carnivora	AML *Ailuropoda melanoleuca*	Giant panda	Y08521; Ledje and Arnason (1996)
Carnivora	PVI *Phoca vitulina*	Harbor seal	X63726; Arnason and Johnsson (1992)
Carnivora	HGR *Halichoerus grypus*	Grey seal	X72004; Arnason and Gullberg (1993)
Carnivora	LWE *Leptonychotes weddelli*	Weddell seal	Y08522; Ledje and Arnason (1996)
Carnivora	MLE *Mirounga leonina*	Southern elephant seal	Y08523; Ledje and Arnason (1996)
Carnivora	MSC *Monachus schauinslandi*	Hawaiian monk seal	Y08524; Ledje and Arnason (1996)
Carnivora	ZCA *Zalophus californianus*	California sea lion	Y08525; Ledje and Arnason (1996)
Carnivora	AGA *Arctocephalus gazella*	Antarctic fur seal	Y08526; Ledje and Arnason (1996)
Carnivora	AFO *Arctocephalus forsteri*	New Zealand fur seal	Y08527; Ledje and Arnason (1996)
Cetacea	SCO *Stenella coeruleoalba*	Striped dolphin	X78168; Douzery (1993)
Cetacea	BPH *Balaenoptera physalus*	Finback whale	X61145; Arnason *et al.* (1991)
Cetacea	BMU *Balaenoptera musculus*	Blue whale	X72204; Arnason and Gullberg (1993)
Chiroptera			
Megachiroptera	NAL *Nyctimene albiventer*	Tube-nosed fruit bat	U61077; Springer and Douzery (1996)
Megachiroptera	RLE *Rousettus leschenaulti*	Leschenault's rousette	AF153000; McNiff and Allard (1998)
Microchiroptera	EFU *Eptescius fuscus*	Brown bat	U61082; Springer and Douzery (1996)

TABLE 1—*Continued*

Order	Abbreviation / species	Common name	GenBank Accession No; reference
Dermoptera	CVA *Cynocephalus variegatus*	Malayan flying lemur	AF152999; McNiff and Allard (1998)
Edentata	CVI *Chaetophractus villosus*	Hairy armadillo	U61080; Springer and Douzery (1996)
Hyracoidea	PCA *Procavia capensis*	Rock hyrax	U60184; Lavergne *et al.* (1996)
Hyracoidea	DDS *Dendrohyrax dorsalis*	Tree hyrax	X86945; Douzery and Catzeflis (1995)
Insectivora	AAL *Atelerix albiventris*	Middle-African hedgehog	M95109; Allard and Miyamoto (1992)
Insectivora	AHO *Amblysomus hottentotus*	Golden mole	M95108; Allard and Miyamoto (1992)
Insectivora	BBR *Blarina brevicauda*	Short-tailed shrew	M95110; Allard and Miyamoto (1992)
Insectivora	EEU *Erinaceus europaeus*	Western European hedgehog	X88898; Krettek *et al.* (1995)
Insectivora	PBR *Parascalops brewerii*	Hairy-tailed mole	AF153004; This article
Insectivora	OTA *Oryzorictes talpoides*	Rice tenrec	AF153005; This article
Insectivora	TEC *Tenrec ecaudatus*	Common tenrec	AF153002; McNiff and Allard (1998)
Insectivora	SPA *Solenodon paradoxus*	Solenodon	AF153006; This article
Lagomorpha	OCU *Oryctolagus cuniculus*	European rabbit	http / / www.ba.cnr.it \ guineapig
Macroscelidea	ERU *Elephantulus rufescens*	Elephant shrew	U97339; Springer *et al.* (1997)
Marsupialia	DGL *Dromiciops gliroides*	Monito del mote	U61073; Springer and Douzery (1996)
Marsupialia	DVI *Didelphis virginiana*	North American Opossum	Z29573; Janke *et al.* (1994)
Marsupialia	MGI *Macropus giganteus*	Eastern Gray kangaroo	X86941; Douzery and Catzeflis (1995)
Marsupialia	MRO *Macropus robustus*	Wallaroo	Y10524; Janke *et al.* (1997)
Perissodactyla	EGR *Equus grevyi*	Grevy's zebra	X86943; Douzery and Catzeflis (1995)
Perissodactyla	ECA *Equus caballus*	Horse	X79547; Xu and Arnason (1994)
Perissodactyla	EAS *Equus asinus*	Donkey	X97337; Xu *et al.* (1996)
Perissodactyla	CSI *Ceratotherium simum*	Rhinoceros	X86942; Douzery and Catzeflis (1995)
Perissodactyla	RUN *Rhinoceros unicornis*	Greater Indian rhinoceros	X97336; Xu *et al.* (1996)
Pholidota	MAN *Manis* sp	Pangolin	U61079; Springer and Douzery (1996)
Primates	HSA *Homo sapiens*	Man	J01415; Anderson *et al.* (1981)
Primates	PPY *Pongo pygmaeus*	Orangutan	X97707; Xu and Arnason (1996)
Primates	PPA *Pan paniscus*	Pygmy chimpanzee	D38116; Hixson and Brown (1986)
Primates	PTR *Pan troglodytes*	Chimpanzee	X93335; Arnason and Gulberg (1996)
Primates	GGO *Gorilla gorilla*	Gorilla	X93347; Xu and Arnason (1995)
Primates	HLA *Hylobates lar*	Common gibbon	X99256; Arnason *et al.* (1996)
Primates	TBA *Tarsius bancanus*	Western tarsier	AF153001; McNiff and Allard (1998)
Proboscidea	LAF *Loxodonta africana*	African elephant	U60182; Lavergne *et al.* (1996)
Proboscidea	EMA *Elephas maximus*	Indian elephant	X93602; Lavergne *et al.* (1996)
Rodentia	ACA *Acomys cahirinus*	Egyptian spiny mouse	X84387; Hanni *et al.* (1995)
Rodentia	CGA *Cricetomys gambianus*	Gambian giant pouched rat	X99461; Dubois *et al.* (1996)
Rodentia	CMI *Cricetulus migratorius*	Armenian hamster	X84389; Hanni *et al.* (1995)
Rodentia	GGL *Glis glis*	Fat dormouse	X84385; Hanni *et al.* (1995)
Rodentia	HST *Hylomyscus stella*	African soft-furred rat (1)	X85953; Sourrouille *et al.* (unpublished)
Rodentia	MER *Mastomys erythroleucus*	African soft-furred rat (2)	X85952; Sourrouille *et al.* (unpublished)
Rodentia	LED *Leopoldamys edwarsi*	Long-tailed giant rat	X84386; Hanni *et al.* (1995)
Rodentia	MAU *Mesocricetus auratus*	Golden hamster	X84390; Hanni *et al.* (1995)
Rodentia	MNI *Microtus nivalus*	Snow vole	X99464; Dubois *et al.* (1996)
Rodentia	MMU *Mus musculus*	House mouse	J01420; Bibb *et al.* (1981)
Rodentia	MSE *Mus setulosus*	African pygmy mouse (1)	X85949; Sourouille *et al.* (unpublished)
Rodentia	MMA *Mus mattheyi*	African pygmy mouse (2)	X85950; Sourrouille *et al.* (unpublished)
Rodentia	MCO *Mus cookii*	Cook's mouse	X85946; Sourouille *et al.* (unpublished)
Rodentia	MCR *Mus crociduroides*	Shrew mouse (1)	X85951; Sourouille *et al.* (unpublished)
Rodentia	MPA *Mus pahari*	Shrew mouse (2)	X84383; Hanni *et al.* (1995)
Rodentia	MSA *Mus saxicola*	Spiny mouse	X85948; Sourrouille *et al.* (unpublished)
Rodentia	MPL *Mus platythrix*	Flat-haired jungle mouse	X85947; Sourrouille *et al.* (unpublished)
Rodentia	MAV *Muscardinus avellanarius*	Hazel mouse	X84384; Hanni *et al.* (1995)
Rodentia	NRU *Nesomys rufus*		X99462; Dubois *et al.* (1996)
Rodentia	PLU *Peromyscus leucopus*	White-footed mouse	X99463; Dubois *et al.* (1996)
Rodentia	RNO *Rattus norvegicus*	Norway rat	X14848; Gadaleta *et al.* (1989)
Rodentia	TGA *Tatera kempi gambiana*	Large naked-sole gerbil	X84391; Hanni *et al.* (1995)
Rodentia	HHY *Hydrochaeris hydrochaeri*	Capybara	U61081; Springer and Douzery (1996)
Rodentia	CPO *Cavia porcellus*	Domestic guinea pig	L35585; Frye and Hedges (1995)

TABLE 1—*Continued*

Order	Abbreviation/species	Common name	GenBank Accession No; reference
Rodentia	URU *Uranomys ruddi*	White-bellied brush-furred rat	X84388; Hanni *et al.* (1995)
Scandentia	TGL *Tupaia glis*	Common tree shrew	AF153003; McNiff and Allard (1998)
Sirenia	DDU *Dugong dugon*	Dugong	U60185; Lavergne *et al.* (1996)
Sirenia	TMA *Trichechus manatus*	Caribbean manatee	U60183; Lavergne *et al.* (1996)
Tubulidentata	OAF *Orcyteropus afer*	Aardvark	U97338; Springer *et al.* (1997)

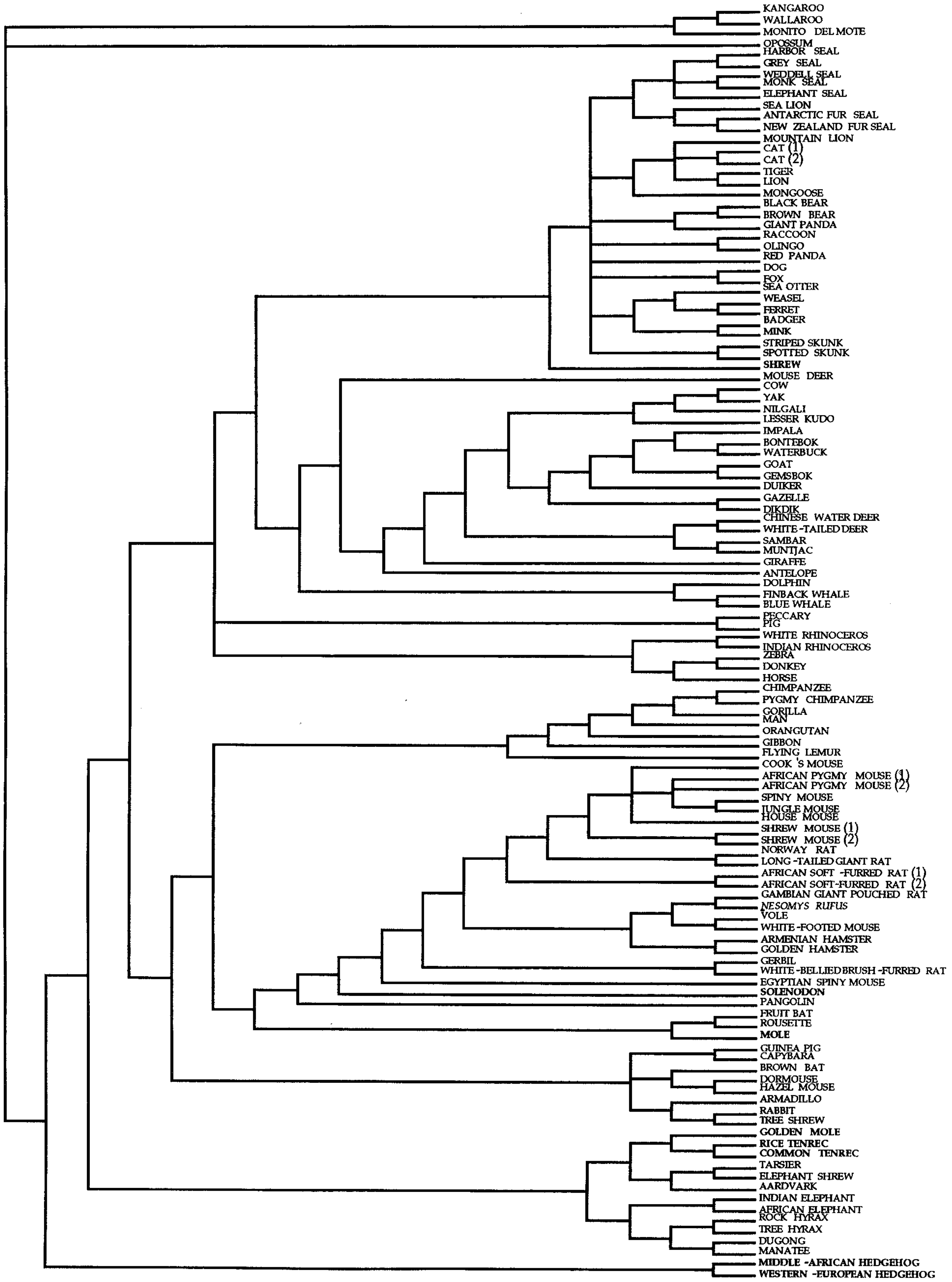
(flying lemur) with an incomplete primate group (lacking the tarsier) at 69, and a group consisting of Lagomorpha (rabbit) and Scandentia (tree shrew) at 62. Cetacea and Perissodactyla are well supported as distinct monophyletic clades. Carnivora also appears as monophyletic, although the group is supported less at a frequency of 70. The tenrec–golden mole clade is the only supported association between insectivoran families (70), while all affinities with other orders are dissolved in parsimony jackknifing.

Because of the large number of equally parsimonious trees recovered, a second analysis was performed using successive weighting, in which the characters were reweighted according to their relative rescaled consistency values using a base weight of 10. After four iterations of successive weighting, this analysis produced eight equally parsimonious trees, the consensus of which (not shown) demonstrates that these eight trees are not a subset of the original 30 but contain structure and associations not found in those trees. For instance, the mole and shrew form a sister group to the Primate–Dermopteran clade after successive weighting. This and other discrepancies provide yet another demonstration of instability and character incongruence throughout the data and corroborate the low support seen in the parsimony jackknifing analysis.

One of the more problematic issues involving analysis of the 12S rRNA gene is constructing a multiple sequence alignment. Specifically, the 12S rRNA gene has several highly variable regions, thus making it difficult to align and ensure that characters are homologous. Many studies have chosen to exclude these regions from analysis for this reason. Others have referred to the complex secondary structure of the gene product to prioritize regions for gap placement based on evidence of covariation in stem regions and the preferential occurrence of indels within loop regions (Springer *et al.*, 1996; Lavergne *et al.*, 1996). An assessment of the effect of different alignment strategies on tree topology with regard to 12S rRNA may provide more information on the utility of this gene in addressing questions at the ordinal level. In this study we analyzed only one alignment.

Many molecular studies use various *a priori* weighting strategies to reduce the effects of homoplasy and increase the amount of congruence within and among data sets. This approach has been examined (Allard and Carpenter, 1996; Allard *et al.*, 1999) and shown to be unnecessary in that identical trees were found in both transversion weighted and equally weighted parsimony analyses of complete mitochondrial genomes (see also Eernisse and Kluge, 1993; Honeycutt and Adkins, 1993). However, it also was demonstrated that tree topologies derived from individual mitochondrial genes varied greatly. The results of the analysis presented here suggest that an increased number of taxa is not enough to either overcome the ambiguity present in sequences of the 12S rRNA gene or provide well-supported groups of eutherian orders. Instead, the observed low support may be an indication of weak or conflicting signals within the 12S sequences.

FIG. 1. Strict consensus of 30 most parsimonious trees found through heuristic searches of 100 random stepwise addition replicates. Tree lengths = 8446, CIs = 0.154, HIs = 0.846, RIs = 0.543, and RCs = 0.090 for the 30 trees found.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SEA LION
ANTARCTIC FUR SEAL
NEW ZEALAND FUR SEAL
MOUNTAIN LION
CAT (1)
CAT (2)
TIGER
LION
MONGOOSE
BLACK BEAR
BROWN BEAR
GIANT PANDA
RACCOON
OLINGO
RED PANDA
DOG
FOX
SEA OTTER
WEASEL
FERRET
BADGER
MINK
STRIPED SKUNK
SPOTTED SKUNK
SHREW
MOUSE DEER
COW
YAK
NILGALI
LESSER KUDO
IMPALA
BONTEBOK
WATERBUCK
GOAT
GEMSBOK
DUIKER
GAZELLE
DIKDIK
CHINESE WATER DEER
WHITE-TAILED DEER
SAMBAR
MUNTJAC
GIRAFFE
ANTELOPE
DOLPHIN
FINBACK WHALE
BLUE WHALE
PECCARY
PIG
WHITE RHINOCEROS
INDIAN RHINOCEROS
ZEBRA
DONKEY
HORSE
CHIMPANZEE
PYGMY CHIMPANZEE
GORILLA
MAN
ORANGUTAN
GIBBON
FLYING LEMUR
COOK'S MOUSE
AFRICAN PYGMY MOUSE (1)
AFRICAN PYGMY MOUSE (2)
SPINY MOUSE
JUNGLE MOUSE
HOUSE MOUSE
SHREW MOUSE (1)
SHREW MOUSE (2)
NORWAY RAT
LONG-TAILED GIANT RAT
AFRICAN SOFT-FURRED RAT (1)
AFRICAN SOFT-FURRED RAT (2)
GAMBIAN GIANT POUCHED RAT
NESOMYS RUFUS
VOLE
WHITE-FOOTED MOUSE
ARMENIAN HAMSTER
GOLDEN HAMSTER
GERBIL
WHITE-BELLIED BRUSH-FURRED RAT
EGYPTIAN SPINY MOUSE
SOLENODON
PANGOLIN
FRUIT BAT
ROUSETTE
MOLE
GUINEA PIG
CAPYBARA
BROWN BAT
DORMOUSE
HAZEL MOUSE
ARMADILLO
RABBIT
TREE SHREW
GOLDEN MOLE
RICE TENREC
COMMON TENREC
TARSIER
ELEPHANT SHREW
AARDVARK
INDIAN ELEPHANT
AFRICAN ELEPHANT
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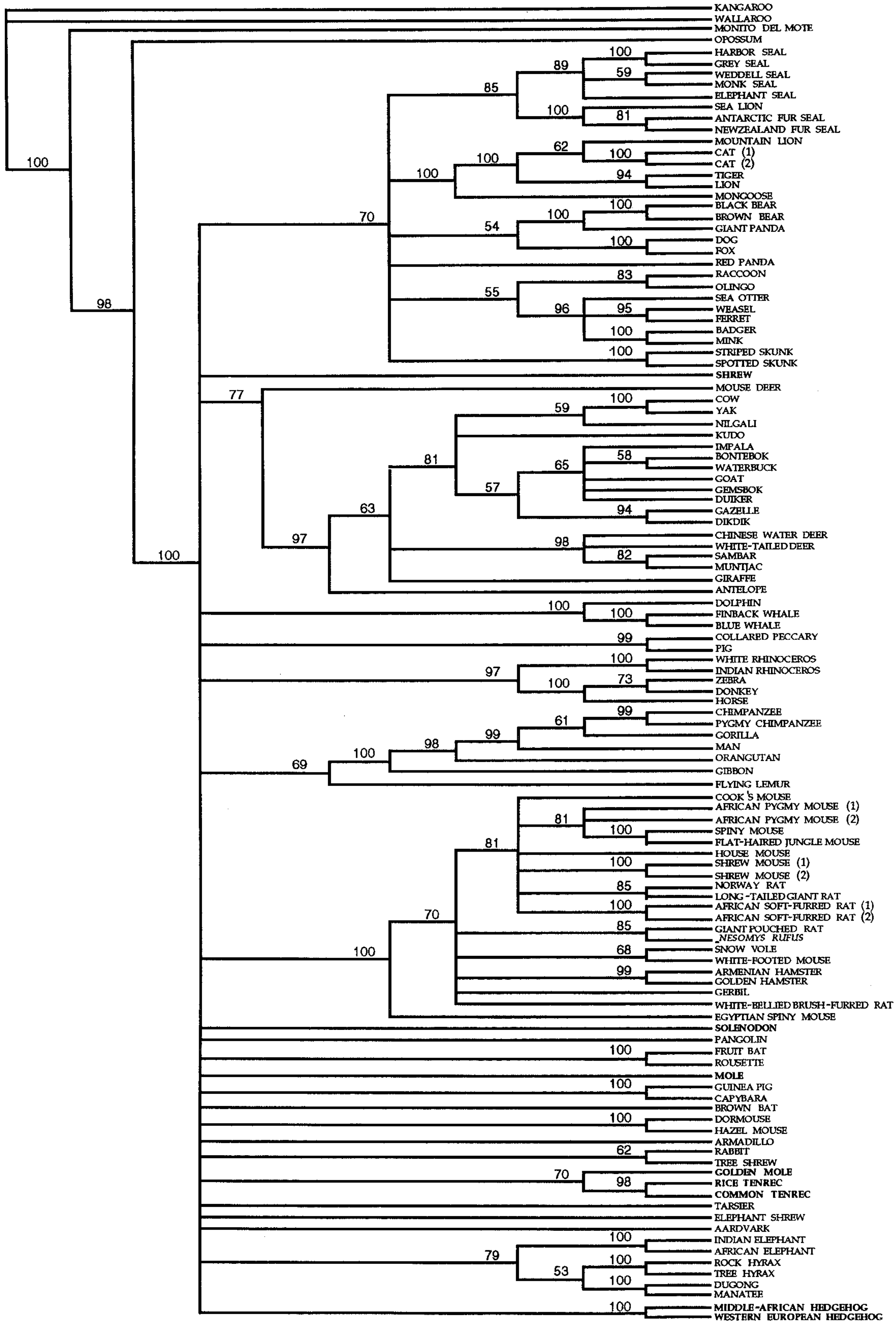
TREE HYRAX
DUGONG
MANATEE
MIDDLE-AFRICAN HEDGEHOG
WESTERN-EUROPEAN HEDGEHOG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OPOSSUM
HARBOR SEAL
GREY SEAL
WEDDELL SEAL
MONK SEAL
ELEPHANT SEAL
SEA LION
ANTARCTIC FUR SEAL
NEWZEALAND FUR SEAL
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CAT (1)
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TIGER
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SPOTTED SKUNK
SHREW
MOUSE DEER
COW
YAK
NILGALI
KUDO
IMPALA
BONTEBOK
WATERBUCK
GOAT
GEMSBOK
DUIKER
GAZELLE
DIKDIK
CHINESE WATER DEER
WHITE-TAILED DEER
SAMBAR
MUNTJAC
GIRAFFE
ANTELOPE
DOLPHIN
FINBACK WHALE
BLUE WHALE
COLLARED PECCARY
PIG
WHITE RHINOCEROS
INDIAN RHINOCEROS
ZEBRA
DONKEY
HORSE
CHIMPANZEE
PYGMY CHIMPANZEE
GORILLA
MAN
ORANGUTAN
GIBBON
FLYING LEMUR
COOK'S MOUSE
AFRICAN PYGMY MOUSE (1)
AFRICAN PYGMY MOUSE (2)
SPINY MOUSE
FLAT-HAIRED JUNGLE MOUSE
HOUSE MOUSE
SHREW MOUSE (1)
SHREW MOUSE (2)
NORWAY RAT
LONG-TAILED GIANT RAT
AFRICAN SOFT-FURRED RAT (1)
AFRICAN SOFT-FURRED RAT (2)
GIANT POUCHED RAT
NESOMYS RUFUS
SNOW VOLE
WHITE-FOOTED MOUSE
ARMENIAN HAMSTER
GOLDEN HAMSTER
GERBIL
WHITE-BELLIED BRUSH-FURRED RAT
EGYPTIAN SPINY MOUSE
SOLENODON
PANGOLIN
FRUIT BAT
ROUSETTE
MOLE
GUINEA PIG
CAPYBARA
BROWN BAT
DORMOUSE
HAZEL MOUSE
ARMADILLO
RABBIT
TREE SHREW
GOLDEN MOLE
RICE TENREC
COMMON TENREC
TARSIER
ELEPHANT SHREW
AARDVARK
INDIAN ELEPHANT
AFRICAN ELEPHANT
ROCK HYRAX
TREE HYRAX
DUGONG
MANATEE
MIDDLE-AFRICAN HEDGEHOG
WESTERN EUROPEAN HEDGEHOG

Therefore, these data may be more useful in combination with other molecular and/or morphological data sets.

DISCUSSION

This analysis is in agreement with a recent examination (Springer *et al.*, 1997), demonstrating that the order Insectivora is not monophyletic and corroborates the grouping of a so-called African clade (consisting of paenungulates, aardvarks, elephant shrews, and golden moles), which in this study also includes tarsiers and tenrecs. However, other established orders, as well as the superorder Archonta, also appear to be polyphyletic, while few are shown to be monophyletic. This may be due in part to limited taxon sampling despite the large number of taxa included in the analysis. Springer *et al.* (1997) presented consistent results using several different genes; however, the sampling in that study was not as extensive as that utilized here and involved only two or three insectivore representatives. Similar to results shown here, few interordinal relationships were recovered, and still fewer were well supported. Most interordinal associations remained unresolved at bootstrap values greater than 75%. If the Insectivora radiated simultaneously with Mammalia, it would stand to reason that the resolution of both groups would prove troublesome. It appears that the 12S rRNA sequences lack the ability to resolve deeper relationships among divergent eutherian lineages. This would explain the polyphyly, unusual affinities, and low support derived in this and other recent molecular studies also using 12S rRNA sequences and describing similar results (Douzery and Catzeflis, 1995; Lavergne *et al.*, 1996; Springer *et al.*, 1997; McNiff and Allard, 1998). Additional genes and species sampling will be necessary to clearly elucidate whether the Insectivora form a monophyletic group and determine if the African clade will withstand further scrutiny. The results of this analysis suggest that an increased number of taxa is insufficient to provide well-supported groups among eutherian orders by overcoming the ambiguity present in sequences of the 12S rRNA gene. To provide better resolution, these sequences will need to be analyzed together with other genes and/or morphological data in a combined analysis.

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FIG. 2. Supported groups assessed by 10,000 parsimony jackknifing replicates. Numbers to the left of each node indicate group frequencies. Only groups with frequencies higher than 0.5 are shown. Taxa in bold are members of the lipotyphlan Insectivora.

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