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Molecular Phylogenetics and Evolution 27 (2003) 247–258

MOLECULAR PHYLOGENETICS AND EVOLUTION

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Molecular phylogenetic relationships of moles, shrew moles, and desmans from the new and old worlds

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Received 28 January 2002; revised 9 September 2002

Abstract

A rich variety of anatomical and physiological specializations has enabled members of the family Talpidae (moles, shrew moles, and desmans) to exploit a diverse range of habitats: terrestrial, semi-aquatic, aquatic/fossorial, semi-fossorial, and fossorial. While numerous morphological and biochemical studies pertaining to the origin and radiation of the Talpidae have been completed, phylogenetic hypotheses remain controversial. To address this shortcoming we sequenced the mitochondrial DNA cytochrome *b* gene (1140 bp) from 29 individuals spanning 12 talpid species. Phylogenetic trees incorporating 12 New and Old World genera (18 species; all 3 extant subfamilies) were then constructed using NJ, MP, ML, and NJ–ML (NJ with ML parameters) methods. Our results provide molecular support for a mononphyletic Talpidae, and suggest that the 12 genera are clustered into seven major clades; (1) Asiatic shrew-like moles (*Uropsilus*), (2) North American aquatic/fossorial moles (*Condylura*), (3) North American fossorial moles (*Parascalops*, *Scalopus*, and *Scapanus*), (4) North American semi-fossorial shrew moles (*Neurotrichus*), (5) Japanese semi-fossorial shrew moles (*Dymecodon* and *Urotrichus*), (6) European semi-aquatic desmans (*Desmana*), and (7) Eurasian fossorial moles (*Euroscaptor*, *Mogera*, and *Talpa*). None of these groupings comprised mole species from both continents. In fact, North American moles and shrew moles do not appear to have specific affinities with Asian moles and shrew moles, respectively. Although low bootstrap support was generally found for evolutionary nodes uniting the major talpid clades, all gene trees constructed identified fossorial North American and Eurasian mole lineages as nonmonophyletic groups, suggesting subterranean specializations arose independently at least twice during the evolution of the Talpidae. Additionally, our data set provides molecular support for a basal divergence and long independent history of *Uropsilus* from the main talpid line, and refutes the traditional taxonomic status and secondarily basal phylogenetic placement of the subfamily Desmaninae within the Talpidae.

Keywords: Phylogeny; Cytochrome *b*; Talpidae; Eulipotyphla; Shrew moles; Moles; Desmans

1. Introduction

The late Tertiary was an important epoch of adaptive radiation and lineage expansion for moles, shrew moles, and desmans of the family Talpidae (Mammalia, Eulipotyphla). During this period, talpids evolved a remarkable variety of locomotor patterns and life history traits including ambulatory (shrew-like moles), semi-aquatic (desmans), aquatic/fossorial (star-nosed mole), semi-fossorial (shrew moles), and highly fossorial (moles in the strict sense) (Hutchison, 1976; Yates and Moore, 1990). Recent classifications recognize anywhere from 12 to 17 genera and 31–42 species (Corbet and Hill, 1991; Nowak, 1999; Yates, 1984) grouped into three extant subfamilies: Uropsilinae (*Uropsilus*), Desmaninae (*Desmana* and *Galemys*), and Talpinae (all remaining genera). The latter subfamily is further subdivided into 5 well-defined tribes; Talpini (Old World fossorial moles), Scalopini (North American fossorial moles), Urotrichini (shrew moles), Scaptonychini (*Scaptonyx*; long-tailed mole), and Condylurini (*Condylura*) (McKenna and Bell, 1997).

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doi:10.1016/S1055-7903(02)00416-5

The oldest fossils assigned to the Talpidae are from late Eocene and late Oligocene sediments of Europe and North America, respectively (McKenna and Bell, 1997). Based on these specimens and geological evidence, it has been suggested that the family originated in Europe and spread by way of multiple dispersal events to Asia and North America (Hutchison, 1976; Moore, 1986; Whidden, 2000). Today, highly fossorial talpids are distributed continuously worldwide from the Eurasian continent, including the Japanese Islands, to North America. Less-fossorial talpids tend to show limited geographic distributions. Desmans, for instance, are limited to drainage basins in north-eastern (*Desmana*) and north-western (*Galemys*) Europe, while shrew moles are restricted to Japan (*Dymecodon* and *Urotrichus*) and western North America (*Neurotrichus*). The observation that both living and fossilized talpids exhibit noncontiguous distributions within and between Europe, Asia and North America is a major source of controversy among phylogenetic hypotheses relating to the evolution and radiation of the family (e.g., Hutchison, 1968, 1976; Whidden, 2000; Yates and Moore, 1990). Despite a general lack of phylogenetically informative data, the subfamilies Uropsilinae and Desmaninae are commonly placed at the basal and intermediate nodes, respectively, in cladograms for the family (Whidden, 2000; Yates and Moore, 1990). However, it is the relationships among the ecologically diverse tribes of the subfamily Talpinae that are most contentious.

Based on combined allozymic, chromosomal and morphological data sets, Yates and Moore (1990) recognized two major groups of extant fossorial moles, one from the Old World (*Mogera*, *Euroscaptor*, and *Talpa*) and the other from the New World (*Parascalops*, *Scalopus*, and *Scapanus*) and China (*Scapanulus*). Interestingly, these authors place semi-fossorial and aquatic/fossorial talpids between these two strictly fossorial tribes. In contrast, Whidden (2000) grouped the fossorial Eurasian (represented by *Talpa*) and North American moles as a monophyletic, highly derived assemblage based on his study of comparative myology. A monophyletic group of semi-fossorial shrew moles from Japan (*Urotrichus* and *Dymecodon*) and North American (*Neurotrichus*) is also generally supported by studies of osteology (Campbell, 1939; Hutchison, 1976), myology (Whidden, 2000), and cytogenetics and morphology (Moore, 1986; Yates and Moore, 1990). However, studies based on allozymes (Yates and Greenbaum, 1982) and dental homologies (Ziegler, 1971) have raised the possibility that these morphologically similar forms may have arisen separately.

To our knowledge there is no molecular phylogenetic data published for the Talpidae except for two studies limited to seven Eurasian species (Okamoto, 1999; Tsuchiya et al., 2000) and a recent study investigating lipotyphlan relationships that incorporated four talpid genera (Douady et al., 2002). To establish an explicit molecular phylogeny of the Talpidae and hopefully resolve whether semi-aquatic, semi-fossorial and fossorial Eurasian, and North American talpids evolved independently or represent monophyletic assemblages, we conducted a detailed phylogenetic analysis of the mitochondrial DNA cytochrome *b* (cyt *b*) gene from 18 species of moles, shrew moles, and desmans from both the New and Old Worlds. Our second goal was to evaluate traditional talpid classification schemes based upon our mtDNA data set.

2. Materials and methods

2.1. Biological materials

We followed talpid nomenclature and classification schemes commonly used in the literature (Abe, 1994, 1995; Corbet and Hill, 1991; Motokawa and Abe, 1996). Data were collected from 29 talpid specimens (12 species) from both the New and Old Worlds (Table 1). Additional cyt *b* sequence data from *Mogera wogura*, *Mogera imaizumii*, *Mogera tokudae*, *Mogera insularis*, *Euroscaptor mizura*, *Talpa altaica*, *Talpa europaea*, and *Uropsilus gracilis* were obtained from DNA databases (DDBJ/EMBL/GenBank; Table 1). For use in phylogenetic analyses (see below), the Japanese white-toothed shrew (*Crocidura dsinezumi*) was sequenced and employed together with the long-clawed shrew (*Sorex unguiculatus*) and musk shrew (*Suncus murinus*) as an outgroup. Selected outgroups were all from the family Soricidae, a commonly accepted sister taxon of the family Talpidae (see Campbell, 1939; Nikaido et al., 2001; Stanhope et al., 1998; but see Douady et al., 2002, McKenna and Bell, 1997).

2.2. Laboratory methods

Genomic DNA was prepared from ethanol fixed liver or spleen samples by proteinase K digestion, phenol–chloroform extraction and ethanol precipitation. A 1.2-kb fragment of the cyt *b* gene was first amplified using the universal primers L14724 and H15915 (Irwin et al., 1991); the letters L and H refer to the light and heavy strands, respectively, while the associated numbers refer to the 3′ position of the primer within the complete sequence of human mtDNA (Anderson et al., 1981). Three overlapping gene fragments were then independently amplified from the initial PCR product using species'specific primer sets (Table 2). All PCR reactions were carried out for 35 cycles as follows: 30 s at 96 °C for denaturation, 30 s at 50 °C for annealing, and 30 s at 60 °C for extension. Both DNA strands from the second PCR reaction were directly sequenced using the Dye Primer Cycle Sequencing Kit (ABI) and an automated sequencer (model 373A, ABI).

Table 1
List of specimens analyzed

Species	Common name	Life style	DDBJ Accession No.	Code	Collecting site
Condylura cristata	Star-nosed mole	Semi-aquatic/Fossorial	AB076810, AB076811	Ccr 1, 2	Piney, Manitoba, Canada
			AB076812	Ccr 3	Potter County, Pennsylvania, USA
Desmana moschata	Russian desman	Semi-aquatic	AB076836	Dmo	Rockovskoye lake, ad Vladimirskiy, Russia
Dymecodon pilirostris	Lesser Japanese shrew-mole	Semi-fossorial	AB076830	Dpi 1	Mt. Tsurugi, Tokushima Pref., Japan
			AB076831	Dpi 2	Oku-Tateshina, Chino, Nagano Pref., Japan
Euroscaptor mizura	Japanese mountain mole	Fossorial	AB076828	Emi 1	Towadako, Aomori Pref., Japan
			AB037604	Emi 2	Tsuchiya et al. (2000)
Mogera imaizumii	Lesser Japanese mole	Fossorial	AB037609	Mim	Tsuchiya et al. (2000)
Mogera insularis	Insular mole	Fossorial	AB037606	Min	Tsuchiya et al. (2000)
Mogera tokudae	Tokuda's mole	Fossorial	AB037607	Mto	Tsuchiya et al. (2000)
Mogera wogura	Greater Japanese mole	Fossorial	AB037646	Mwo 1	Tsuchiya et al. (2000)
			AB037623	Mwo 2	Tsuchiya et al. (2000)
Neurotrichus gibbsii	American shrew-mole	Semi-fossorial	AB076821–AB076824	Ngi 1–4	Seattle, Washington, USA
			AB076825	Ngi 5	Surrey, British Columbia, Canada
			AB076826	Ngi 6	Vancouver, British Columbia, Canada
			AB076827	Ngi 7	Coquitlam, British Columbia, Canada
Parascalops breweri	Hairy-tailed mole	Fossorial	AB076808	Pbr	Albany, New York, USA
Scalopus aquaticus	Eastern mole	Fossorial	AB076809	Saq	Davidson County, Tennessee, USA
Scapanus latimanus	Broad-footed mole	Fossorial	AB076813, AB076814	Sla 1, 2	San Francisco, California, USA
Scapanus orarius	Coast mole	Fossorial	AB076815	Sor 1	Coquitlam, British Columbia, Canada
			AB076816, AB076817	Sor 2, 3	Abbotsford, British Columbia, Canada
Scapanus townsendii	Townsend's mole	Fossorial	AB076818–AB076820	Sto 1–3	Sumas, Washington, USA
Talpa altaica	Siberian mole	Fossorial	AB037602	Tal	Tsuchiya et al. (2000)
Talpa europaea	European mole	Fossorial	AB076829	Teu 1	Aarhus, Denmark
			AB037601	Teu 2	Tsuchiya et al. (2000)
Urotrichus talpoides	Greater Japanese shrew-mole	Semi-fossorial	AB076832	Uta 1	Mt. Gomadan, Wakayama Pref., Japan
			AB076833	Uta 2	Mt. Tsurugi, Tokushima Pref., Japan
			AB076834	Uta 3	Shitada, Niigata Pref., Japan
			AB076835	Uta 4	Tomioka, Gunma Pref., Japan
Uropsilus gracilis	Gracile shrew mole	Ambulatory	AB076699	Ugr	Shinohara et al. (unpublished data)
Crocidura dsinezumi	Japanese white-toothed shrew	⟨Out group⟩	AB076837	—	Kami-Koani, Akita Pref., Japan
Sorex unguiculatus	Long-clawed shrew	⟨Out group⟩	AB061527	—	Nikaido et al. (2001)
Suncus murinus	Musk shrew	⟨Out group⟩	AB033610	—	Onuma et al. (2000)

Table 2
Nested primers for secondary PCR of the mitochondrial cytochrome *b* gene

Primer	Primer sequence	Species specificity[a]
N-L14724[b]	5′-CAGGAAACAGCTATGACCGATATGAAAAACCATCGTTG-3′	Universal
N-H15155[b]	5′-TGTAAAACGACGGCCAGTTGCCCCTCAAAAGGATATTTG-3′	Universal (except Sla, Saq, Pbr)
N-H15155′	5′-TGTAAAACGACGGCCAGTTGCACCTCAGAATGATATCTG-3′	Sla, Saq, Pbr
N-L15135[c]	5′-CAGGAAACAGCTATGACCGCTATAATAGCAACAGCATTCATAGG-3′	Uta, Dpi, Ngi, Sto
N-L15135′	5′-CAGGAAACAGCTATGACCGCCGTAATAGCCACTGCATTCATAGG-3′	Universal (except Uta, Dpi, Ngi, Sto, Cds)
N-L15135″	5′-CAGGAAACAGCTATGACCATAGCTACTGCCTTTATAGG-3′	Cds
N-H15599[c]	5′-TGTAAAACGACGGCCAGTGAGTCCTCCTAGTTTGTTGGG-3′	Saq, Pbr, Sto
N-H15567	5′-TGTAAAACGACGGCCAGTGATCGTAGGATTGCGTATGCGAATAGG-3′	Uta, Dpi, Ngi
N-H15599′	5′-TGTAAAACGACGGCCAGTAGTACACCTCCTAGTTTATTAGG-3′	Teu, Emi, Ccr, Sor, Sla, Ugr, Dmo
N-H15582	5′-TGTAAAACGACGGCCAGTAGGGATTGATCGTAAGATTG-3′	Cds
N-L15561[d]	5′-CAGGAAACAGCTATGACCCCACATATTAAACCAGAATG-3′	Universal
N-H15916[d]	5′-TGTAAAACGACGGCCAGTGTCATCTCCGGTTTACAAGA-3′	Universal

[a] Species abbreviations listed in Table 1.
[b] Suzuki et al. (1997).
[c] Iwasa et al. (2000).
[d] Suzuki et al. (2000).

2.3. Data analyses

2.3.1. Sequence alignment

Because no insertions or deletions were evident in the cyt *b* data set, sequences were aligned visually and verified with deduced amino acid sequences. Gene sequences determined for this study have been deposited in DDBJ/EMBL/GenBank under Accession Nos. AB076808–AB076837.

2.3.2. Phylogenetic analyses

We plotted the transition and transversion distances versus total pairwise distances (*p*-distance) for all taxa and examined this relationship within species, within genera, among genera, and between families. Base composition and base composition bias were analyzed according to Irwin et al. (1991). Values from this test range from 0 to 1, with zero indicating no bias and one indicating complete bias.

To infer molecular phylogenetic relationships among the 18 talpid species of our data set, we conducted neighbor-joining (NJ; Saitou and Nei, 1987), maximum parsimony (MP; Swofford and Olsen, 1990) and maximum likelihood (ML; Felsenstein, 1981) analyses using PAUP* 4.0b8 software (Swofford, 2001). Optimal parameters for maximum-likelihood searches were obtained with Modeltest 3.06 (Posada and Crandall, 1998). Both the hierarchical likelihood ratio tests and the Akaike information criterion selected the GTR (I + G) model (Rodriguez et al., 1990) with the following parameters: empirical base frequencies ($A = 0.37$, $C = 0.33$, $G = 0.06$, $T = 0.24$), gamma distribution shape = 0.9, number of rate categories = 4, and proportion of invariable sites = 0.52. For the NJ analyses, we used the Kimura-2-parameter model with transversions only (Kimura, 1980). This analysis was repeated using ML parameters estimated from the ML analysis (NJ–ML). The MP analyses were performed with the heuristic search algorithm (1000 replicates of random taxon additions, TBR branch swapping) using a priori weighting (transversions only) to search for the most parsimonious trees. Character optimizations were performed using both the ACCTRAN and DELTRAN settings.

The statistical confidence of groupings in the NJ, NJ–ML, and MP trees was evaluated by the bootstrap test (Felsenstein, 1985). In addition, we assessed the level of confidence with decay indices (Bremer, 1988) for the MP trees using TreeRot. v2 (Sorenson, 1999). Confidence intervals for the ML tree were assessed using quartet-puzzling scores (Strimmer and von Haeseler, 1996; Strimmer et al., 1997).

3. Results

3.1. Cytochrome b sequence variation

We determined the cyt *b* gene sequences (1140 bp) from 29 individuals spanning 12 talpid species. The resultant sequence data were analyzed together with *Uropsilus gracilis* (Shinohara et al., unpublished data) and those previously published for seven species of Eurasian moles (Tsuchiya et al., 2000). The base composition of the resulting data set was shown to have a bias ranging from 0.152 to 0.296; these values are notably low in G content for both variable (0.058) and informative sites (0.060; Table 3). The percentage and level of variable and informative sites of our data set are shown in Table 4. Comparative sequence alignments revealed that 44.9% of sites (512 of 1140 bp) were variable, while 40.4% (460 bp) were parsimoniously informative. Of the

Table 3
Base composition and bias of all variable and phylogenetically informative sites

	All		Variable		Informative	
	Mean	SD	Mean	SD	Mean	SD
A	0.313	0.011	0.384	0.024	0.359	0.027
C	0.265	0.019	0.338	0.042	0.361	0.047
G	0.136	0.005	0.058	0.013	0.060	0.013
T	0.286	0.023	0.220	0.051	0.221	0.056
Bias[a]	0.152		0.296		0.293	

[a] The bias is calculated using the formula of Irwin et al. (1991).

Table 4
Amount of variable and informative sites

		All taxa	(%)	Without outgroup	(%)
Variable		512	44.9	484	42.5
	1st	113	9.9	104	9.1
	2nd	32	2.8	25	2.2
	3rd	367	32.2	355	31.1
Informative		460	40.4	433	38.0
	1st	97	8.5	90	7.9
	2nd	21	1.8	17	1.5
	3rd	342	30.0	326	28.6

460 informative positions, 97 (21.1%) were at the first-codon position, 21 (4.6%) at the second-codon position, and 342 (74.3%) at the third-codon position (Table 4).

In pairwise comparisons among genera, the rates of transitions were shown to be saturated (Fig. 1a). In contrast, transversions were not fully saturated, even in deep lineage comparisons (Fig. 1b). Consequently, for the NJ and MP tree building methods, transversions only were employed to assess phylogenetic relationships.

3.2. Phylogenetic tree comparisons

Reasonably congruent phylogenetic trees in terms of branching and clustering of taxa were generated with the NJ (Fig. 2), MP (Fig. 3), ML (Fig. 4), and NJ–ML (Fig. 5) methods. In each of these topologies, the 12 genera were generally aligned into seven clades; (1) Asiatic shrew-like moles (*Uropsilus*), (2) North American aquatic/fossorial moles (*Condylura*), (3) North American fossorial moles (*Parascalops*, *Scalopus*, and *Scapanus*), (4) North American semi-fossorial shrew moles (*Neurotrichus*), (5) Japanese semi-fossorial shrew moles (*Dymecodon* and *Urotrichus*), (6) European semi-aquatic desmans (*Desmana*), and (7) Eurasian fossorial moles (*Euroscaptor*, *Mogera*, and *Talpa*). Notably, no phylogenetic grouping was found to comprise mole species from both continents. In fact, both Eurasian and North American fossorial moles and Eurasian and North American shrew moles appear to represent nonmonophyletic assemblages (Figs. 2–5).

3.3. Phylogenetic relationships within the Talpidae

All topologies supported the basal placement of Asiatic shrew-like moles (*Uropsilus*) within the family Talpidae (Figs. 2–5). In contrast, the phylogenetic position of *Condylura* varied substantially depending upon tree building method, and all cases support values for evolutionary associations of *Condylura* with other talpid clades were perilously low (12–32%).

A monophyletic North American (*Parascalops* (*Scalopus, Scapanus*)) clade was moderately supported (43–66% bootstrap support) in all topologies save the ML analyses (16% support). Within this fossorial clade, the geographically isolated *Scalopus* (eastern North America) and *Scapanus* (western North American) were consistently clustered with high support (bootstrap values ranging from 68–98%). Within the genus *Scalopus*, *S. orarius* and *S. townsendii* formed a well-supported cluster distinct from *S. latimanus* without fail (Figs. 2–5).

All phylogenetic analyses strongly backed a Japanese shrew-mole clade (*Urotrichus* and *Dymecodon*; bootstrap support = 100% in NJ, MP, and NJ–ML) to the exclusion of the North American *Neurotrichus* (Figs. 2–5). The level of sequence divergence between Japanese and North American semi-fossorial genera was substantial ($p = 0.17$; data not shown). Intraspecific variation was found to be relatively high in *Urotrichus talpoides* and *Dymecodon pililostris*, but not in *Neurotrichus gibbsii* (Figs. 2–5).

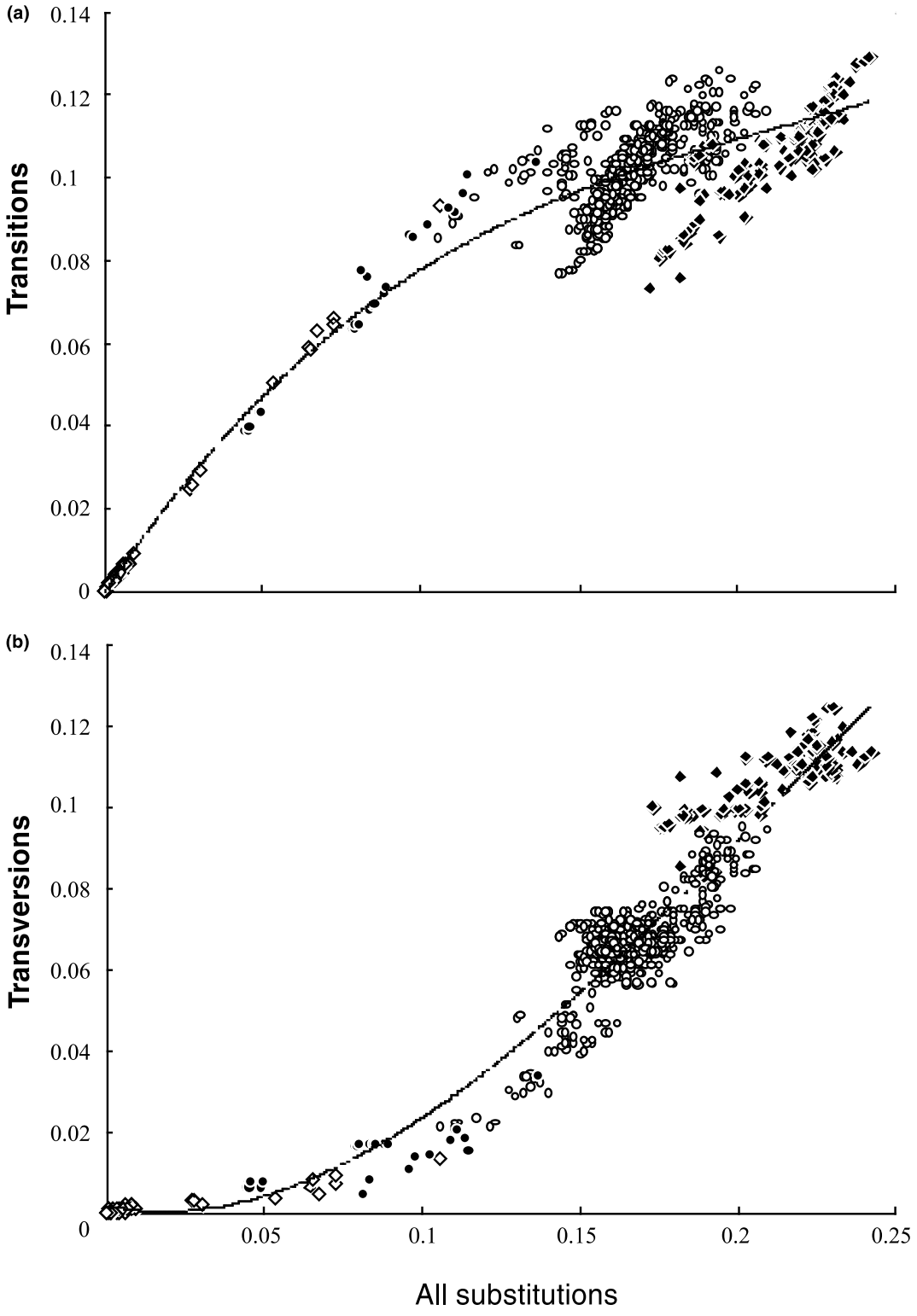

Fig. 1. Pairwise comparisons (*p*-distances) of: (a) transitions versus all substitutions, and (b) transversions versus all substitutions. Intraspecific comparisons (◇), intrageneric comparisons (●), intergeneric comparisons (○), and comparisons between members of the family Soricidae (see Materials and methods) and each talpid species (◆) are plotted.

Within all topologies, *Desmana* was nested between a basal clade of shrew moles and the more derived fossorial Eurasian mole clade. In fact, with the exception of the MP analysis (Fig. 3), the semi-aquatic desmans were grouped together with Eurasian fossorial moles with modest (63–78%) bootstrap support. The NJ, ML, and

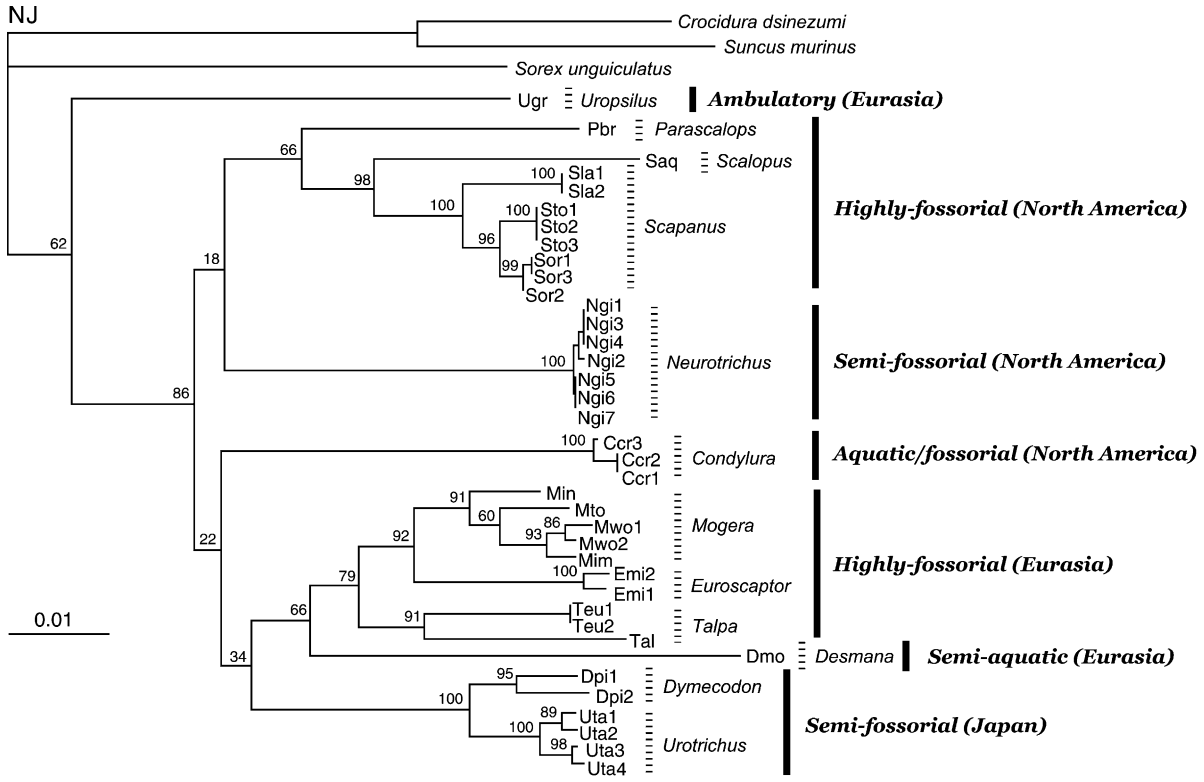

Fig. 2. Neighbor-joining tree based on cyt *b* gene sequences using Kimura-2-parameter model with transversions only. Bootstrap values expressed as percentages of 1000 replications are given at each node.

NJ–ML analyses supported a monophyletic relationship of the Eurasian fossorial genera (*Mogera*, *Euroscaptor*, and *Talpa*), while the MP analysis was unable to resolve the deeper evolutionary nodes of the Eurasian fossorial mole clade (Fig. 3). However, a monophyly of the East Asian moles *Mogera* and *Euroscaptor* was consistently well supported in all topologies.

4. Discussion

Despite numerous phenetic and cladistic analyses, hypotheses pertaining to the evolution, biogeography and morphological diversification of Eurasian and North American moles, shrew moles, and desmans remain equivocal (Hutchison, 1976; Moore, 1986; Whidden, 2000; Yates and Moore, 1990). Results of this study suggest that the 12 talpid genera examined can be resolved into 7 major clades (Figs. 2–5), with intergeneric relationships within each cluster typically well supported regardless of tree building method. Interestingly, with the exception of the Urotrichini, the generic makeup of each cluster closely matched that previously ascribed to each tribe or subfamily (McKenna and Bell, 1997). However, relatively low support was generally found for internal branching patterns uniting the major clades (Figs. 2–5). Our inability to resolve phylogenetic relationships among these identified clusters with certainty may reflect limitations of the cyt *b* gene, which is generally suitable for investigating divergence events only within the last 20 million years (Harrison, 1989; Irwin et al., 1991). In this regard, it is notable that fossil data suggest radiation events leading to the three recognized subfamilies occurred from the Late Eocene to Middle Oligocene (Hutchison, 1976). Alternatively, the observed polytomy may be the result of several independent, relatively contemporaneous divergence events among the various clusters. Additional molecular data with slower evolving mitochondrial and nuclear gene markers will be required to clarify phylogenetic relationships among these groupings.

4.1. Phylogenetic relationships

Endemic to the mountainous highlands of south-central Asia, members of the genus *Uropsilus* are thought to represent an early talpid offshoot (probably Eocene; Hutchison, 1968; Yates and Moore, 1990), in that they possess morphological features reminiscent of both shrews and moles (Allen, 1938). Indeed, histological findings indicate the presence of Eimer's organ-like structures (a unique talpid specialization) and a nasal epidermal morphology intermediate to that of both families (Catania, 2000). However, because previous investigations were not designed to formally assess talpid monophyly, the traditional basal placement of

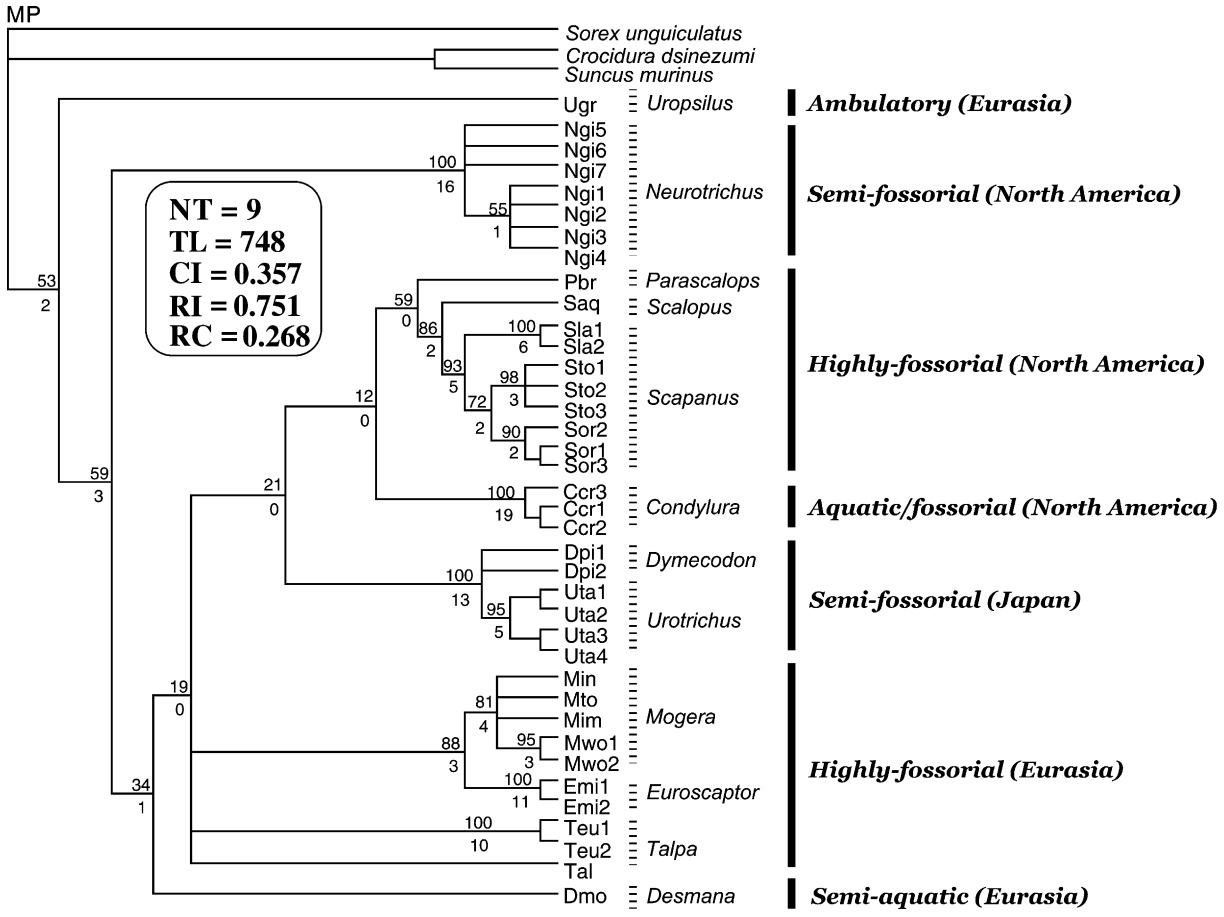

Fig. 3. Maximum parsimony tree based on cyt *b* gene sequences considering only transversions. Consensus tree was constructed by the strict method. Bootstrap values (percentages of 1000 replications) and decay indexes are shown at each node. The number of most-parsimonious trees recovered (NT), total tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency index (RC) are also presented.

Uropsilus within the Talpidae relies almost completely upon the retention of primitive characters (Whidden, 2000). Including representative members from all 3 extant subfamilies and associated soricid outgroups, our analyses placed *Uropsilus* at the basal node of a monophyletic Talpidae in all cases, with bootstrap support for this clade ranging from 33% (ML) to 65% (NJ-ML). Additional molecular support for both a basal *Uropsilus* and a monophyletic Talpidae were recently found in a robust mammalian analysis that included 4 talpid genera (Douady et al., 2002). On balance, these findings corroborate earlier notions identifying this species as the first offshoot on the talpid tree.

The phylogenetic position of *Condylura* within the Talpidae was found to vary substantially depending upon tree building method (Figs. 2–5), with the highest bootstrap support uniting *Condylura* with any other talpid clade being only 32% (ML analysis). Interestingly, this topology suggests that the divergence of *Condylura* from the main talpid line predated the diversification of the subfamily Talpinae (Fig. 4). Although this early divergence of *Condylura* was not recognized by Hutchison (1976), Moore (1986), or Yates and Moore (1990), it was well supported in an explicit phylogenetic analysis utilizing morphological data (Whidden, 2000). Nonetheless, the significant level of sequence divergence (mean *p*-distance = 0.171; range = 0.144–0.194) and low affinity observed between *Condylura* and all other talpid clades, suggests a long independent history of this lineage within the Talpidae. Inclusion of additional nucleotide data and taxa (e.g., *Scaptonyx* and *Scapanulus*; see below) will be required to elucidate the relationship of this unusual species with other extant talpids in future.

Characterized by slightly broadened fore limbs, a short-thickened tail and distinctly semi-fossorial habits, Japanese (*Urotrichus* and *Dymecodon*) and North American (*Neurotrichus*) shrew moles are strikingly similar to the Chinese long-tailed mole (*Scaptonyx*) in external form (Allen, 1938; Campbell, 1939; Whidden, 2000). Although the relationships and timing of radiation events among these genera are debated, separate cladistic analyses utilizing fossil (Hutchison, 1976), and genic and chromosomal characters (Yates and Moore, 1990), suggest the 3 shrew-mole genera comprise a nat-

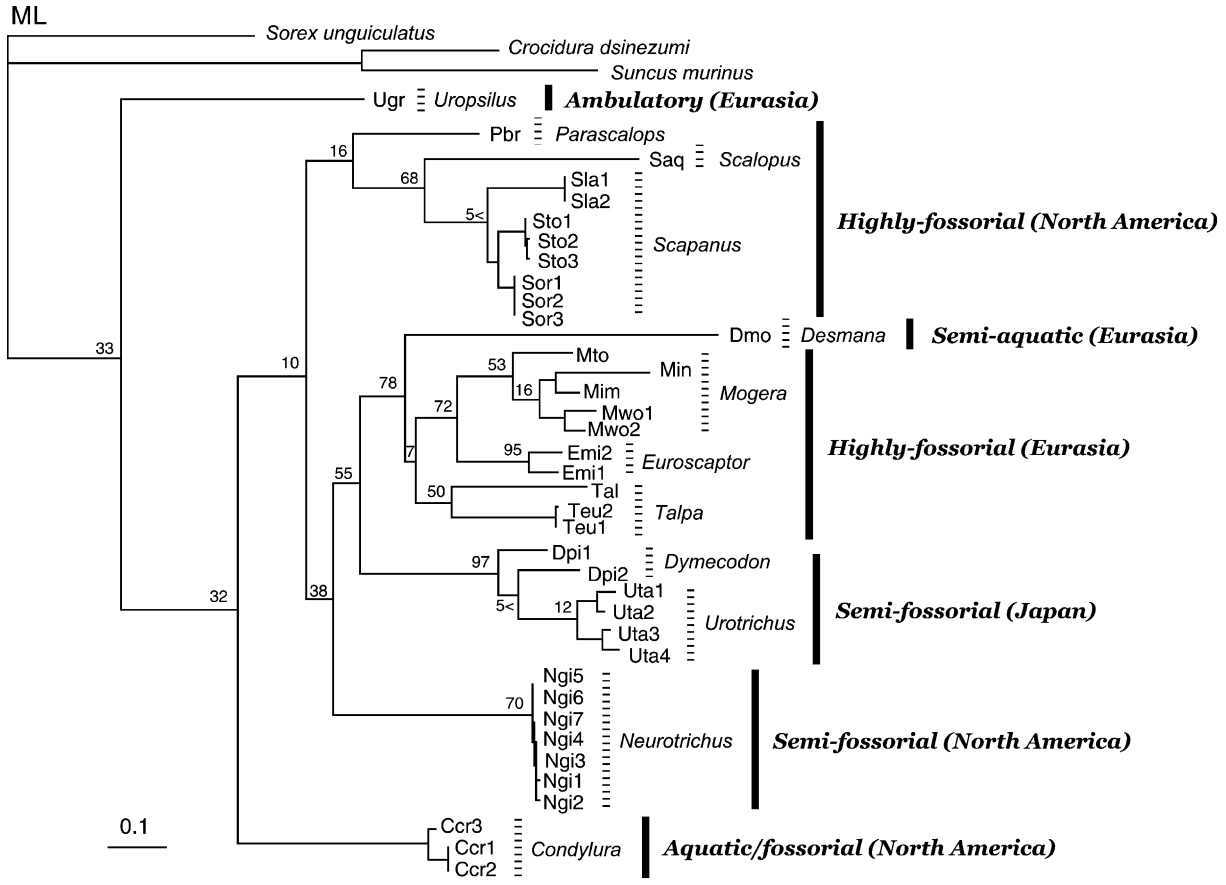

Fig. 4. Maximum likelihood tree based on cyt *b* gene sequences with a selected model (see text). Quartet puzzling scores (percentages of 10,000 steps) are shown at each node. Branch lengths are given in maximum likelihood distance with the selected model.

ural assemblage distinct from *Scaptonyx*. However, Campbell (1939) closely aligned all 4 genera into a monophyletic clade based on his study of comparative osteology and myology, a finding corroborated by Whidden (2000) on the basis of 8 unambiguous myological synapomorphies. While it is unfortunate we were unable to obtain samples from *Scaptonyx*, our present data do not support a close relationship between North American and Japanese shrew moles. In fact, our data suggests the divergence of North American and Japanese shrew moles occurred prior to the diversification of the talpini (Figs. 2–5), and as such represent separate phyletic lineages. It will be essential to include *Scaptonyx* in future studies to clarify the phylogenetic affinities among the semi-fossorial lineages of Asia and North America, and their relationships with fossorial and semi-aquatic talpids.

A recent phylogenetic analysis united fossorial North American and Eurasian moles within a single monophyletic group based on a suite of unambiguous synapomorphic myological characters (Whidden, 2000). Contrary to this conclusion, and in support of the phylogenetic hypothesis forwarded by Douady et al. (2002), Hutchison (1976) and Yates and Moore (1990), our cyt *b* data indicate that fossorial moles endemic to North America and Eurasia represent two distinct assemblages. Indeed, North American moles do not appear to have specific affinities with any Eurasian mole lineage examined (Figs. 2–5), with the possible exception of the Chinese genus *Scapanulus*, which is thought to be closely aligned with the North American scalopine species (Allen, 1938; Campbell, 1939; Hutchison, 1976; Yates and Moore, 1990; but see Ziegler, 1971). This finding is significant as it implies subterranean specializations arose independently at least twice during the evolution of the Talpidae, as suggested by Moore (1986).

The three strictly fossorial genera of North America were consistently separated into two groups with *Scapanus* and *Scalopus* comprising a monophyletic clade distinct from *Parascalops*. This finding harmonizes with the data of Moore (1986) and Yates and Moore (1990) and supports Hutchison's (1968) contention that *Parascalops* represents an early offshoot (Early Miocene) on the branch leading to *Scapanus* and *Scalopus*. In agreement with the conclusions of Hutchison (1968) and Yates and Greenbaum (1982), our mtDNA data indicate that *Scapanus orarius* and *S. townsendii* represent

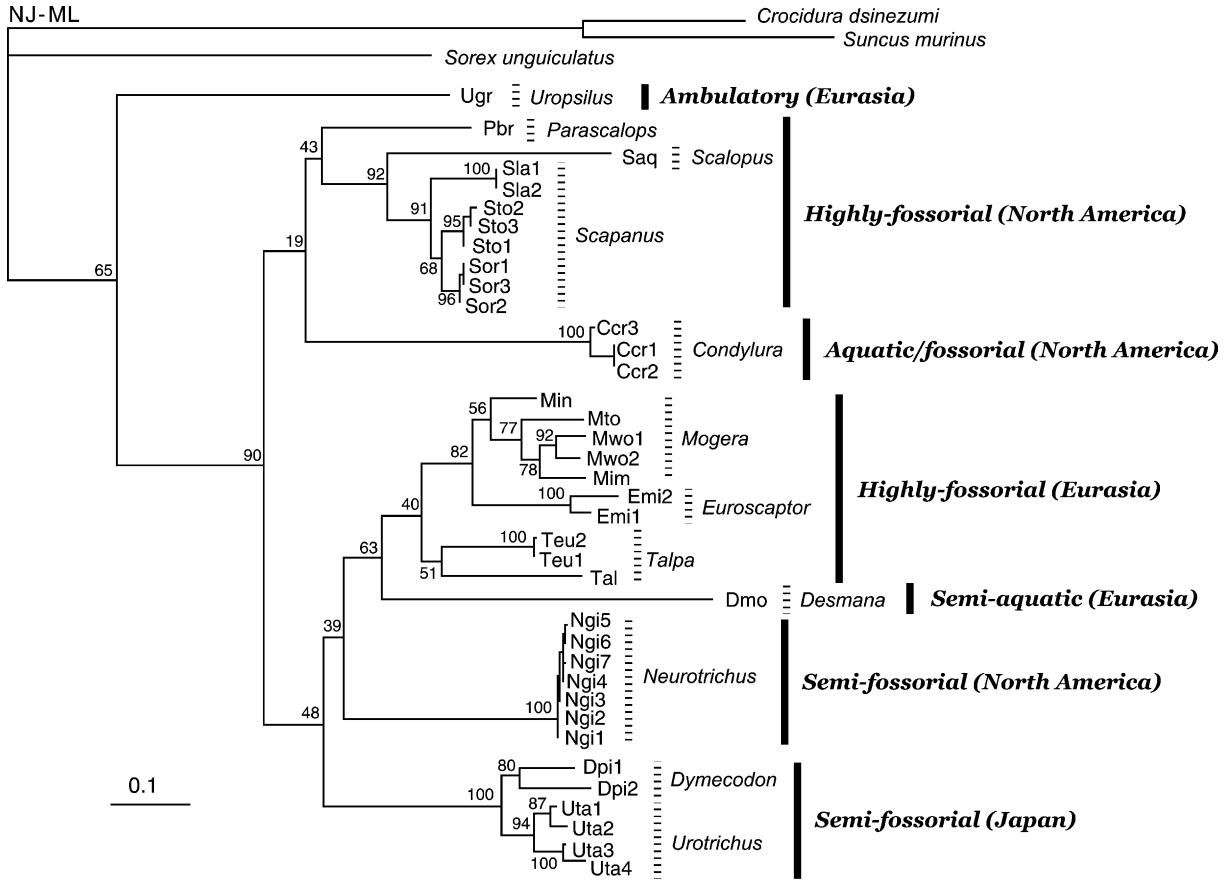

Fig. 5. Neighbor-joining tree based on cyt *b* gene sequences adopting maximum likelihood parameters. Bootstrap values (percentages of 1000 replications) are shown at each node.

sister taxa distinct from the more southerly distributed *S. latimanus*. This affiliation of Pacific coast North American moles was always well supported, and conflicts with genic data of Moore (1986).

A previous study based on the cyt *b* sequences of 7 Eurasian talpini species indicated that the genera *Talpa*, *Mogera*, and *Euroscaptor* were closely related (Tsuchiya et al., 2000). Although this conclusion was potentially biased due to incomplete taxon sampling and limited outgroups the same close relationships among these 3 genera is supported by our more comprehensive molecular analyses. Consonant with other investigations (Moore, 1986; Tsuchiya et al., 2000), it is noteworthy that *Mogera* and *Euroscaptor* form a highly concordant cluster distinct from *Talpa* (Figs. 2–5). This finding supports the suggestion that the spatial subdivision between Europe (including West Asia) and East Asia took place early in the evolution of fossorial Eurasian moles (Tsuchiya et al., 2000).

Based largely on morphological and fossil evidence, the semi-aquatic desmans of Europe (represented in this study by *Desmana*) are afforded both subfamilial status and a secondarily basal position within the Talpidae (Hutchison, 1976; McKenna and Bell, 1997; Whidden, 2000). However, as cautioned by Yates and Moore (1990), most hypotheses pertaining to the taxonomic status and placement of desmans suffer from a basic lack of phylogenetically useful data (especially genetic), and thus are potentially biased by several uniquely derived semi-aquatic specializations. Similar to the topology of Douady et al. (2002), we found no support for an early independent radiation of *Desmana* from the main talpid line (Figs. 2–5). Although the comprehensive molecular analyses of Douady et al. (2002) incorporated only 4 talpid genera, these authors found significant bootstrap support for a derived desman-fossorial Eurasian mole grouping to the exclusion of North American fossorial moles (I.e. *Scalopus* (*Galemys, Talpa*)). Interestingly, our results also provide moderate support for a derived monophyletic desman-fossorial Eurasian mole clade, with bootstrap scores for this association ranging from 63 to 78% in the NJ, ML and NJ-ML analyses. In addition, it is noteworthy that each of our phylogenetic analyses nested *Desmana* between a clade of ancestral semi-fossorial shrew moles and a more derived grouping of fossorial Eurasian moles (Figs. 2–5). This implied evolutionary pattern is of interest considering Reed (1951) viewed "the desman to be a semi-fossorial animal

which has secondarily become aquatic''. This interpretation, however, has been questioned (Whidden, 1999). Regardless, the putative relationships we observed among these groups question the current subfamily status of the Desmaninae, and indicate that the subfamily Talpinae, as presently defined, is paraphyletic. Clearly, additional morphological and molecular evidence together with comparable data from the Iberian desman (*Galemys pyrenaicus*) are necessary to refute (or support) the subfamily status and relatively basal phylogenetic position of desmans within the talpid line.

Acknowledgments

We wish to express our appreciation to Drs. N.R. Lovejoy (University of Manitoba, Canada), K. Tsuchiya (Tokyo University of Agriculture, Japan), H.P. Whidden (Augustana College, USA), and R.E. Weber (University of Aarhus, Denmark) for valuable suggestions and co-operation with the collection of animals. We are deeply grateful to M. Harada, B. Gitzen, B. Masuda, K.C. Catania, N. Rubenstein, A. Bannikova, and S.T. Sheehan for providing tissue samples and specimens. We are indebted to H. Abe, M.A. Iwasa, A.P. Kryukov, Y.-P. Zhang, X. Jiang, M. Motokawa, K. Koyasu, and S.-I. Kawada for their valuable advice. We also thank K. Serizawa for technical assistance and S.G. Goto for his kind support in phylogenetic analyses. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan (HS). Additional funding by the Danish Natural Science Research Council and Natural Sciences and Engineering Research Council of Canada (KLC) are gratefully acknowledged.

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