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Evolution and Biogeography of Talpid Moles from Continental East Asia and the Japanese Islands Inferred from Mitochondrial and Nuclear Gene Sequences

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ABSTRACT—We sequenced the cytochrome *b* gene from two little-studied mammal species from the highlands of Southwest China, the long-tailed mole *Scaptonyx fusicaudus* and the gracile shrew-like mole *Uropsilus gracilis*. This data was used to examine the phylogenetic relationships among 19 talpid species within the family Talpidae (Mammalia: Eulipotyphla). Cytochrome *b* gene trees supported a basal placement of shrew-like moles (*Uropsilus*) within the Talpidae, and suggested that fossorial specializations arose twice during talpid evolution. To assess the evolutionary relationships of moles endemic to this region, we additionally sequenced the 12S rRNA gene and the nuclear recombination-activating gene-1 from eight and ten East Asian taxa, respectively. Analyses of these single and concatenated data sets suggested that East Asian shrew moles diverged prior to the evolution of fossorial Eurasian moles. However, we were unable to determine whether semi-fossorial shrew moles are monophyletic. In contrast, fossorial Eurasian genera (*Talpa*, *Mogera* and *Euroscaptor*) were consistently found to form a monophyletic clade, with *Mogera and Euroscaptor* representing sister taxa. Furthermore, this fossorial clade grouped with the semi-aquatic *Desmana*, although with fairly low (35–62%) bootstrap support. *Mogera imaizumii* was found to be more closely related to *M. wogura* than to *M. tokudae*. This implies that the ancestors of these three species entered Japan from the Asian continent in this order via a series of migration events, suggesting that the Japanese Islands have played an important role in preserving mole lineages from ancient to recent times.

Key words: Talpidae, cyt *b*, RAG-1, 12S rRNA, molecular phylogeny

INTRODUCTION

East Asian moles of the Family Talpidae can be divided into three distinct ecological and morphological groups: (1) highland endemic terrestrial shrew-like moles, (2) small semi-fossorial shrew moles with slightly broadened forefeet, and (3) larger-bodied, strictly fossorial moles with highly specialized forelimbs. A major topic of interest regarding this family concerns the evolution of morphological and ecological traits that enabled the adoption of subterranean habits by fossorial moles. It has been hypothesized that talpids evolved from ambulatory surface dwellers to semi-fossorial forms and finally to fossorial groups (Reed, 1951). However,

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this “surface to subterranean” evolutionary progression has not been rigorously tested by molecular phylogenetic analyses. Since representatives from each of these three phenotypes inhabit East Asia, phylogenetic analysis of East Asian moles may help to resolve the evolutionary history of this family. Moreover, as East Asia is comprised of both a continental mainland and nearby island chains (i.e. the Japanese Islands and Taiwan), each of which might act as source of lineage expansion (cradle) and/or lineage preservation (museum), this data may be useful to help decipher the biogeography of talpids endemic to this region.

According to fossil evidence, terrestrial and semi-fossorial talpids were once broadly distributed across the Eurasian continent (McKenna and Bell, 1997). However, compared to the ranges of fossorial moles, which today range widely throughout Europe, Asia and North America, the current distribution of extant non-fossorial and semi-fossorial species is very limited. Two such genera, *Scaptonyx* and *Uropsilus,* first described by Milne-Edwards in 1871, are found in the mountainous highlands of central Asia (Allen, 1938). Externally, *Uropsilus* is shrew-like in appearance, possessing a long cartilaginous snout, a long slender tail, conspicuous external ears extending beyond the fur of the head, and forefeet that are not specialized for burrowing (Allen, 1938; Hoffmann, 1984; Nowak, 1999). *Scaptonyx* is a monotypic genus with more mole-like features than *Uropsilus*, having slightly broadened fore limbs with large, nearly straight claws, no external ear and a tail only thinly covered with short, stiff hairs (Allen, 1938; Nowak, 1999). Milne-Edwards (in Allen, 1938) noted *Scaptonyx* resembles a mole with the feet of a shrew mole, or a shrew mole with the head of a mole. Allen (1938) further suggested it represents an annectant stage between semi-fossorial and wholly subterranean life. Not surprisingly, *Scaptonyx* has been grouped closely with both shrew moles (Campbell, 1939; Whidden, 2000) and fossorial moles (Hutchison, 1976; Yates and Moore, 1990).

We previously examined the phylogenetic relationships of East Asian (Tsuchiya *et al*., 2000) and North American talpids (Shinohara *et al*., 2003) using the mitochondrial cytochrome *b* (cyt *b*) gene. Both studies recovered a monophyletic fossorial Eurasian mole clade, and revealed a close relationship between large-sized (*Mogera*) and small-sized (*Euroscaptor*) Asian moles, to the exclusion of large-sized European (*Talpa*) moles. Additionally, data of Shinohara *et al*. (2003) indicated that the fossorial Eurasian and North American (*Scapanus*, *Parascalops* and *Scalopus*) clades arose separately. Similarly, shrew moles from the Japanese Islands and North America did not appear to show a close phylogenetic affinity (Shinohara *et al*., 2003). Unfortunately, Asiatic shrew-like moles (*Uropsilus*) and long-tailed moles (*Scaptonyx*) are rarely captured and have rarely been studied, and sequence data from *Scaptonyx* has not been available. To elucidate the evolutionary history of extant talpids, it clearly is essential to determine the phylogenetic positions of these genera in relation to the present day desman, shrew mole and fossorial mole lineages. Thus, we sequenced the mitochondrial cyt *b* gene (1140bp) from *U. gracilis* and *S. fusicaudus*, and utilized these sequences with the cyt *b* data set for 17 talpids reported by Shinohara *et al*. (2003) to assess the phylogeny and radiation of the Talpidae.

One of the intriguing features of talpids in East Asia is the high degree of species richness in the Japanese Islands. Five species are endemic to Honshu, Shikoku and Kyushu islands, comprising two shrew moles (*Urotrichus talpoides*, *Dymecodon pilirostris*), one mountain mole (*Euroscaptor mizura*), and two plains moles (*Mogera imaizumii* and *M. tokudae*). A sixth Japanese species, *M. wogura* (also referred to as *M. kobeae*, see Hutterer, 1993) is distributed in Kyushu, Shikoku and the eastern part of Honshu. However, it is also found in adjacent areas of the Asian continent, specifically the Korean Peninsula, East China and the Primorye region of Russia (Tsuchiya *et al*., 2000). Thus, to identify episodes of lineage expansion into (or from) this island chain, it is important to clarify the phylogenetic relationships among the Japanese lineages. Additionally, interrelationships among the three large-sized Asian mole species are controversial. Mitochondrial sequence data support a sister-taxon relationship for *M. wogura* and *M. imaizumii* (Okamoto, 1999; Tsuchiya *et al*., 2000), whereas chromosomal banding analyses place *M. imaizumii* together with *M. tokudae* to the exclusion of *M. wogura* (Kawada *et al*., 2001). The former phylogenetic hypothesis suggests a series of migration events occurred from the Asian mainland to Japan, with *M. tokudae*, *M. imaizumii*, and *M. wogura* independently entering Japan from the continent in this order. The latter finding may suggest parapatric lineage differentiation in the northern part of Honshu between *M. tokudae* living on Sado island and the Niigata plain, and *M. imaizumii*, which is primarily distributed in the remaining part of northern Honshu with several small relict populations in western Honshu. To test these hypotheses and better understand the biogeography of Eurasian talpid species, robust phylogenetic analyses are required. Thus, our second goal was to construct molecular phylogenetic trees for ten species of Eurasian moles, including *Uropsilus* and *Scaptonyx* from both mitochondrial (cyt *b* and 12S rRNA) and nuclear gene sequences (recombination-activating gene-1 or RAG-1).

MATERIALS AND METHODS

The species examined in this study are listed in Table 1. We followed the classifications of Corbet and Hill (1991) and Hutterer (1993) for continental species, and the more recent classifications of Abe (1995) and Motokawa and Abe (1996) for Japanese species. Specimens of *Uropsilus* were identified to species following Hoffmann (1984).

Gene amplification and direct sequencing

Genomic DNA from ethanol-preserved liver samples of each specimen was extracted using proteinase K digestion and phenol-

Table 1. List of samples used in this study

Species	Accession No.		
	Cytochrome *b*	12S rRNA	RAG1
Uropsilus gracilis	AB076699*, AB076700*	AB106230*, AB106231*	AB106240*
Talpa europaea	AB037601[1]	Y19192[3]	AB106246*
Talpa altaica	AB037602[1]	AY012100[4]	AB176542*
Euroscaptor mizura	AB037604[1]	AB106233*	AB176543*
Mogera insularis	AB037606[1]	AB106234*	AB176544*
Mogera tokudae	AB037607[1]	AB106235*	AB106243*
Mogera imaizumii	AB037609[1]	AB106236*	AB106242*
Mogera wogura	AB037623[1], AB037646[1]	AB106237*, AB106238*	AB106244*
Urotrichus talpoides	AB076833[2]	AB106239*	AB106245*
Scaptonyx fusicaudus	AB106229*	AB106232*	AB106241*

Asterisks denote new sequences determined in this study. Other sequences presented are from [1]Tsuchiya *et al*. (2000), [2]Shinohara *et al*. (2003), [3]Mouchaty *et al*. (2000) and [4]Murphy *et al*. (2001). For the large cyt *b* gene tree analyses (see text for details) the following sequences of Shinohara *et al*. (2003) were included: *Dymecodon pilirostris* (AB076830), *Neurotrichus gibbsii* (AB076827), *Parascalops breweri* (AB076808), *Scapanus townsendii* (AB076820), *S. latimanus* (AB076813), *S. orarius* (AB076816), *Scalopus aquaticus* (AB076809), *Condylura cristata* (AB076812), *Desmana moschata* (AB076836) and *Crocidura dsinezumi* (AB076837). Outgroup sequences utilized were: *Crocidura dsinezumi* (cyt *b*: AB076837; Shinohara *et al*., 2003), *Suncus murinus* (cyt *b*: AB033610: Onuma *et al*., 2000) and *Sorex unguiculatus* (cyt *b*: AB061527; 12S: AB061527; Nikaido *et al*., 2001).

chloroform-isoamyl alcohol extraction procedures. The complete mitochondrial cyt *b* gene was first amplified using the universal primer pair L-14724 and H-15915 (Irwin *et al*., 1991). Secondary amplification of this product was carried out with three primer pairs: (1) H-15916 (Suzuki *et al*., 2000) and L-15525 (Hosoda *et al*., 2000), (2) L-15135 and H-15599 (Shinohara *et al*., 2003), and (3) H-15155 (5'-TGTAAAACGACGGCCAGTTGCACCTCAAAATGATATTTG-3') and L-14724 (Suzuki *et al*., 1997). Alternatively, the cyt *b* product was re-amplified with two primer pairs: (1) L-14724 (Suzuki *et al*., 1997) and H-15401 (5'- TGTAAAACGACGGCCAGT TGTGTAGTATGGGTGGAATGG -3'), and (2) H-15916 (Suzuki *et al*., 2000) and L-15423 (5'- CAGGAAACAGCTATGACCCCTAGTAGAATGAATCTGAGG -3').

An amplicon of the mitochondrial 12S rRNA gene (ca. 900 bp) was obtained using the universal primer pair L-613 (Mindell *et al*., 1991) and H-1478 (Kocher *et al*., 1989). Following this step, a heminested PCR was carried out using two primer pair sets: (1) R-L613 (Yamada *et al*., 2002) and U-H1066 (Suzuki *et al*., 1997) and (2) R-L 946 (5'-CAGGAAACAGCTATGACCGACATACGGCGTAAAGAGTGT-3') and U-H1478 (Suzuki *et al*., 1997).

A partial exon sequence of the RAG-1 gene was amplified using the primer pair RAG1-F1851 (Sato *et al*., 2004) and RAG1-R2951 (5'- GAGCCATCCCTCTCAATAATTTCAGG-3'; = RAG1-R2864, Teeling *et al*., 2000); Numbers in primer names indicate the position of the 3' end of the primer in the published human sequence (accession number M29474, Schatz *et al*., 1989). The PCR product was then re-amplified with the following primer sets: RAG1-F1851 and RAG1-R2486 (Sato *et al*., 2004), and RAG1-F2401 (5'-CCGAGAACCTGGAGCGCTATGAGGTCTGG-3') and RAG1-R2951.

The second PCR product of each reaction was primed using either a Big-Dye Primer or Big-Dye Terminator Cycle Sequencing Kit (ABI) and both strands directly sequenced (Model 310, Applied Biosystems).

Sequence alignment

We determined nucleotide sequences of the cyt *b*, 12S rRNA and RAG-1 genes in two, eight, and ten Asian mole species, respectively (Table 1). These gene sequences have been deposited in the GenBank/EMBL/DDBJ nucleotide sequence database. These sequences, together with cyt *b* and 12S rRNA nucleotide data from the GenBank/EMBL/DDBJ database, were used to construct larger data sets for phylogenetic analyses (see Table 1 for list). No insertions or deletions were found in either the cyt *b* or RAG-1 data sets, thus sequences were aligned visually and verified using deduced amino acid sequences. Because sequence lengths of the 12S rRNA products varied between species, they were first aligned together using Clustal-X (Thompson *et al*., 1997) with default options, then manually adjusted with Se-Al v2.0 (Rambaut, 2002). Due to ambiguities in the resulting 874 bp alignment, we excluded 45 indel sites from this data set. Thus, the final alignment length of our 12S rRNA data set was 829 bp.

Phylogenetic analyses

All phylogenetic analyses were carried out with the computer software program package PAUP*4.0 (Swofford, 2001) and visualized with Tree View 1.6 (Page, 1996). For each data set, phylogenetic trees were constructed using neighbor-joining (NJ; Saitou and Nei, 1987), maximum parsimony (MP; Swofford and Olsen, 1990) and maximum likelihood (ML; Felsenstein, 1981) algorithms. MP trees were obtained by heuristic searches with 100 random addition replicates utilizing the Tree-Bisection-Reconnection (TBR) swapping algorithm. ML trees were determined using a heuristic search with the TBR swapping algorithm. The nucleotide substitution model for ML criteria was selected by the Akaike Information Criterion (AIC; Akaike, 1974) using Modeltest 3.06 (Posada and Crandall, 1998). NJ trees were constructed with the Kimura-2-parameter model (Kimura, 1980) and repeated with ML parameters estimated from the ML analyses (NJ-ML). The statistical confidence of branching patterns was evaluated by the bootstrap test (Felsenstein, 1985). Prior to constructing phylogenetic trees for the ten species of Eurasian talpids from the concatenated cyt *b*, 12S and RAG-1 data sets, we conducted a partition homogeneity test (Farris *et al.,* 1995) to determine if nucleotide data for each gene were representative of the same underlying phylogeny using PAUP*4.0. When constructing cyt *b* and 12S rRNA gene trees for these ten species,

we employed *Sorex unguiculatus* as an outgroup. We were unable to obtain RAG-1 gene sequences from Eulipotyphlan outgroups, and thus employed *U. gracilis* as an outgroup for both the RAG-1 and concatenated gene trees; *Uropsilus* is invariably found to the basal talpid lineage in phylogenetic analyses (Hutchison, 1976; Yates and Moore 1990; Whidden, 2000; Shinohara *et al*., 2003; this study). To test the conflicting hypotheses relating to the phylogenetic position of *M. tokudae* within the Japanese mole clade (Tsuchiya *et al*., 2000; Kawada *et al*., 2001), we employed the Kishino-Hasegawa test (K-H test; Kishino and Hasegawa, 1989) with RELL distribution settings on all alterative phylogenetic trees constructed from individual and concatenated gene data sets with our ML criteria. Finally, to assess talpid phylogeny from the large (19 species) cyt *b* data set, we constructed a strict consensus tree from our NJ, MP, ML and NJ-ML analyses. For this topology, the long-clawed shrew (*Sorex unguiculatus*), Japanese white-toothed shrew (*Crocidura dsinezumi*) and musk shrew (*Suncus murinus*) were used as outgroups.

RESULTS

Large data set analyses

A consensus phylogeny was generated for 19 species (13 genera) of talpids and three outgroup taxa (Fig. 1) using sequences of the complete mitochondrial cyt *b* gene (1140 bp). All analyses (NJ, MP, ML and NJ-ML) supported a basal position for Chinese shrew-like moles (*U. gracilis*) within the Talpidae, with bootstrap values ranging from 40 to 69%. The other 12 genera were clustered into three groups. Group A included Eurasian fossorial and semi-fossorial talpids, European desmans and North American shrew moles. Group B consisted of the three genera of fossorial North American moles, with the semi-aquatic/semi-fossorial North American star-nosed mole flanking these two groups. However, bootstrap scores supporting the monophyly of each node were not high, especially for group A (18–58%). The four genera of shrew moles (*U. talpoides*, *D. pilirostris*, *N. gibbsii* and *S. fusicaudus*) were consistently placed into the Eurasian clade (Fig. 1). However, we found little if any evidence supporting the monophyly of shrew moles, with only the NJ analysis recovering this clade (14% bootstrap support). The semi-aquatic Russian desman formed a monophyletic clade with the fossorial Eurasian moles, with bootstrap support for this association ranging from 35 to 62% (Fig. 1).

Small data set analyses

No significant differences were found between the cyt *b* (1140 bp), 12S rRNA (829 bp) and RAG-1 (1010 bp) data sets in the partition homogeneity test (p-value=0.43; cyt *b* vs. 12S rRNA vs. RAG-1), allowing us to use the single and concatenated (2979 bp) data sets for phylogenetic inference. Sixteen phylogenetic trees incorporating ten Eurasian talpid species were constructed from these four data sets using NJ, MP, ML and NJ-ML methods (details for each of these topologies are shown in Appendix). Maximum likelihood trees were obtained from cyt *b* (Fig. 2A), 12S rRNA (Fig. 2B), RAG-1 (Fig. 2C) and a concatenation of these sequences (Fig. 2D). In contrast to the relatively low boot-

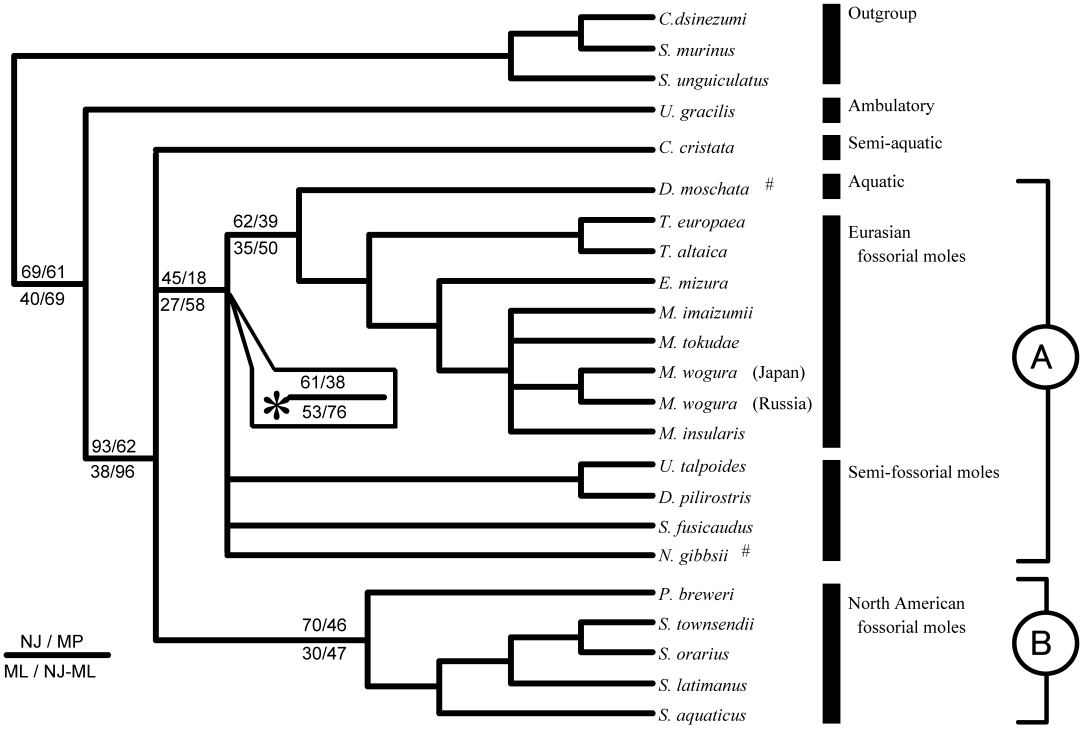

Fig. 1. Strict consensus tree of NJ, MP, ML and NJ-ML analyses using mitochondrial cytochrome *b* gene sequences (1140 bp). Bootstrap scores (percentages of 1000 replications in NJ, MP, NJ-ML analyses and of 100 replications in the ML analysis) are presented for each node. Numbers associated with the asterisk denote bootstrap support values for group A when both *Desmana* and *Neurotrichus* were excluded from the analyses.

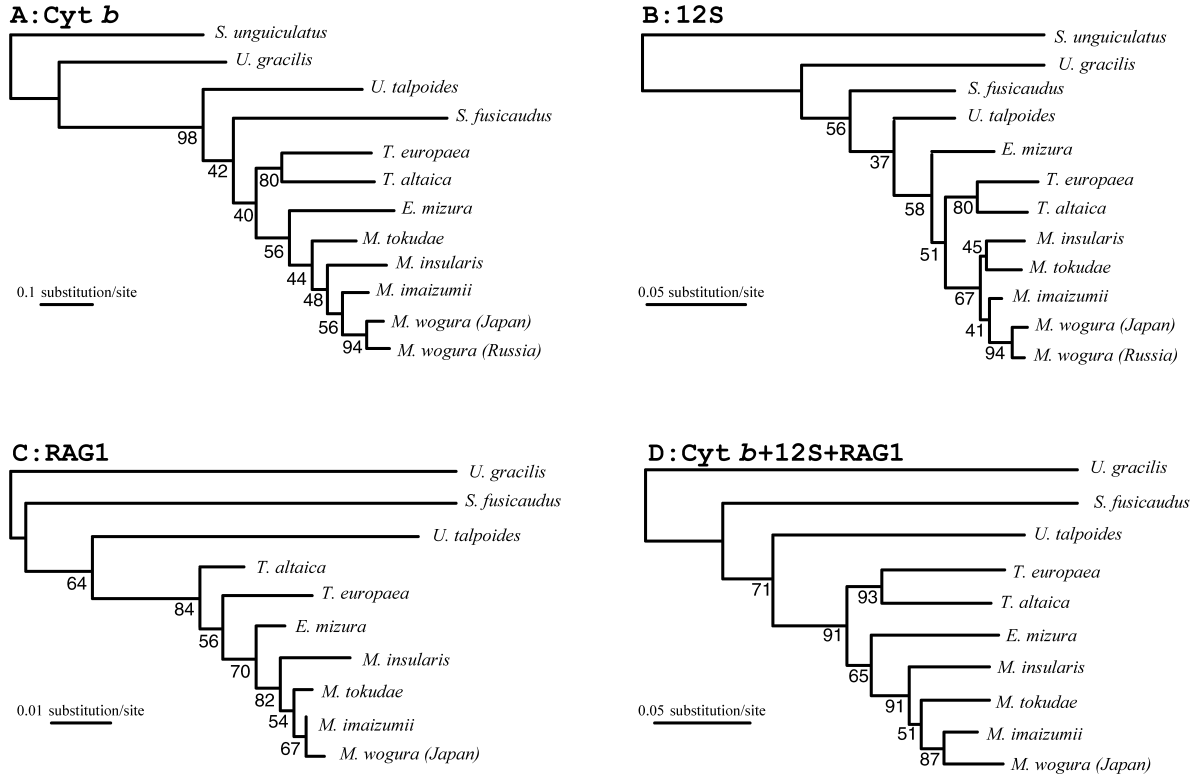

Fig. 2. Maximum likelihood trees for ten East Asian talpid species based on variation in (A) cyt *b*, (B) 12S rRNA, (C) RAG-1, and (D) the concatenation of these gene sequences. Bootstrap values, expressed as a percentage of 100 replications, are given at each node.

strap scores uniting the nodes of our mono-gene trees (Figs. 2A–C), the concatenated ML tree typically exhibited higher support values (Fig. 2D). All topologies tended to exhibit the same general features, and suggested shrew moles diverged prior to the fossorial mole radiation. However, minor differences were found among the 16 trees. For instance, the ML cyt *b* tree (Fig. 2A) indicated Japanese shrew moles (*U. talpoides*) diverged from the main talpid line before *S. fusicaudus*, whereas the other ML trees (Figs. 2B-D) suggested the opposite. Additionally, our cyt *b*, RAG-1 and concatenated data sets placed *E. mizura* as a sister group to the *Mogera* clade to the exclusion of *Talpa* with 56 to 65% bootstrap support (Figs. 2A, C and D). However, the 12S rRNA gene tree grouped *Mogera* and *Talpa* together with 58% support (Fig. 2B). A final incongruence was found for the phylogenetic relationships within the genus *Mogera*. Both the RAG-1 and concatenated gene trees tended to indicate a close relationship for *M. wogura*, *M. imaizumii* and *M. tokudae* to the exclusion of *M. insularis* (Fig. 2D, Appendix 1) with 51 to 54% bootstrap support in the ML trees. Alternatively, ML trees constructed with 12S rRNA placed *M. tokudae* and *M. insularis* together in a monophyletic clade with similarly low (45%) support (Fig. 2B, Appendix).

Kishino-Hasegawa test

We executed the K-H test with ML criteria using ten Eurasian talpid species. In general, K-H tests utilizing both single and concatenated data sets supported a *M. wogera* /

Table 2. Kishino-Hasegawa test of three alternative evolutionary hypotheses for the genus *Mogera*.

Topological Constraint	cyt *b*		12S rRNA		RAG-1[#]		cyt *b*+12S rRNA+RAG-1[#]	
	ΔlnL	P	ΔlnL	P	ΔlnL	P	ΔlnL	P
Best tree	**<6050.06197>**		**<2719.71226>**		**<2596.81216>**		**<10558.68369>**	
(((MwoJ,MwoR),Mim),Mto)	**=best**		**=best**		**=best**		**=best**	
(((MwoJ, MwoR), Mto), Mim)	6056.46795	0.335	2722.97095	0.437	2602.86043	0.256	10571.24236	0.083*
((MwoJ, MwoR),(Mim, Mto))	6056.73502	0.303	A) 2723.45378 B) 2723.42853	0.337 0.348	2600.35096	0.581	10572.29701	0.046*

Asterisk denotes a significant difference between the best and alternative tree (P<0.1). For the 12S rRNA analysis, two equivalent tree scores (A and B) were found for one of the topological constraints using the heurestic search algorithm (see text for details). Species abbreviations are Mto (*M. tokudae*), Mim (*M. imaizumii*), MwoJ (*M. wogura*, Japan) and MwoR (*M. wogura*, Russia). [#]The RAG-1 gene sequence for MwoR was not obtained, and hence, omitted from these analyses.

M. imaizumii sister-taxon relationship (Table 2). However, only the concatenated analyses revealed a significant difference ($P<0.1$) between this tree and the other topological constraints. We also conducted the Shimodaira-Hasegawa test (S-H test; Shimodaira and Hasegawa, 1999) with the concatenated gene data set. In this test, the constrained ((*M. wogera* / *M. imaizumii*) / *M. tokudae*) topology was significantly better than the alternative trees at the 5% level (data not shown).

DISCUSSION

Based upon complete mitochondrial cyt *b* gene sequences, our analyses establish a phylogenetic framework for 19 talpid species from Europe, East Asia and North America (Fig. 1). Moreover, we present the results of detailed phylogenetic relationships among ten Eurasian talpids using single (cyt *b*, 12S rRNA and RAG-1) and concatenated gene sequences. Together, these analyses provide valuable clues to the evolution of the various morphological phenotypes found in this intriguing group of mammals and resolve conflicting hypotheses relating to the biogeographical history of the genus *Mogera* in Japan.

While little is known about the ecology of Asiatic shrew-like moles, they maintain a suite of primitive characters (Whidden, 2000), and presumably have undergone little morphological change since their origin. Indeed, these high-alpine insectivores are poorly adapted for digging (Allen, 1938; Hoffmann, 1984), and probably root among the leaf litter in search of prey (Nowak, 1999). Consistent with prevailing views of talpid evolution and systematics (Yates and Moore, 1990; McKenna and Bell, 1997; Whidden, 2000), results of this study consistently placed *Uropsilus* as the first offshoot from the talpid tree (Figs. 1–2, Appendix). In the future, it would be insightful to obtain comparative molecular data from all three recognized species of *Uropsilus* (Hoffmann, 1984) to better evaluate the antiquity and evolution of this basal talpid group.

Chinese (*Scaptonyx*), Japanese (*Urotrichus* and *Dymecodon*) and North American (*Neurotrichus*) shrew moles closely resemble each other in external appearance and habits (Allen, 1938; Reed, 1951). These diminutive animals commonly excavate shallow tunnel systems in the leaf mold of soft loamy soils and appear to occupy an ecological niche between those of the shrew-like and fossorial talpids. Although differences exist in the dental formulas of extant and extinct species (Ziegler, 1971; Hutchison, 1974), they share several osteological and myological synapomorpies (Campbell, 1939; Whidden, 2000). Despite the clear similitude of extant shrew moles, results of our cyt *b* analyses did not support the monophyly of this group (Fig. 1). In fact, only the NJ tree (topology not shown) recovered a monophyletic relationship for this group, and that with very low support (14%). In the smaller 10 species analysis, for which *Urotrichus* and *Scaptonyx* were the only shrew moles included, eight of 16 trees (primarily 12S rRNA and RAG-1) supported a paraphyletic relationship for these species (Fig. 2 and Appendix). Taken together, these findings suggest that semi-fossorial habits may have arisen in parallel several times early in the evolution of the Talpidae. If so, the morphological characters of each of these lineages has changed remarkable little since their divergence from the main talpid line. This notion is consistent with the recovery of fossilized shrew mole specimens ascribed to the genera *Urotrichus* and *Scaptonyx*, respectively, from the late Oligocene (25–35 million years ago; Hutchison, 1974; McKenna and Bell, 1997). However, it should be stressed that the support for each node of semi-fossorial moles in our topologies was low (14–45% in NJ, 9–23% in MP, 15–27% in ML, 40–59% in NJ-ML; trees not shown). Thus, we currently are unable to refute the hypothesis that shrew moles represent a monophyletic grouping.

Interestingly, our analysis placed shrew moles, Eurasian fossorial moles and the Russian desman into a monophyletic clade (group A of Fig. 1). However, this finding should be viewed with caution as slightly higher bootstrap values (38–76%) were obtained for this clade if both *N. gibbsii* and *D. moschata* were excluded from the analysis (tree not shown). This caution notwithstanding, our cyt *b* data clearly support the independent origin of fossorial North American moles (group B of Fig. 1) from their subterranean Eurasian counterparts. In addition, cyt *b* gene trees constructed using four different criteria placed *Desmana* as a sister group to the Eurasian fossorial mole clade with marginal (35–62%) support (Fig. 1). While we noted this close affinity in an earlier study based upon cyt *b* sequences (Shinohara *et al*., 2003), other studies have placed desmans in a more basal position within the Talpidae (Hutchison, 1976; Whidden, 2000; but see Yates and Moore, 1990). One previously accepted piece of evidence supporting this putatively basal position was based upon the “primitive” dental formula of desmans: D. moschata had been thought to possess the fundamental dental formula of proto-eutherians (I 3/3, C1/1, P4/4, M3/3) and not that of the derived type common to other talpids (Hutchison, 1976). However, recent work based upon the pattern of tooth eruption in *D. moschata* suggests that the upper jaw dental formula of this species corresponds closely to the derived formula (I2, C1, P5, M3) of other talpids (Kawada *et al*., 2002). Additional data is clearly needed to support or refute the early independent radiation of desmans from the main talpid line.

Eurasian fossorial moles are poorly differentiated morphologically from one another, and as such, both *Euroscaptor* and *Mogera* have traditionally been considered to be subgenera or synonyms of *Talpa* (Nowak, 1999). Recently, however, Yates and Moore (1990) and Hutterer (1993) recognized the three groups as being generically distinct. Our results support this separation, with sequence variability suggesting these groups diverged in the distant past. All topologies except those based on the variable 12S rRNA gene (Fig. 2B, Appendix), placed *Euroscaptor* and *Mogera* as sister taxa to the exclusion of *Talpa*. Within the Far-East

Asian clade, small-sized Japanese mountain moles (*Euroscaptor mizura*) are distributed throughout the mountainous regions of central and southern Honshu, and are thought to be the oldest fossorial lineage presently in Japan (e.g. Abe, 1967; Abe *et al*., 1991; Tsuchiya *et al*., 2000). Consistent with this interpretation, both nuclear (Fig. 2C) and mitochondrial sequences (Figs. 1, 2A–B, Okamoto, 1999; Tsuchiya *et al*., 2000, Shinohara *et al*., 2003), place *Euroscaptor* as the most basal species of fossorial mole in Japan. Because the genus *Euroscaptor* (e.g. *E. klossi*, *E. micrura* and *E. longirostris*) has a spotty distribution in peripheral locations of East Asia, including the Japanese Islands, it is reasonable to conclude that they are relict species, as is probably also the case for the Japanese shrew moles. Phylogenetic relationships among the members of the genus *Euroscaptor,* however, are poorly understood, and consequently the taxonomic status of this group is still under debate. To elucidate the origin and radiation of Far-East Asian fossorial moles, future molecular studies incorporating additional species of *Euroscaptor* are required.

Our analyses included four species of *Mogera* from the continental mainland, Taiwan and the Japanese Islands. A close relationship of *M. imaizumii* (Japanese eastern mole) with *M. wogura* (Japanese western mole), to the exclusion of *M. tokudae* (endemic to Sado Island), was evident in topologies constructed from single and concatenated gene data sets (Fig. 2). Statistical support for this relationship was also found from both the Shimodaira-Hasegawa (data not shown) and Kishino-Hasegawa tests (Table 2). This relationship supports the suggestion that the ancestors of *M. tokudae*, *M. imaizumii*, and *M. wogura*, which exhibit a north to south distribution in Japan, respectively, evolved on the continent and entered Japan from the Korean Peninsula (where today only *M. wogura* is found) in this order during separate Pleistocene glacial periods (Tsuchiya *et al*., 2000). This finding is in contrast to the phylogenetic hypothesis of Kawada *et al*. (2001) that suggested a parapatric divergence of *M. tokudae* from *M. imaizumii* in northern Japan. Judging from our molecular evidence, the pericentric inversion of chromosome No. 11 found in *M. imaizumii* and *M. tokudae* but not seen in *M. wogura* or other outgroup taxa (Kawada *et al*., 2001), should be considered to be an example of homoplasy (karyotypic orthoselection) as speculated by Kawada (2002). This, in turn, suggests that inversions near the terminal portions of chromosomes may have occurred in high incidence during the course of talpid evolution, as is found in humans (see Mefford and Trask, 2002 for review).

Finally, our data imply that the Japanese Islands may have played a key role in preserving several East Asian talpid lineages following their emigration from the continental mainland. Indeed, an emerging concept from this and earlier studies (Tsuchiya *et al*., 2000), is the notion that the central domain of the Asian continent fostered the evolution of novel talpid phenotypes and was the cradle of multiple dispersion events. Conversely, peripheral parts of the continent, especially the nearby island chains, helped to preserve these distinct mole lineages over time. A similar procession has been documented in other small mammal groups from continental to insular regions of East Asia (Suzuki *et al*., 1997, 1999; Iwasa *et al*., 2000; Serizawa *et al*., 2000; Iwasa and Suzuki 2002; Yamada *et al*., 2002). Consequently, the Japanese Islands, home to six talpid species, can best be regarded as a species refugia, preserving important biological materials integral to tracing the biogeographical and evolutionary history of the family Talpidae.

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Appendix: NJ, MP, ML and NJ-ML phylogenetic trees for 10 Eurasian talpid species based on variation in cyt *b*, 12S rRNA, RAG-1 gene sequences.

Gene marker	Criteria	Substitution model	Topology
cyt *b*	NJ	K2P (Tv)	(Sorex,(Ugr,((((Teu,Tal),(Emi,**((Min,(Mim,(MwoJ,MwoR))**),Mto))),Uta),Sfu)))
	MP	Tv	(Sorex,(Ugr,(((Teu,Tal),**(**Emi,**Min,Mto,Mim,(MwoJ,MwoR))**),Sfu,Uta)))
	ML	GTR+I+G	**=Fig. 2A**
	NJ-ML	ML parameters	(Sorex,(Ugr,((((Teu,Tal),(Emi,**(Min,(Mto,(HSMim,(MwoJ,MwoR))**))),Uta),Sfu)))
12S	NJ	K2P	(Sorex,(Ugr,((((Teu,Tal),**((Min,Mto),(Mim,(MwoJ,MwoR)))**),Emi),(Sfu,Uta))))
	MP	Ti+Tv	(Sorex,(Ugr,((((Teu,Tal),**((Min,Mto),(Mim,(MwoJ,MwoR)))**),Emi),(Sfu,Uta))))
	ML	GTR+I+G	**=Fig. 2B**
	NJ-ML	ML parameters	(Sorex,(Ugr,((((Teu,Tal),**((Min,Mto),(Mim,(MwoJ,MwoR)))**),Emi),(Sfu,Uta))))
RAG-1	NJ	K2P	(Ugr,(((Teu,Tal),(Emi,**(Min,(Mto,(Mim,MwoJ))**))),(Sfu,Uta)))
	MP	Ti+Tv	(Ugr,((Teu,Tal,(Emi,**(Min,(Mto,(Mim,MwoJ))**))),(Sfu,Uta)))
	ML	GTR+I+G	**=Fig. 2C**
	NJ-ML	ML parameters	(Ugr,(((Teu,Tal),(Emi,**(Min,(Mto,(Mim,MwoJ))**))),(Sfu,Uta)))
cyt *b*+12S+RAG-1	NJ	K2P	(Ugr,(((Teu,Tal),(Emi,**(Min,(Mto,(Mim,MwoJ))**))),(Sfu,Uta)))
	MP	cyt b:Tv, 12S+RAG-1:Ti+Tv	(Ugr,((((Teu,Tal),(Emi,**(Min,Mto,(Mim,MwoJ))**)),Uta),Sfu))
	ML	GTR+I+G	**=Fig. 2D**
	NJ-ML	ML parameters	(Ugr,((((Teu,Tal),(Emi,**(Min,(Mto,(Mim,MwoJ))**))),Uta),Sfu))

Abbreviations are NJ (neighbor joining), MP (maximum parsimony), ML (maximum likelihood), NJ-ML (neighbor joining with maximum likelihood parameters), cyt *b* (cytochrome *b*), 12S (12S rRNA), RAG-1 (recombination activating gene-1), K2P (Kimura 2 parameter: Kimura, 1980), GTR (general time reversal: Rodriguez *et al*., 1990), Tv (transversions only) and Ti (transitions only). Species abbreviations are Sorex (*Sorex unguiculatus*), Ugr (*U. gracilis*), Teu (*T. europaea*), Tal (*T. altaica*), Emi (*E. mizura*), Mto (*M. tokudae*), Mim (*M. imaizumii*), MwoJ (*M. wogura*, Japan), MwoR (*M. wogura*, Russia), Uta (*U. talpoides*) and Sfu (*S. fucicaudus*).