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An evolutionary view on the Japanese talpids based on nucleotide sequences

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Abstract. Japanese talpid moles exhibit a remarkable degree of species richness and geographic complexity, and as such, have attracted much research interest by morphologists, cytogeneticists, and molecular phylogeneticists. However, a consensus hypothesis pertaining to the evolutionary history and biogeography of this group remains elusive. Recent phylogenetic studies utilizing nucleotide sequences have provided reasonably consistent branching patterns for Japanese talpids, but have generally suffered from a lack of closely related South-East Asian species for sound biogeographic interpretations. As an initial step in achieving this goal, we constructed phylogenetic trees using publicly accessible mitochondrial and nuclear sequences from seven Japanese taxa, and those of related insular and continental species for which nucleotide data is available. The resultant trees support the view that four lineages (*Euroscaptor mizura*, *Mogera tokuade* species group [*M. tokudae* and *M. etigo*], *M. imaizumii*, and *M. wogura*) migrated separately, and in this order, from the continental Asian mainland to Japan. The close relationship of *M. tokudae* and *M. etigo* suggests these lineages diverged recently through a vicariant event between Sado Island and Echigo plain. The origin of the two endemic lineages of Japanese shrew-moles, *Urotrichus talpoides* and *Dymecodon pilirostris*, remains ambiguous. Further analyses on intra-species diversity are necessary to fully solve the evolutionary histories of Japanese moles and shrew-moles.

Key words: molecular phylogeny, talpidae, cytochrome *b*, 12S rRNA, RAG1.

The family Talpidae consists of more than 40 species (Hutterer 1993), and is widely distributed across the temperate regions of Eurasia and North America. In East Asia, a notably high level of species richness and diversity is evident, with the nearly 20 species recognized (Hutterer 1993; Nowak 1999) exploiting three distinct ecological niches: high-alpine terrestrial (shrew-like moles), semi-fossorial (shrew-moles) and strictly-fossorial (true moles). In particular, the Japanese islands, where we would like to focus here, possess an outstanding degree of species richness. In fact, of the eight Japanese taxa, which include two shrew-moles and six true moles, at least seven are endemic (Abe 2005). Each of these species has specific distribution ranges, though some affinities among species are evident. For instance, the lesser Japanese shrew-mole (*Dymecodon pilirostris*) and Japanese mountain mole (*Euroscaptor mizura*) are largely restricted to high-mountain regions and exhibit fragmented distribution patterns on Honshu, Shikoku, and Kyushu. Conversely, the greater Japanese shrew-mole (*Urotrichus talpoides*) is widely distributed in the lowlands and peripheral islands of Japan. This species possesses two chromosomal races identified by a single pericentric inversion (e.g. Kawada and Obara 1999; Harada et al. 2001), which are geographically separated by the two rivers of Kurobe and Fuji in central Honshu (Harada et al. 2001). The lesser Japanese mole (*Mogera imaizumii*) and large Japanese mole (*M. wogura*) follow the similar distribution pattern as these two chromosomal races, occurring in eastern and western Japan, respectively. The 'eastern' species, *M. imaizumii*, however, has several relic populations in western Japan, including the Kii Peninsula (e.g. Abe 2005). The remaining true moles (Sado mole, *M. tokudae*, Etigo mole, *M. etigo*, and

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Senkaku mole, *M. uchidai*) have restricted ranges in Sado Island, Echigo plain and Senkaku Island, respectively (Abe 2005). These species exhibit limited distribution ranges and are cited in the Japanese Mammalian Red List as endangered (Ministry of the Environment, Government of Japan 2002).

The evolutionary histories of the Japanese talpids, with their outstanding species richness and geographic complexities, have attracted much research by morphologists, cytogenetists, and molecular biologists, leading to numerous phylogenetic hypotheses for this group. However, unqualified consensus among the practitioners of these disciplines is lacking. In this review, we have tried to provide a comprehensive overview on phylogeny and biogeography on the Japanese talpids, taking into account phylogenetic data of isozymes, karyotypes, restriction fragment length polymorphisms (RFLP), and nucleotide sequences of mitochondrial and nuclear DNA. We also present several novel phylogenetic trees constructed from the publicly accessible mitochondrial and nuclear sequences of seven Japanese taxa together with those of related insular and continental species for which nucleotide data is available.

Evolutionary views based on traditional analyses

A consensus morphology based phylogenetic hypothesis has not been achieved in part because of the taxonomic confusion (Motokawa and Abe 1996) and considerable morphological variety among Japanese species (Abe 1996; Abe 1999). The two Japanese shrew-moles are classified as different genera, based mainly on differences in dental formula; *D. pilirostris* has one more pair of teeth in the lower jaw compared to *U. talpoides*, and is considered the more ancestral species (e.g. Imaizumi 1970; Ziegler 1971). The genus *Euroscaptor* also possesses a 'primitive' dental formula, with one additional pair of teeth in the lower jaw, and as such, is considered distinct at the generic level from the taxon *Mogera* (e.g. Imaizumi 1964; Abe 1988). Phylogenetic relationships among the genus *Mogera* have long been poorly understood, though a morphological study on vertebral formula suggested that *M. tokudae* was the first to branch from the *Mogera* lineage (Abe 1988 based on Yoshiyuki 1986). Beginning the 1990s, genetic based studies utilizing isozymes (Fig. 1A; Tsuchiya 1992), restriction fragment length polymorphisms (RFLP) of mitochondrial DNA (Fig. 1B; Tsuchiya 1992), the nuclear ribosomal RNA genes (rDNA) (Fig. 1C; Tsuchiya 1992), and chromosome-banding patterns (Fig. 1D; Kawada et al. 2001) were used to assess the evolutionary relationships among Japanese talpids. These phylogenetic hypotheses consistently reveal a close affinity between *M. etigo* and *M. tokudae*, but exhibit markedly different branching patterns for the other species. For instance, the mtDNA-RFLP and karyotype data (Fig. 1B and D) support a basal position for *M. wogura*, and suggest a close association between *M. imaizumii* and the *etigo*/*tokudae* group. Conversely, isozyme data favor a basal position of the *etigo*/*tokudae* group (Fig. 1A), while the rDNA-RFLP analyses group *M. etigo/tokudae* into a clade with *M. wogura* and separate from *M. imaizumii* (Fig. 1C). The source of incongruence among these studies thus appears to be most attributable to the phylogenetic position of the *M. etigo*/*tokudae* group.

Evolutionary views from DNA sequence data

Early molecular phylogenetic studies on Japanese talpids utilizing mitochondrial cytochrome oxidase 1 (CO1) (Okamoto 1999) and cytochrome *b* (cyt *b*) (Tsuchiya et al. 2000) gene sequences provided an additional framework to assess traditional and genetic based phylogenetic hypotheses among Japanese taxa. Unfortunately, these studies generally lacked closely related South-East Asian species to make sound biogeographic interpretations. Since publication of these molecular based studies, complete mitochondrial genome sequences of *U. talpoides* and *M. wogura* (Nikaido et al. 2003), plus additional sequence data from cyt *b*, 12S rRNA (12S) and nuclear recombination activating gene-1 (RAG1) have become available for Japanese and numerous other Eurasian and North American talpid moles (Shinohara et al. 2003; Shinohara et al. 2004a, 2004b). In order to reevaluate the molecular phylogenetic relationships of Japanese talpids, we utilized these published DNA sequences from the DNA databases DDBJ, EMBL, and GenBank (Fig. 2, see also Appendix 1 for methods), though were unable to use the CO1 data of Okamoto (1999), because of general lack of available sequences in DNA databases. The resultant phylogenetic trees obtained (Fig. 2) are in good accord with those of previous studies (Okamoto 1999; Tsuchiya et al. 2000; Shinohara et al. 2004a).

While little support current exists for a monophyletic relationship among the four genera of shrew moles (*Dymecodon*, *Urotrichus*, *Scaptonyx*, and *Neurotrichus*; Shinohara et al. 2004a), the two Japanese shrew-mole lineages exhibit a close affinity (Shinohara et al. 2003;

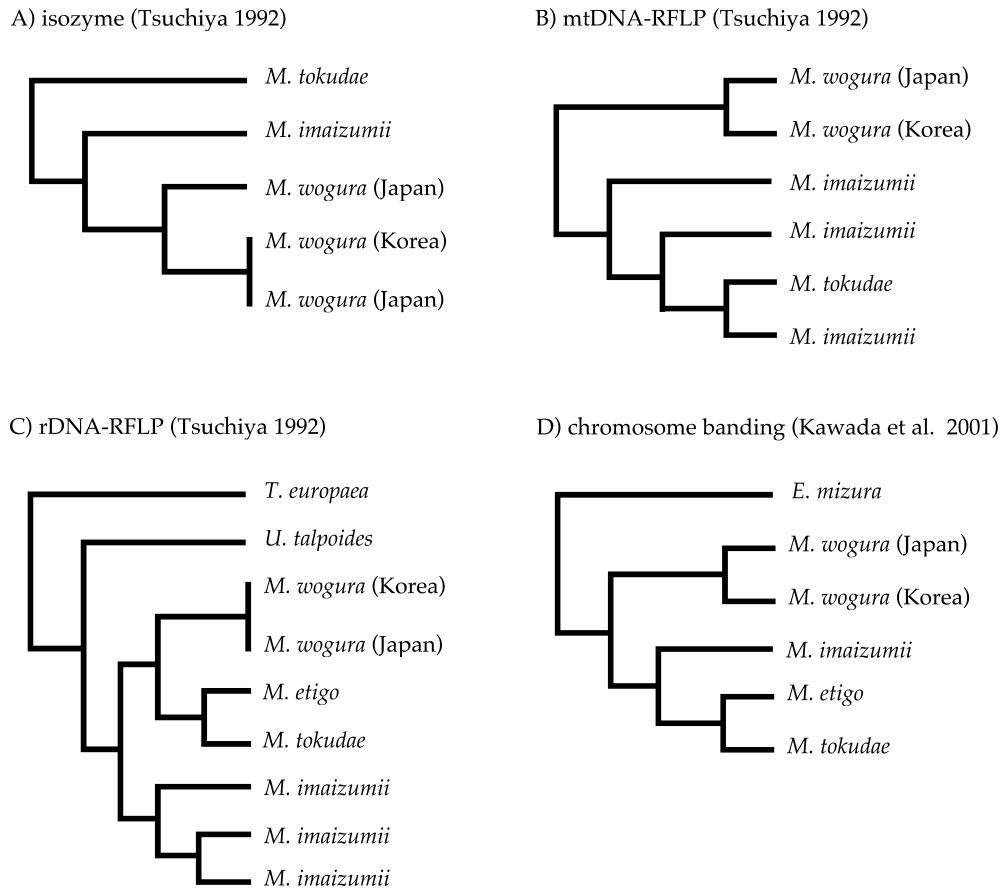

Fig. 1. Phylogenetic relationships among Japanese talpid moles proposed by Tsuchiya (1992) based on isozymes (A), mtDNA-RFLP (B), and rDNA-RFLP (C), and Kawada et al. (2001) based on chromosome banding patterns (D). The scientific names of Tsuchiya (1992) are revised on this figure from the original paper according to the collecting localities.

Fig. 2). However, *D. pilirostris* is believed to retain primitive morphological characters compared to other shrew-moles, but possesses a karyotype similar to that of *U. talpoides* (especially with regard to its western chromosome race; Hamada and Yoshida 1980; Kawada and Obara 1999). Unfortunately, molecular phylogenetic studies have not addressed the significance of this latter finding yet.

Consistent with traditional taxonomic views, the maximum likelihood (ML) nuclear RAG1 gene tree (Fig. 2) supports the monophyly of Malaysian and Japanese *Euroscaptor* with moderate bootstrap support (78%). However, ML trees based on concatenated data sets (cyt *b* + 12S, Fig. 2; and cyt *b* + 12S + RAG1, Shinohara et al. 2004b) suggest *Euroscaptor* is paraphyletic with respect to *Mogera*. Similarly, Motokawa (2004) recently indicated a possible paraphyly of *Euroscaptor* based on his morphological analyses of skulls that included two species of *Euroscaptor* and two additional East Asian mole genera (*Parascaptor* and *Scaptochirus*). Further molecular studies with larger taxon sets and additional sequence data are required to resolve the phylogenetic affinities among members of the genus *Euroscaptor*.

Our ML phylogenetic trees support the monophyly of the genus *Mogera*, and consistently place *M. tokudae* (including *M. etigo*) basal with respect to the monophyletic *M. wogura*/*M. imaizumii* grouping (Fig. 2). This phylogenetic hypothesis is in harmony with isozyme data (Fig. 1A), but is inconsistent with mtDNA-RFLP (Fig. 1B) and rDNA-RFLP tree (Fig. 1C), probably due to lack of resolving power in these latter analyses. The discrepancy with the chromosome banding pattern topology (Fig. 1D) is likely explained by homoplacy of only one pericentric inversion in chromosome No. 11 of *E. mizura* and *M. wogura*, as predicted by Kawada (2002). Indeed, inversions near the terminal portions of chromosomes have been found to occur with high incidence during the course of evolution (see Mefford and Trask 2002 for review in human cases).

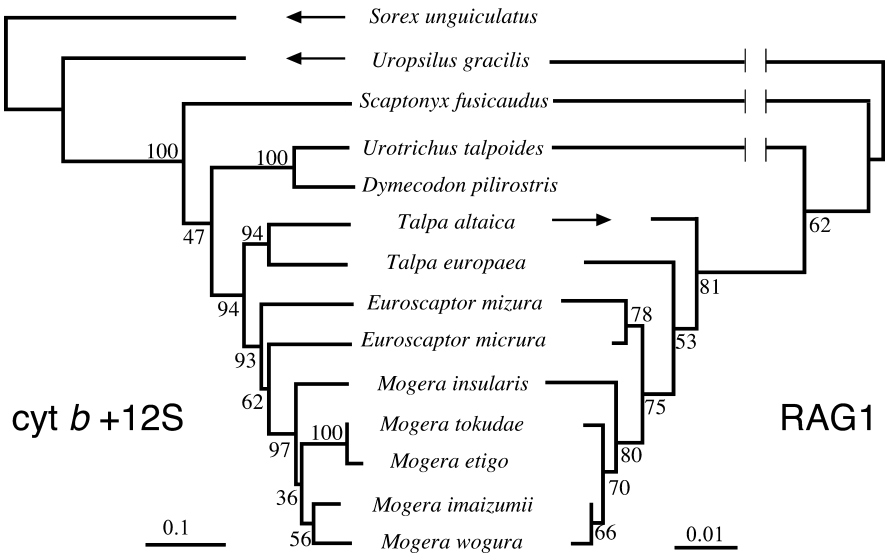

Fig. 2. Phylogenetic hypotheses of Japanese talpid moles inferred from mitochondrial (cyt *b* + 12S; left side) and nuclear (RAG1; right side) gene sequences. For the mitochondrial analysis the complete cyt *b* (1140 bp) and partial 12S (about 900 bp) gene sequences for each species, except *M. etigo* (402 bp of the cyt *b* gene) and *D. pilirostris* (complete cyt *b* gene sequence), were concatenated. Accession numbers and detailed description of our methods of analysis are presented in Appendix 1.

Biogeographic view on the Japanese talpids

Molecular phylogenetic studies presented here and in the literature (Tsuchiya et al. 2000; Shinohara et al. 2004a) imply that intermittent, stepwise migrations of talpid lineages occurred from the Eurasian continent to the Japanese islands. Moreover, Imaizumi et al. (1969) and Imaizumi (1972) forwarded the hypothesis, based on their ecological and biogeographic studies, that *D. pilirostris* was once distributed in the lowlying areas of Japan. According to this scenario, one can speculate that the lineage leading to *D. pilirostris* (with primitive morphological characters) was then largely displaced to isolated highland areas following the migration of *U. talpoides* from the Eurasian continent. On the contrary, taking into account the close relationship between these taxa and their endemism to Japan, we cannot exclude the possibility that lineage differentiation occurred in the Japanese islands by an as yet unknown vicariant event. In the case of true moles, Tsuchiya (1990) was the first to propose that the four current Japanese lineages (*Euroscaptor mizura*, *Mogera tokudae* species group [*M. tokudae* and *M. etigo*], *M. imaizumii*, and *M. wogura*) migrated separately, and in this order, from the continental Asian mainland. Our data provide support for this hypothesis and suggest the Japanese islands have played a key role in preserving species lineages of East Asian talpids that had previously been subjected to phenotypic changes including dental morphology and body size (Tsuchiya et al. 2000; Shinohara et al. 2004a). In this context, it is interesting to note that the more ancestral *Mogera* lineages appear to inhabit islands and coastal regions. To better understand mole evolution and diversification, therefore, future molecular phylogenetic studies need to include additional species from peripheral insular domains, i.e., Taiwan (*M. insularis*), Senkaku (*M. uchidai*) and Hainan (*M. hainana*) islands. Then, overall, the evolutionary features of the Japanese talpids can be characterized not only by subsequent migration events from the continent to Japan but also by vicariant events probably due to ancient occurrence and topographic complexities of the Japanese islands. In this respect, we also need to pay more attention to the problems of intraspecies diversification. For example, *U. talpoides* has a high level of intraspecific genetic diversity between the two chromosomal races, the extent of which is comparable to that between the two common Japanese moles, *M. wogura* and *M. imaizumii* (Shinohara et al. unpublished). In addition, it has been shown that the two widely distributed moles *M. imaizumii* and *M. wogura* have substantial extents of sequence divergence among local populations (Okamoto 1999; Tsuchiya et al. 2000). Within this context, *D. pilirostris* and *E. mizura* should be expected to possess a large degree of genetic diversity among their remotely isolated populations. Acquisition of additional detailed information from these

(and other) species should contribute to assessing the role of ancient climate change and other factors played in shaping the phylogeny and population genetic structures of Japanese moles.

In conclusion, molecular phylogenetic analyses using DNA sequences appear to have been successful in obtaining a reliable understanding of the interrelationships among Japanese moles. These molecular results show well affinity with morphological and isozyme data and new insights for chromosome rearrangements of the Japanese moles. While development of molecular phylogenies utilizing phylogenomics (see Delsuc et al. 2005 for review) may further clarify our understanding the evolutionary history of moles and shrew-moles, many issues still remain unresolved. Indeed, further integrated studies using morphological, cytogenetic, and DNA analyses are needed to reorganize the confused taxonomy in South-East Asian moles. Specifically, the genus *Euroscaptor* will be a key taxonomic group to understanding the connection between ancestral and derived moles species. In addition, comparative phylogenetic community ecological analyses (see Webb et al. 2002 for review) of continental subterranean mammals occupying similar niches as talpids, such as mole-shrews of the genus *Anourosorex*, may provide interesting insights to the past niche competition that lead to the exploitation of semi- and strictly-fossorial life in this group. Together, these phylogenetic and biogeographical approaches will most likely contribute to necessary taxonomic rearrangements, and provide a further understanding of the evolution, diversity, and species richness of Japanese moles.

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Appendix 1.

Detail of the analyses and accession numbers used for phylogenetic trees.

The scientific names used were according the original papers, except that the mole inhabiting Echigo plain was renamed in this study as *M. etigo* (AB037608; Tsuchiya et al. 2000) based on Abe (2005). The sequence length of the 12S rRNA gene varied among species, hence, they were first aligned together using ClustalX (Thompson et al. 1997) with default options, then manually adjusted by eye. Phylogenetic trees were constructed with maximum likelihood (ML; Felsenstein 1981) algorithms using the computer program PAUP* (Swofford 2001) and visualized by Tree View 1.6 (Page 1996). The best trees were searched by the heuristic method utilizing the Tree-Bisection-Reconnection (TBR) swapping algorithm. With consideration to both invariable sites (+I) and rate variation among sites (+G), GTR (Tarvaré 1986) and SYM (Zharkikh 1994) nucleotide substitution models were selected by Modeltest 3.7 (Posada and Crandall 1998) for mitochondrial and nuclear analyses, respectively. The statistical confidence of branching patterns was evaluated by the bootstrap test (Felsenstein 1985) with 100 replications.

Accession numbers of sequences for phylogenetic analyses used in this study.

Sources of data: 1, Tsuchiya et al. (2000); 2, Mouchaty et al. (2000); 3, Murphy et al. (2001); 4, Nikaido et al. (2001); 5, Shinohara et al. (2003); 6, Shinohara et al. (2004a); 7, Shinohara et al. (2004b). ***M. wogura*** cyt *b:* AB037623 (ref. 1); 12S: AB106237 (ref. 6); RAG1: AB106244 (ref. 6). ***M. imaizumii*** cyt *b*: AB037609 (ref. 1); 12S: AB106236 (ref. 6); RAG1: AB106242 (ref. 6). ***M. tokudae*** cyt *b*: AB037607 (ref. 1); 12S: AB106235 (ref. 5); RAG1: AB106243 (ref. 6). ***M. etigo*** cyt *b* (402 bp): AB037608 (ref. 1). ***E. mizura*** cyt *b*: AB037604 (ref. 1); 12S: AB106233 (ref. 6); RAG1: AB176543 (ref. 6). ***E. micrura*** cyt *b*: AB185152 (ref. 7); 12S: AB185154 (ref. 7); RAG1: AB185156 (ref. 7). ***U. talpoides*** cyt *b*: AB076833 (ref. 5); 12S: AB106239 (ref. 6); RAG1: AB106245 (ref. 6). ***D. pilirostris*** cyt *b*: AB076830 (ref. 5). ***T. altaica*** cyt *b*: AB037602 (ref. 1); 12S: AY012100 (ref. 3); RAG1: AB176542 (ref. 6). ***T. europaea*** cyt *b*: AB037601 (ref. 1); 12S: AY19192 (ref. 2); RAG1: AB106246 (ref. 6). ***U. gracilis*** cyt *b*: AB076700 (ref. 6); 12S: AB106231 (ref. 6); RAG1: AB106240 (ref. 6). ***S. unguiculatus*** cyt *b* and 12S: AB061527 (ref. 4).