

Molecular phylogenetic relationships and intra-species diversities of three *Euroscaptor* spp. (Talpidae: Lipotyphla: Mammalia) from Vietnam

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Abstract. Until 2008, three species of fossorial moles (tribe Talpini, Talpidae, Lipotyphla, Mammalia) were recognised in Vietnam: *Euroscaptor longirostris* from the northern highlands, *E. parvidens* from the southern Annamese mountains and *Mogera latouchei* from the northern lowlands. Recently, a new species (*Euroscaptor subanura*) was described from northern Vietnam. This new mole is externally similar to *E. parvidens*, but the skull and dental characters are rather similar to *E. longirostris*. In this study, we determined the complete mitochondrial cytochrome *b* (*Cytb*; 1140 bp), partial mitochondrial 12S ribosomal RNA (12S; ca. 850 bp) and partial nuclear recombination activating gene 1 (*Rag1*; 1010 bp) gene sequences of 19 specimens of *E. subanura* collected from three localities in northern Vietnam and estimated the phylogenetic relationships among the Southeast Asian moles. Our results strongly support the full species status of *E. subanura* and that it is a sister taxon to *E. parvidens* of central Vietnam, but not *E. longirostris* of northern Vietnam. Although we analysed samples from only three localities, intraspecific diversity in *E. subanura* was lower than in the other two species. These results suggest that *E. subanura* diverged from *E. parvidens* and that it is a relict species that survived past climatic changes.

Key words. *Euroscaptor*, Talpidae, *Mogera*, Molecular phylogeny, Vietnam

INTRODUCTION

Southeast Asia is a major hotspot of biodiversity, and has the highest relative rate of mammalian biodiversity (Sodhi et al., 2004; Ceballos & Ehrlich, 2006). Vietnam is located on the eastern margin of the Indo-Burma hotspot, and includes many islands and high mountainous regions. Partly due to these geographic features, the fauna of Vietnam is highly diverse (Can et al., 2008). Notably, many new mammalian species from this region were described in the twentieth century, such as the saola antelope (*Pseudoryx nghetinhensis*: Dung et al., 1993) and the giant muntjac (*Megamuntiacus vuquangensis*: Schaller & Vrba, 1996). Even in the twenty-first century, many new species have been found. These include mainly small mammals, such as shrews (*Chodsigoa*

caovansunga: Lunde et al., 2003; *Crocidura kegoensis*: Lunde et al., 2004; *Crocidura phanluongi*: Jenkins et al., 2010), bats (*Hipposideros griffini*: Thong et al., 2012) and rats (*Pseudoberylmys muongbangensis*: Tran et al., 2009). These discoveries are indicative of the high mammalian species richness in Vietnam and suggest the potential for further discoveries of new mammal species from Vietnam.

In the latest checklist (Can et al., 2008), three species of talpine mole, belonging to the tribe Talpini (Talpidae, Lipotyphla, Mammalia), are recognised in Vietnam: the long-nosed mole *Euroscaptor longirostris* from the northern highlands, the small-toothed mole *E. parvidens* from the southern Annamese mountains (central Vietnam) and Latouche's mole *Mogera latouchei* from the northern lowlands (see Kawada et al., 2009). In addition to these three species, at least five additional talpine moles are distributed in adjacent regions of Vietnam: Kloss's mole *E. klossi* in Thailand, the Malaysian mole *E. malayana* in peninsular Malaysia, the greater Chinese mole *E. grandis* from Mt. Emei, China, the Himalayan mole *E. micrura* in the Himalayas and the white-tailed mole *Parascaptor leucura* in north-eastern India to south-western China (Hutterer, 2005; Kawada et al., 2008).

In addition to these moles, Kawada et al. (2012) described a new species of mole (*Euroscaptor subanura*) from northern Vietnam. The holotype of this species was collected from Tam Dao National Park, located about 150 km north of Hanoi city. In this same mountainous district, *Euroscaptor*

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longirostris is also present in the highland forest (about 1000 m above sea level: Kawada et al., 2009), but the new species was collected from the lowland forests (200–300 m above sea level: Kawada et al., 2012). Compared to other species of the genus *Euroscaptor*, *E. subanura* is characterised by small body size, an extremely short tail and a different karyotype (Kawada et al., 2012). Interestingly, the new mole is externally similar to *E. parvidens* from central Vietnam, but the skull and dental characters are similar to those of *E. longirostris* from northern Vietnam. Therefore, the authors commented, “*E. subanura* is characterised by a mosaic of features similar to the external morphology of *E. parvidens* and the skull of *E. longirostris*” (Kawada et al., 2012), though no molecular data were used in their discussion of relationships as no comparable molecular data then existed.

Recently, several molecular phylogenetic studies on the evolutionary relationships of Southeast Asian talpine moles have been published (Zemlemerova et al., 2013; Kai et al., 2014; Shinohara et al., 2014). These studies revealed that the Southeast Asian mole lineage has evolved in long isolation and the species richness of moles in this area has probably been underestimated. However, these studies did not include *E. subanura* in their analyses. Hence, the molecular phylogenetic relationships among the three Vietnamese *Euroscaptor* species (*E. longirostris*, *E. parvidens* and *E. subanura*) remain unresolved. Here, we report the phylogenetic position of *E. subanura* using complete mitochondrial cytochrome *b* (*Cytb*: 1140 bp), partial mitochondrial 12S rRNA (12S: ca. 850 bp) and partial nuclear recombination activating gene 1 (*Rag1*: 1010 bp) gene sequences and discuss the evolutionary history of Vietnamese moles. Since the habitat of *E. subanura* is highly fragmented, it is not unlikely that the population size is decreasing. Therefore, we also examine the intra-species genetic diversity of *E. subanura* in comparison to other Vietnamese moles to exhibit conservation significance, and to estimate their evolutionary histories.

MATERIAL AND METHODS

Laboratory experiments. We sequenced the complete mitochondrial cytochrome *b* (*Cytb*: 1140 bp), partial mitochondrial 12S ribosomal RNA (12S: ca. 850 bp) and partial nuclear recombination activating gene 1 (*Rag1*: 1010 bp) gene sequences from 19 specimens of *E. subanura* collected from three localities in northern Vietnam (Table 1). The holotype and three paratypes of *E. subanura* were collected from the northwestern slope of Tam Dao mountain, Tuyen Quang Province, Vietnam and nine samples were collected from Na Hang, Tuyen Quang Province, Vietnam (Fig. 1, Table 1). These samples were identified by Kawada et al. (2012). The remaining six samples were collected in July 2012 from Xuan Son National Park, Phu Tho Province, located 100 km west of Hanoi city (Fig. 1), and morphologically identified as *E. subanura* by the second author (SK). Genomic DNA from preserved liver was extracted using proteinase-K digestion and phenol-chloroform-isoamyl alcohol extraction procedures, followed by purification by ethanol precipitation. The polymerase

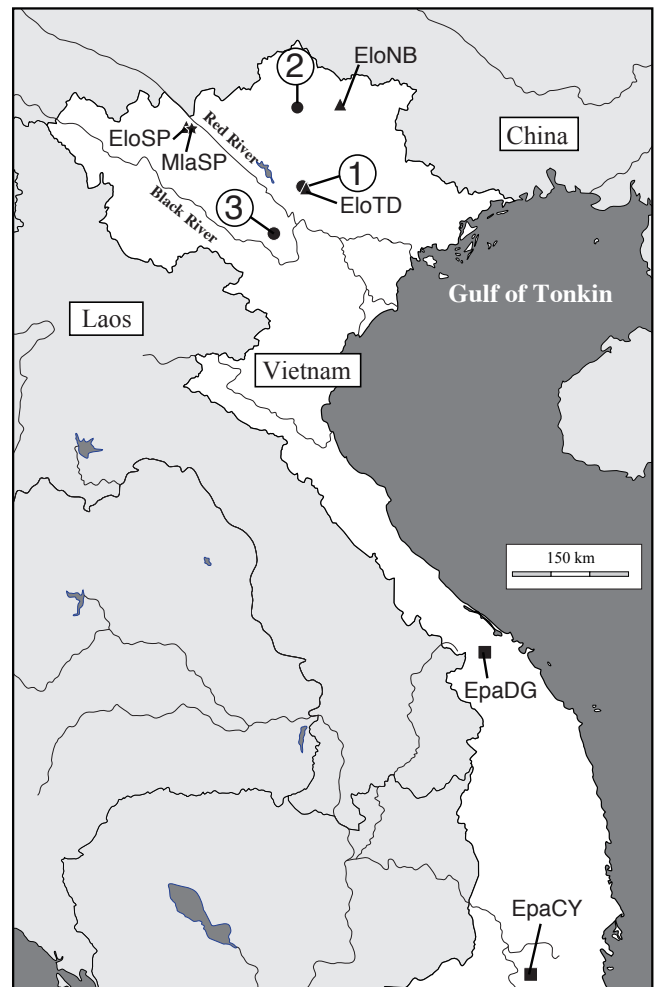


Fig. 1. The three collecting localities of *Euroscaptor subanura* (closed circle): (1) Tam Dao National Park, Tuyen Quang Province (sample codes; EsuTD1-4); (2) Na Hang, Tuyen Quang Province (sample codes; EsuNH1-9); and (3) Xuan Son National Park, Phu Tho Province (sample codes; EsuXS1-6). Additionally, collection localities of other Vietnamese moles (closed triangle: *E. longirostris*, closed square: *E. parvidens* and closed star: *Mogera latouchei*) are indicated. The sample codes correspond to Fig. 2 and Appendix 1. The original map was obtained from the d-maps.com (http://d-maps.com/carte.php?num_car=978&lang=en).

chain reaction (PCR) method for amplification of *Cytb*, 12S and *Rag1* genes followed our previous study (Shinohara et al., 2014). The obtained sequences were deposited in DDBJ under the accession numbers LC013279–LC013335.

Data analysis. To reveal the phylogenetic position of *E. subanura* among the Southeast Asian moles, we collected appropriate additional sequences of moles from DNA databases (DDBJ/GenBank/EMBL). In addition, homologous gene sequences from the large Japanese shrew mole (*Urotrichus talpoides*) and gracile shrew like mole (*Uropsilus gracilis*) were also retrieved from DNA databases for use as the outgroup in the phylogenetic analysis. In total, 69 sequences were collected from DNA databases for phylogenetic analysis (Appendix 1).

We aligned the 12S sequences using MUSCLE (Edgar, 2004) implemented in MEGA v5 (Tamura et al., 2011) with default settings (gap open = –400, gap extend = 0) since

Table 1. *Euroscaptor subanura* samples used in this study.

Collecting Locality	Voucher	Sex	Code	DNA Database Accession Number		
				<i>Cytb</i> (1140bp)	12S (ca. 850 bp)	<i>Rag1</i> (1010 bp)
Tam Dao (Alt. 250m), Tuyen Quang, Vietnam	IEBR-M-1734/SIK0875**	male	EsuTD1	LC013279	LC013298	LC013317
	IEBR-M-1735/SIK0876**	male	EsuTD2	LC013280	LC013299	LC013318
	IEBR-M-1798/SIK0882*	male	EsuTD3	LC013281	LC013300	LC013319
	IEBR-M-1799/SIK0883**	female	EsuTD4	LC013282	LC013301	LC013320
Na Hang, Tuyen Quang, Vietnam	IEBR-M-3250/SIK0913	male	EsuNH1	LC013283	LC013302	LC013321
	IEBR-M-3251/SIK0914	male	EsuNH2	LC013284	LC013303	LC013322
	IEBR-M-3252/SIK0915	female	EsuNH3	LC013285	LC013304	LC013323
	IEBR-M-3253/SIK0916	female	EsuNH4	LC013286	LC013305	LC013324
	IEBR-M-3254/SIK0917	male	EsuNH5	LC013287	LC013306	LC013325
	IEBR-M-3255/SIK0918	male	EsuNH6	LC013288	LC013307	LC013326
	IEBR-M-3256/SIK0919	male	EsuNH7	LC013289	LC013308	LC013327
	IEBR-M-3259/SIK0922	male	EsuNH8	LC013290	LC013309	LC013328
	IEBR-M-3261/SIK0924	male	EsuNH9	LC013291	LC013310	LC013329
Xuan Son NP, Phu Tho, Vietnam	IEBR-M-4107/SIK0930	male	EsuXS1	LC013292	LC013311	LC013330
	IEBR-M-4109/SIK0932	female	EsuXS2	LC013293	LC013312	LC013331
	IEBR-M-4113/SIK0936	female	EsuXS3	LC013294	LC013313	LC013332
	IEBR-M-4114/SIK0937	female	EsuXS4	LC013295	LC013314	LC013333
	SIK0938	female	EsuXS5	LC013296	LC013315	LC013334
	SIK0939	female	EsuXS6	LC013297	LC013316	LC013335

Note: All voucher specimens were deposited in the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. Asterisks indicate holotype (*) and paratype (**) specimens (Kawada et al., 2012). The code is labelled on the map and phylogenetic trees (Figs. 1, 2).

the sequence lengths varied among the samples. The *Cytb* and *Rag1* gene sequences did not include any insertions or deletions. The final alignment lengths of the *Cytb*, 12S and *Rag1* gene sequences were 1140, 865 and 1010 bp, respectively. The phylogenetic trees were estimated using the maximum-likelihood (ML) method and Bayesian inference. The concatenated data set was divided into seven partitions: protein-coding genes (*Cytb* and *Rag1*) were divided into first, second and third positions, and the rRNA gene (12S) was treated as a single partition. The best substitution models for each partition, and partitioning schemes were estimated by PartitionFinder v1.1.1 (Lanfear et al., 2012) under the AIC, and by Kakusan 4 (Tanabe, 2011) under the BIC, respectively, and implemented in the ML and Bayesian phylogenetic analyses (Table 2). The ML analysis was carried out using Garli 2.0 (Zwickl, 2006) with five replicated searches. The statistical confidences of the clades of the ML tree were evaluated with 1000 bootstrap replicates using Garli 2.0. The Bayesian analysis was conducted using MrBayes v3.2.1 (Ronquist et al., 2012) with 10 million generations of two independent runs of four Markov chains, sampling one tree every 100 generations. Sampled parameters were checked for convergence using Tracer version 1.6 (Rambaut et al., 2014). The final average standard deviation of split frequencies (ASDSF) was 0.00546, and the average effective sample size (avg ESS) for all parameters was more than 200. To find a maximum credibility tree and summarise posterior probabilities, we manually combined the tree files of the two runs after discarding the first 25% of trees, and then

used the software TreeAnnotator (part of the BEAST v1.7.5 package) (Drummond et al., 2012).

Even though we collected from three localities, we found that all specimens of *E. subanura* have little genetic diversity among the samples. Therefore, we compared the intra-species genetic diversity of the four Vietnamese moles (*E. subanura*, *E. parvidens*, *E. longirostris* and *M. latouchi*) to reveal their population histories. We included additional *Cytb* gene sequences deposited in the DNA databases (Appendix 2), and calculated haplotype (*h*) and nucleotide (π) diversities (Nei, 1987) with standard deviations (SD), the number of haplotypes, the number of segregation sites and Tajima's *D* (Tajima, 1989) using DnaSP v.5 (Librado & Rozas, 2009).

RESULTS

Phylogenetic position of *Euroscaptor subanura*. The phylogenetic relationships among the East and Southeast Asian moles were identical between the ML and Bayesian inferences. Therefore, we show only the ML tree in Fig. 2, including both the ML bootstrap support values and posterior probabilities.

The phylogenetic relationships among the genera observed in our analyses were for the most part similar to previous studies (Zemlemerova et al., 2013; Kai et al., 2014; Shinohara et al., 2014); *Mogera* is monophyletic, but *Euroscaptor* is not monophyletic (Fig. 2). The genus *Euroscaptor* was divided

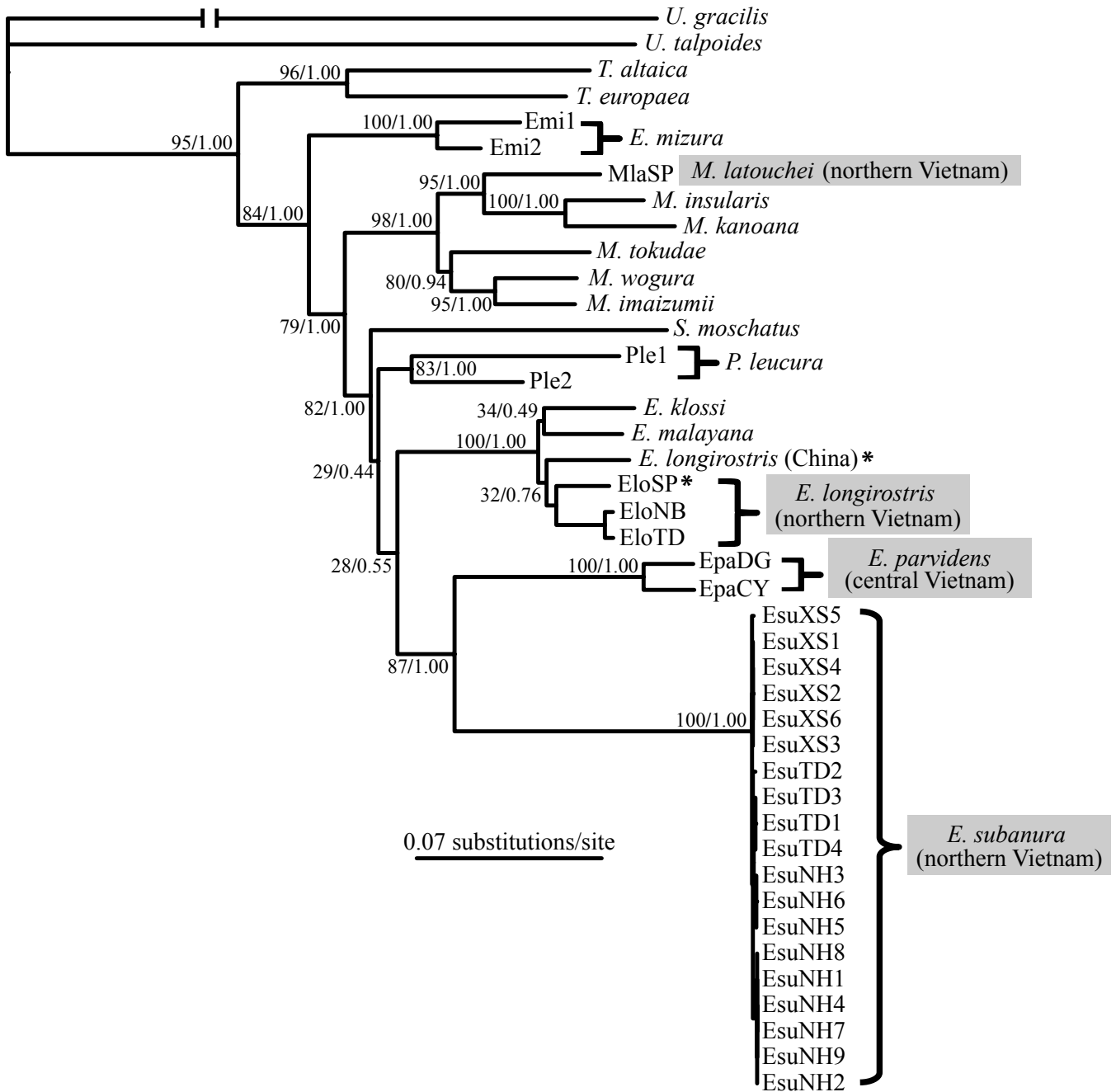


Fig. 2. Molecular phylogenetic tree constructed using the maximum-likelihood (ML) method with the complete mitochondrial cytochrome *b* (*Cytb*: 1140 bp), partial mitochondrial 12S rRNA (12S: ca. 800 bp) and partial nuclear recombination activating gene-1 (*Rag1*: 1010 bp) sequences. Node labels indicate the bootstrap support values of the ML method/posterior probabilities of the Bayesian analysis. Sample codes correspond to Table 1 for *Euroscaptor subanura* and Appendix 1 for the other moles.

Table 2. Substitution models for seven partitions of the dataset in the maximum-likelihood method and Bayesian inference.

Gene	<i>Cytb</i>			12S	<i>Rag1</i>		
	1st	2nd	3rd	-	1st	2nd	3rd
Maximum likelihood	SYM+I+G	TrN+I	HKY+I+G	GTR+I+G	GTR+I	TVM+I	TrNef+G
Bayesian inference	SYM+G	HKY85+I	HKY85+G	GTR+G	JC69+G	F81+G	K80+G

Note: SYM = symmetrical model (Zharkikh, 1994), TrN = Tamura-Nei model (Tamura & Nei, 1993), HKY = Hasegawa-Kishino-Yano model (Hasegawa et al., 1985), GTR = general time reversible model (Tavarê, 1986), TVM = transversion model, TrNef = TrN model with equal base frequencies, JC69 = Jukes and Cantor's one-parameter model (Jukes & Contor, 1969), F81 = Felsenstein 1981 model (Felsenstein, 1981), K80 = Kimura-2-parameter model (Kimura, 1980), +I = proportion of invariable sites, +G = gamma distribution.

Table 3. Intraspecific genetic diversity of mitochondrial cytochrome *b* gene sequences for four Vietnamese mole species.

Species	N1	N2	Diversity Estimated				Tajima's <i>D</i>
			<i>Nh</i>	<i>S</i>	<i>hd</i> ± SD	π ± SD	
<i>E. subanura</i>	19	3	6	12	0.819 ± 0.050	0.004 ± 0.000	0.675
<i>E. longirostris</i>	16	6*	12	153	0.958 ± 0.036	0.045 ± 0.006	0.345
<i>E. parvidens</i>	13	4	7	150	0.910 ± 0.049	0.061 ± 0.004	1.278
<i>M. latouchei</i>	15	4	8	33	0.867 ± 0.067	0.009 ± 0.001	0.016

Note: N1 = number of sequences, N2 = number of collection localities, *Nh* = number of haplotypes, *S* = number of segregating sites, *hd* = haplotype diversity, π = nucleotide diversity.

*One deposited sequence (GenBank ID: EU122226) has no detailed information on collecting locality; therefore, N2 of *E. longirostris* is at least six.

into two groups, one comprising only a species from Japan (*E. mizura*), and the other group with the remaining species (*E. klossi*, *E. malayana*, *E. longirostris*, *E. parvidens*, and *E. subanura*). The latter group clusters together with a clade comprising the short-faced mole (*Scaptochirus moschatus*), which is widely distributed in China and is classified as a monospecific genus based on its specific morphological and chromosomal characters (Allen, 1938; Kawada et al., 2002), and with the white-tailed mole (*Parascaptor leucura*), which is also widely distributed from northeast India to southwest China and is classified as a monospecific genus based on its dental formula (Allen, 1938). The support values for this clade are moderate (1.00 posterior probability and 82% bootstrap support values). Further, this group is potentially divided into four lineages: 1) *S. moschatus*; 2) *P. leucura*; 3) *E. klossi*, *E. malayana* and *E. longirostris*; and 4) *E. parvidens* and *E. subanura*. Although the relationships among the four lineages were unresolved in our analyses, the Southeast Asian *Euroscaptor* spp. were clearly divided into two groups with very high support values (1.00 posterior probability and 87–100% bootstrap support values). One group was very widely distributed in Thailand (*E. klossi*), Malaysia (*E. malayana*) and southwest China to northern Vietnam (*E. longirostris*), whilst the other is distributed in Vietnam from the central (*E. parvidens*) to northern (*E. subanura*) areas. Interestingly, *E. subanura* from northern Vietnam is a sister taxon of *E. parvidens* from central Vietnam, and they do not form a group with *E. longirostris* from northern Vietnam. All of the 19 individuals morphologically identified as *E. subanura* collected from three localities in northern Vietnam were robustly grouped as a monophyletic clade (1.00 posterior probability and 100% bootstrap support values).

Intraspecific diversity of *Euroscaptor subanura*. In our tree (Fig. 2), small genetic differences were observed among the samples of *E. subanura*. Therefore, we compared the intraspecific genetic diversity with the three other Vietnamese moles with additional sampling (Appendix 2). Even though we collected from three localities, we found that the nucleotide diversity of *E. subanura* is extremely low with very few segregating sites, but the haplotype diversity is high (Table 3). Interestingly, these trends are similar to *M. latouchei*, but differ from *E. longirostris* and *E. parvidens*, which show both higher haplotype diversity and much

higher nucleotide diversity (Table 3). These results suggest that the population history is somewhat similar between *E. longirostris* and *E. parvidens* and between *E. subanura* and *M. latouchei*. The neutrality test using Tajima's *D* was positive in all four species, but very low in *M. latouchei* (Table 3).

DISCUSSION

In line with the recent progress on molecular phylogenetic relationships among Southeast Asian moles (Zemlemerova et al., 2013; Kai et al., 2014; Shinohara et al., 2014), our analysis revealed the phylogenetic position of *E. subanura*, which was newly described from northern Vietnam (Kawada et al., 2012), using complete mitochondrial *Cytb* (1140 bp), partial 12S rRNA (ca. 850 bp) and partial nuclear *Rag1* (1010 bp) gene sequences. All of the 19 *E. subanura* samples were robustly grouped as a monophyletic clade, with substantial genetic distances to the other *Euroscaptor* species in our phylogenetic trees, clearly supporting full species status. Of the three species of Vietnamese *Euroscaptor*, *E. subanura* forms a sister group with *E. parvidens*, but not with *E. longirostris*.

The external morphological characters fit well with the molecular results for the *Euroscaptor* moles studied. The recently described species, *E. subanura*, is morphologically characterised by a small and slender body, very large hips and an extremely short tail (Kawada et al., 2012). These external body features are similar to *E. parvidens* from central Vietnam. In particular, the rump structure, which is extended posteriorly with most of the tail vertebrae embedded within the hip, is only observed in these two species. In addition, the fore and hind feet were similar in size to those of *E. parvidens*, and smaller than in *E. longirostris*, *E. malayana* and *E. klossi* (Kawada et al., 2012). However, the skeletal and dental characters of *E. subanura* clearly differ from *E. parvidens*. The skull characters of *E. subanura* are similar to *E. longirostris* based on 15 skull measurements (Kawada et al., 2012). Therefore, this species is morphologically characterised as having a mosaic of features of *E. parvidens* and *E. longirostris*. The external morphological characters fit well with the molecular results, suggests that the external morphological characters possibly reflect the genetic background, although skull characters are emphasised in

talpid taxonomy in general. However, it is also known that body size in moles is geographically variable (Abe, 1967, 1995) and is affected by habitat condition (Abe, 1996). It was demonstrated that habitat conditions also affect the distribution range of moles (Abe, 2001; Moribe & Yokohata, 2011). Furthermore, within the parapatric talpine species, a similar phenomenon to morphological character displacement is reported in Europe (Loy et al., 2005) and Japan (Abe, 1974). These studies suggest that we should carefully observe not only their morphological characters but also habitat conditions and genetic differences. Recently, DNA based species delimitation analyses (e.g., Yang & Rannala, 2010; Zhang et al., 2013) are often used to find putative cryptic species. Using limited genetic markers, however, delimitation analyses sometimes tend to over- or underestimate species numbers, therefore, we advocate an integrated approach with consideration of life history, geographical distribution, genetics and morphology as best practice (see review in Carstens et al., 2013). To reveal cryptic species and more accurately evaluate the biodiversity in Southeast Asia, such a combined approach is vital.

We hypothesise that *E. subanura* or *M. latouchei*, which are found in lower elevations in Vietnam may have arrived more recently compared to species at higher elevations such as *E. longirostris*. Among the four Vietnamese strict fossorial moles, *E. subanura*, *E. longirostris* and *M. latouchei* are distributed in northern Vietnam and *E. parvidens* in central Vietnam. In northern Vietnam, *E. subanura* or *M. latouchei* is sometimes found in the same mountain district as *E. longirostris*. It seems that *E. subanura* and *E. longirostris*, or *M. latouchei* and *E. longirostris* are segregated by elevation (Kawada et al., 2009, 2012). For example, in Tam Dao National Park, *E. subanura* is found in the lowland forests (200–300 m above sea level) and *E. longirostris* is found in the highland forest (1000 m above sea level). In SAPA, Lao Cai Province, *M. latouchei* is found in the lowland forests (1400 m above sea level) and *E. longirostris* is found in the highland forest (2000 m above sea level). We also observe similar habitat segregation on the Japanese islands, one of the most analysed areas with regard to talpid biogeography and phylogeography; *E. mizura* is mainly distributed in high mountainous regions and *M. wogura* and *M. imaizumii* are mainly distributed in lowland areas (Ohdachi et al., 2009), but they sometimes cohabit at the same elevation (see Sagara et al., 1989; Sagara & Fukasawa, 2014). Based on the combination of molecular phylogeny, molecular phylogeography and biogeography, it was demonstrated that *Mogera* migrated into the Japanese islands from the continent via the Korean Strait multiple times (Tsuchiya et al., 2000; Shinohara et al., 2004b, 2005; Kirihara et al., 2013) and it is believed that the newly arrived species dispersed to the east and north with the simultaneous displacement of indigenous species (Tsuchiya et al., 1990). As a result of this history of species competition, the oldest indigenous species, *E. mizura*, survives in fragmented populations in high mountainous areas. Indeed, *E. mizura* shows large intra-species genetic diversity among the isolated populations, consistent with its long isolated history (Shinohara et al., 2014). Based on the history of multiple dispersals of talpids into the Japanese

islands, we also hypothesise a sequential dispersal order in Vietnam: species in lower elevations (*E. subanura* or *M. latouchei*) may have arrived more recently compared to species at higher elevations (*E. longirostris*).

In this study, we also found large haplotype diversities in all four Vietnamese moles, but much smaller nucleotide diversities in species of lower elevation (*E. subanura* or *M. latouchei*) compared to species of higher elevation (*E. longirostris*) (Table 3). Avise (2000) explained that high haplotype diversity with low nucleotide diversity may suggest rapid population growth from an ancestral population with small effective population size, pointing to recent dispersal events in the lower elevation species (*E. subanura* or *M. latouchei*). Moreover, Tajima's *D* in four Vietnamese moles is positive, but very small in *M. latouchei*. According to Tajima (1993), a positive value of Tajima's *D* indicates a recent decrease in population size (bottleneck) or balancing selection. Although we could not completely exclude the possibility of balancing selection, Tajima's *D* may suggest that bottleneck events occurred in *E. subanura*, *E. longirostris* and *E. parvidens*, but not in *M. latouchei*. From these results, we propose the following evolutionary scenario of the moles in Vietnam: One main lineage consisting of the ancestor of *E. longirostris*, *E. malayana* and *E. klossi* must have once been widely distributed in Southeast Asia. A second dispersal event into Vietnam is represented by *E. parvidens* and *E. subanura*. It is possible that the former lineage survived in southern China to northern Vietnam, peninsular Malaysia and Thailand and speciated into *E. longirostris*, *E. malayana* and *E. klossi*, respectively, and that the latter lineage survived in southern Vietnam and speciated into *E. parvidens*. With regard to the occurrence of *E. subanura*, two hypotheses could be considered: one is that a relic population that survived in northern Vietnam speciated into *E. subanura* and the alternative is that a recently migrated population of *E. parvidens* from southern to northern Vietnam speciated into *E. subanura*. The final dispersal into this area is represented by *M. latouchei*, which shares an ancestral lineage with the Taiwanese moles. To further evaluate these hypotheses, historical biogeographic methods, e.g., like dispersal-extinction-cladogenesis model with founder event speciation (Matzke, 2014) and/or dispersal-vicariance analysis (DIVA; Ronquist, 1997) may represent useful approaches. To conduct such analyses, a better knowledge on the distribution of the Vietnamese moles is essential.

Although we have a limited knowledge on their historical biogeography, recent molecular divergence dating analyses gave further insight into the evolution of Asian moles. Kai et al. (2014) and Shinohara et al. (2014) estimated that global climate changes during the late Miocene (Zachos et al., 2001) and ensuing vegetational turnover (Cerling et al., 1997) might have driven their speciation. In the late Miocene, Southeast Asia was connected to Borneo (Hall, 1998) and further reduction of sea levels during the Pleistocene exposed additional land areas (Voris, 2000; Hanebuth et al., 2011), called Sundaland. The Sundaland had huge river systems (Voris, 2000), and the exposed area of Sundaland fluctuated with sea level changes accompanying glaciation

and deglaciation cycles (Voris, 2000; Hanebuth et al., 2011). Such palaeoriver systems and fluctuating landmass area during the Pleistocene may have shaped their distribution (Inger & Voris, 2001). Further comprehensive molecular phylogeographic approaches will facilitate the determination of this scenario.

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Appendix 1. Obtained sequences from DNA database for phylogenetic analyses.

Species	Collecting Locality	Code	DNA Database Accession Number			Reference
			<i>Cytb</i> (1140bp)	12S (ca. 850 bp)	<i>Rag1</i> (1010 bp)	
<i>Euroscaptor longirostris</i>	Tam Dao, Vinh Phuc, Vietnam	EloTD	AB823108	AB823143	AB823180	Shinohara et al. (2014)
	SAPA, Lao Cai, Vietnam	EloSP	AB823111	AB823146	AB823183	Shinohara et al. (2014)
	Nguyen Binh, Cao Bang, Vietnam	EloNB	AB823114	AB823149	AB823186	Shinohara et al. (2014)
	Sichuan, China	–	HG737870	HG737884	HG737914	Kai et al. (2014)
<i>Euroscaptor parvidens</i>	Chu Yang Sin NP, Dak Lak, Vietnam	EpaCY	AB823117	AB823152	AB823189	Shinohara et al. (2014)
	Dong Giang, Quang Nam, Vietnam	EpaDG	AB823120	AB823155	AB823192	Shinohara et al. (2014)
<i>Euroscaptor klossi</i>	Mae Sa Long, Chiang Rai, Thailand	–	AB823106	AB823141	AB823178	Shinohara et al. (2014)
<i>Euroscaptor malayana</i>	Cameron Highlands, Pahang, Malaysia	–	AB185151	AB185153	AB185155	Shinohara et al. (2004a)
<i>Euroscaptor mizura</i>	Kita-azumi, Nagano, Japan	Emi1	AB823103	AB823138	AB823175	Shinohara et al. (2014)
	Mt. Fuji, Fujinomiya, Sizuoka, Japan	Emi2	AB823104	AB823139	AB823176	Shinohara et al. (2014)
<i>Mogera latouchei</i>	SAPA, Lao Cai, Vietnam	MlaSP	AB823091	AB823124	AB823161	Shinohara et al. (2014)
<i>Mogera wogura</i>	Mishima, Shizuoka, Japan	–	AB037623	AB106237	AB106244	<i>Cytb</i> : Tsuchiya et al. (2000) <i>12S, Rag1</i> : Shinohara et al. (2004b)
<i>Mogera imaizumii</i>	Niigata, Niigata, Japan	–	AB037609	AB106236	AB106242	<i>Cytb</i> : Tsuchiya et al. (2000) <i>12S, Rag1</i> : Shinohara et al. (2004b)
<i>Mogera tokudae</i>	Sado Island, Niigata, Japan	–	AB037607	AB106235	AB106243	<i>Cytb</i> : Tsuchiya et al. (2000) <i>12S, Rag1</i> : Shinohara et al. (2004b)
<i>Mogera insularis</i>	Pingtung, Pingdong, Taiwan	–	AB181616	AB181640	AB823158	<i>Cytb, 12S</i> : Kawada et al. (2007) <i>Rag1</i> : Shinohara et al. (2014)
<i>Mogera kanoana</i>	Kenting NP, Pingtung, Taiwan	–	AB181624	AB181648	AB823159	<i>Cytb, 12S</i> : Kawada et al. (2007) <i>Rag1</i> : Shinohara et al. (2014)
<i>Scaptochirus moschatus</i>	Western Jilin, China	–	AB306502	AB306503	AB353298	Shinohara et al. (2008)
<i>Parascaptor leucura</i>	Yongde, Yunnan, China	Ple1	HG737879	HG737893	HG737923	Kai et al. (2014)

Appendix 1...continued

Species	Collecting Locality	Code	DNA Database Accession Number			Reference
			<i>Cytb</i> (1140bp)	12S (ca. 850 bp)	<i>Rag1</i> (1010 bp)	
<i>Parascaptor leucura</i>	Kunming, Yunnan, China	Ple2	HG737880	HG737894	HG737924	Kai et al. (2014)
<i>Talpa altaica</i>	<i>Cytb, Rag1:</i> Novosibirisk, Russia	–	AB037602	AY012100	AB176542	<i>Cytb:</i> Tsuchiya et al. (2000) <i>12S:</i> Murphy et al. (2001) <i>Rag1:</i> Shinohara et al. (2004b)
<i>Talpa europaea</i>	<i>Cytb, Rag1:</i> Aarhus, Denmark <i>12S:</i> Dalby, Sweden	–	AB076829	Y19192	AB106246	<i>Cytb:</i> Shinohara et al. (2003) <i>12S:</i> Mouchaty et al. (2000) <i>Rag1:</i> Shinohara et al. (2004b)
<i>Urotrichus talpoides</i>	<i>Cytb, 12S:</i> Mt. Tsurugi, Tokushima, Japan <i>Rag1:</i> Mt. Gomadan, Wakayama, Japan	–	AB076833	AB106239	AB106245	<i>Cytb:</i> Shinohara et al. (2003) <i>12S, Rag1:</i> Shinohara et al. (2004b)
<i>Uropsilus gracilis</i>	Mt. Yulong, Lijiang, Yunnan, China	–	AB076700	AB106231	AB106240	Shinohara et al. (2004b)

Note: Code is labeled in phylogenetic trees (Fig. 2).

Appendix 2. DNA database accession numbers of cytochrome *b* gene sequences for comparison of the intraspecific genetic diversities.

Species	<i>N</i>	DNA Database Accession Numbers
<i>E. subanura</i>	19	LC013279–LC013335
<i>E. longirostris</i>	16	HG737870–HG737871, KC481345–KC481348, EU122226, AB823108–AB823116
<i>E. parvidens</i>	13	KC481340–KC481344, KC481338–KC481339, AB823117–AB823122
<i>M. latouchei</i>	15	KC481324–KC481325, AB823090–AB823102