Author's personal copy

Molecular Phylogenetics and Evolution 70 (2014) 513–521

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Multilocus phylogeny of talpine moles (Talpini, Talpidae, Eulipotyphla) and its implications for systematics

Kai He [a,b,1], Akio Shinohara [c,1], Xue-Long Jiang [a,*], Kevin L. Campbell [b,*]

[a] State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China
[b] Department of Biological Sciences, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada
[c] Department of Bio-Resources, Division of Biotechnology, Frontier Science Research Center, University of Miyazaki, Miyazaki 889-1692, Japan

ARTICLE INFO

Article history:
Received 5 June 2013
Revised 13 September 2013
Accepted 3 October 2013
Available online 16 October 2013

Keywords:
Cryptic species
Species tree
Species delimitation
Talpidae
Talpini
Rapid radiation

ABSTRACT

The tribe Talpini is a group of strictly subterranean moles distributed across the Eurasian Continent whose phylogenetic relationships and taxonomy remain unresolved. Here we report a multi-locus nuclear-mitochondrial DNA dataset (9468 bp) from 11 talpine species encompassing all five recognized genera, together with analyses of their divergence times and evolutionary affinities inferred from maximum likelihood and Bayesian approaches. Our results finely resolved all relationships except the root of the four recognized Asian genera, which was placed sister to the genus *Talpa*. With respect to the Asian clade, we moreover provide the first molecular support for a sister-taxon relationship between *Parascaptor* and *Scaptochirus* and confirm that the genus *Euroscaptor* is paraphyletic. Further, and despite a relatively small sample size (22 specimens), our species delimitation analyses support the existence of at least two genetically distinct, and hence potentially cryptic species. Taken together, these findings argue that generic status should be given to *E. mizura* and illustrate that the taxonomic diversity of the tribe Talpini in mountainous regions of southwestern China and Southeast Asia is underestimated. Finally, results of our divergence time analyses support a rapid radiation of the endemic Asian genera in the late-Miocene, which temporally corresponds with enhanced aridity and cooling arising from a significant uplift of the Himalayan–Tibetan plateau.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

As currently circumscribed, the tribe Talpini is classified into five genera of strictly subterranean moles which are distributed throughout the Eurasian Continent (Hutterer (2005). Within this assemblage, the genus *Talpa* ranges extensively from Western Europe and Asia to Siberia (Supplementary Fig. 1), while members of *Euroscaptor*, *Mogera*, *Parascaptor* and *Scaptochirus* are endemic to Far East Asia and exhibit limited distributions (Hutterer, 2005). Although the range of *Talpa* does not overlap with that of the other genera, sympatric occurrences of different Talpini species have been observed in both Europe (Loy et al., 2001) and Asia (Kawada et al., 2001).

Although earlier taxonomic classifications placed all Asian species within *Talpa* (Corbet, 1978; Corbet and Hill, 1992), McKenna and Bell (1997) recognized three Asian genera (*Scaptochirus*, *Euroscaptor* and *Nesoscaptor*). Hutterer (2005) elevated two additional Asian lineages (*Parascaptor* and *Mogera*) to generic status and placed *Nesoscaptor* in *Mogera*. Despite these revisions, phylogenetic relationships among the Asian genera remain controversial (Kawada, 2005). For example, Sanchez-Villagra (2006) placed *Parascaptor* as sister to other Talpini moles (Fig. 1a), while other studies (Crumpton and Thompson, 2012; Motokawa, 2004; Shinohara et al., 2008) supported *Talpa* as the sister taxon of the Asian genera (Fig. 1b–d). These morphological and mitochondrial analyses further suggested that *Euroscaptor* is paraphyletic (Crumpton and Thompson, 2012; Motokawa, 2004; Shinohara et al., 2008), though this assertion was only weakly supported (Fig. 1c and d). Finally, it should be noted that since no molecular data is currently available for the genus *Parascaptor*, its ambiguous phylogenetic placement (cf. Fig. 1a and b) is based solely on morphological characters.

While the most recent revision recognized 22 species in the tribe Talpini (Hutterer, 2005), it is of note that three additional Southeast Asian species (*Euroscaptor malayana*, *E. subanura* and *Mogera kanoana*) have since been described or elevated to full

* Corresponding authors. Addresses: Department of Biological Sciences, 50 Sifton Road, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada (K.L. Campbell), State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, 32 Jiaochang Donglu, Kunming, 650223 Yunnan, China. Fax: +86 0871 65125226 (X.-L. Jiang).

E-mail addresses: hk200131060071@gmail.com (K. He), mogura3@med.miyazaki-u.ac.jp (A. Shinohara), jiangxl@mail.kiz.ac.cn (X.-L. Jiang), campbelk@cc.umanitoba.ca (K.L. Campbell).

[1] These authors contributed equally.

1055-7903/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ympev.2013.10.002

Author's personal copy

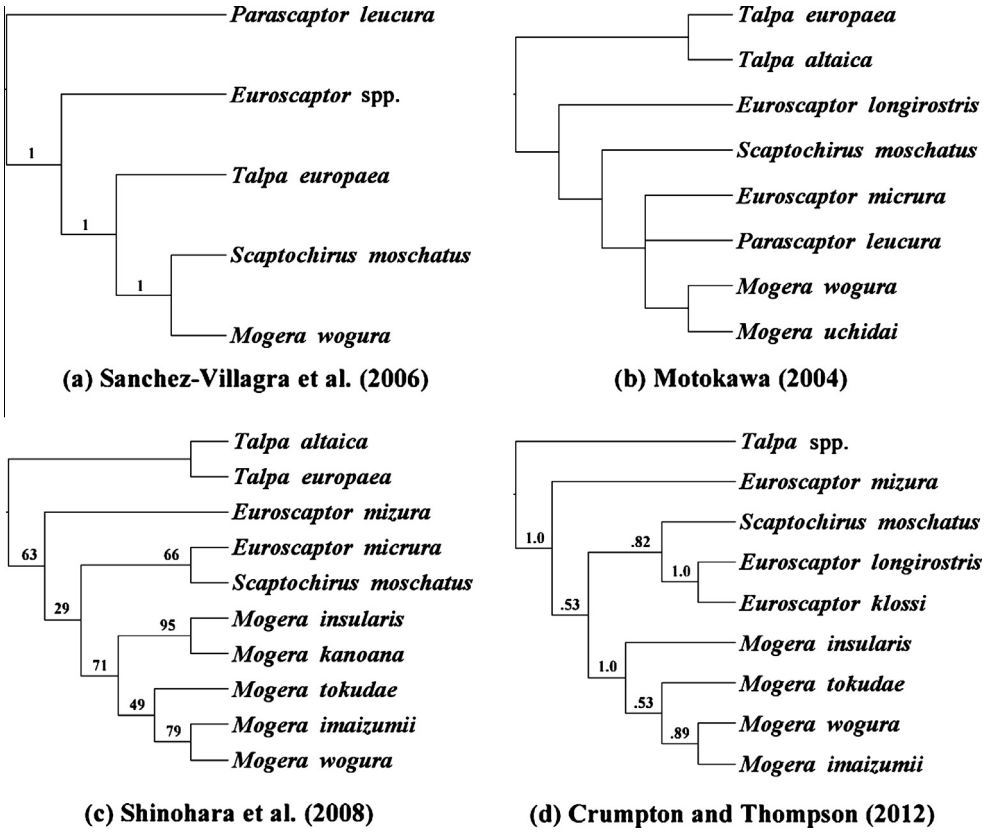

Fig. 1. Phylogenetic hypotheses of the tribe Talpini based on morphological characters (a and b) and molecular data (c and d). Numbers above nodes represent branch support values (a), bootstrap values (c) or posterior probabilities (d).

species status (Kawada et al., 2012, 2008a, 2007). These results imply that talpine species diversity is currently underestimated (i.e., "cryptic species" may be present). The mountains of southwestern China, which are among the most important biodiversity hotspots on earth (Myers et al., 2000), are particularly relevant in this respect. However, to date, only a single talpine specimen from these mountain ranges has been included in any molecular phylogenetic analysis (Zemlemerova et al., 2013).

Climate change has important effects on the evolution of small mammals (Fortelius et al., 2002). For instance, mitochondrial DNA analyses recovered three species pairs of *Talpa*, with divergence times between each pair occurring in the middle-late Pliocene (4.66–2.14 million years ago [Ma]), suggesting that climate change may have shaped the speciation of talpine moles in Europe (Colangelo et al., 2010). Thus we wished to assess whether climate change influenced speciation in Asian species as well.

Here we report the collection and analyses of a multilocus dataset, including two mitochondrial and twelve nuclear loci from 11 talpine species encompassing all five recognized genera, with a number of phylogenetic and taxonomic implications. Briefly, our phylogenetic results clarify the position of *Talpa* as sister to the four recognized Asian genera, place *Scaptochirus* as sister to *Parascaptor*, and provide strong support for the paraphyly of the genus *Euroscaptor*. We also identify genetically distinct and hence potentially undescribed (cryptic) species in the latter two genera. Finally, results of our divergence time analyses support a rapid radiation of the endemic Asian genera following their divergence from *Talpa* in the late-Miocene, that is moreover coincident with enhanced aridity and cooling arising from a significant uplift of the Himalayan–Tibetan plateau. Subsequent intra-generic divergences from the late-Miocene to Pleistocene also appear to be temporally linked to pronounced climate change events in the region.

2. Material and methods

2.1. Taxonomic sampling and nucleotide collection

We collected tissues from 22 specimens (with representatives from all five Talpini genera) from Japan, Southeast Asia, southwestern China, Taiwan, eastern Russia and Denmark (Table 1). Because a robust phylogenetic hypothesis of the genus *Talpa* that includes all but one species (*T. davidiana*) is available (Colangelo et al., 2010), we only included two *Talpa* species to represent this genus. Genomic DNA was extracted from either liver or muscle samples using the DNeasy Tissue Kit (Qiagen) or the phenol/proteinase K/sodium dodecyl sulfate method (Sambrook and Russell, 2001). Two complete mitochondrial loci (cytochrome *b* [*CYT B*] and 12S rRNA) and segments of twelve nuclear genes (adenosine A3 receptor [*ADORA3*], copper-transporting ATPase 1 [*ATP7A*], amyloid precursor protein [*APP*], butyrylcholinesterase [*BCHE*], brain-derived neurotrophic factor [*BDNF*], polycomb ring finger oncogene [*BMI1*], breast cancer type 1 susceptibility protein [*BRCA1*], cAMP responsive element modulator [*CREM*], phospholipase C beta 4 [*PLCB4*], recombination activating protein 1 [*RAG1*], recombination activating protein 2 [*RAG2*] and titin [*TTN*]) were amplified. Each of these genetic markers has been used in previous studies of mammalian phylogeny (Meredith et al., 2011; Murphy et al., 2001; Springer and Douzery, 1996). Primer pairs were either taken from the literature (Dubey et al., 2007; He et al., 2010; Meredith et al., 2011; Teeling et al., 2000) or designed for this study. All primers, PCR cocktails and programs are provided in Supplementary Table 1. Following amplification, PCR products were purified using the Qiaquick PCR Purification Kit (Qiagen, Valencia, CA), prepared for sequencing using the BigDye Terminator (version 3.1) Cycle Sequencing Kit, and sequenced in both directions on either an

Author's personal copy

Table 1
Species, sample locality and abbreviations used in the present study. Locality numbers refer to specimen collection sites presented in Supplementary Fig. 1.

Species	Sample locality	Locality no.	Original code	Abbreviation
Condylura cristata	Pennsylvania, USA		–	*C. cristata*_0
Euroscaptor longirostris	Sichuan, China	1	0905172	*E. longirostris*_1
Euroscaptor longirostris	Sichuan, China	2	0905290	*E. longirostris*_2
Euroscaptor longirostris	Vinh Phuuc, Vietnam	3	SIK0775	*E.* sp_1
Euroscaptor malayana	Pahang, Malaysia	4	SIK0550	*E. malayana*_1
Euroscaptor mizura	Aomori, Japan	5	SAS3	*E. mizura*_1
Euroscaptor parvidens	Quang Nam, Vietnam	6	SIK0859	*E. parvidens*_1
Mogera imaizumii	Shizuoka, Japan	7	SAS105	*M. imaizumii*_1
Mogera insularis	Taiwan, China	8	SIK0587	*M. insularis*_1
Mogera wogura	Liaoning, China	9	LN111104	*M. wogura*_1
Mogera wogura	Liaoning, China	9	LN111102	*M. wogura*_2
Mogera wogura	Liaoning, China	9	LN111103	*M. wogura*_3
Mogera wogura	Nagasaki, Japan	10	SAS64	*M. wogura*_4
Parascaptor leucura	Yunnan, China	11	DY0054	*P. leucura*_1
Parascaptor leucura	Yunnan, China	12	0412179	*P. leucura*_2
Parascaptor leucura	Yunnan, China	12	226065	*P. leucura*_3
Parascaptor leucura	Yunnan, China	13	0810072	*P.* sp_4
Parascaptor leucura	Yunnan, China	13	0810390	*P.* sp_5
Parascaptor leucura	Yunnan, China	13	0810480	*P.* sp_6
Scaptochirus moschatus	Shandong, China	14	SD111101	*S. moschatus*_1
Talpa altaica	–	–	–	*T. altaica*_0
Talpa altaica	Novosibirsk, Russia	15	HS1037	*T. altaica*_1
Talpa europaea	Aarhus, Denmark	16	EM1	*T. europaea*_1

ABI 3130 or an ABI 3730 Genetic Analyzer (Applied Biosystems, Tokyo, Japan).

2.2. Phylogenetic analyses and molecular dating

2.2.1. Concatenated gene tree reconstruction

Sequenced amplicons for each specimen/loci were assembled and edited using DNASTAR Lasergenev7.1. An additional 46 sequences from 9 specimens were downloaded from GenBank and included in the final dataset. Each locus was then separately aligned in MUSCLE (Edgar, 2004) and refined by eye using MEGA5 (Tamura et al., 2011). For all subsequent phylogenetic analyses, the star-nosed mole (*Condylura cristata*) was used as the outgroup.

We concatenated all genes and calculated maximum likelihood (ML) and Bayesian trees using RAxML v7.4.2 (Stamatakis et al., 2008) and BEAST v1.7.4 (Drummond et al., 2012), respectively. For the ML analyses, the best-fit partitioning scheme was determined in PartitionFinder v1.0.0 (Lanfear et al., 2012) under the Bayesian Information Criterion (BIC) (Luo et al., 2010; Schwarz, 1978). We defined data blocks based on genes and codon positions, treating each rRNA or non-coding gene as a single data block. An eight-partition scheme was determined and subsequently used for the ML analyses (Supplementary Table 2). ML tree reconstruction was performed on the CIPRES Science Gateway (Miller et al., 2010). For this analysis, we selected the GTR + gamma model for each partition, and ran 500 bootstrap replicates using the rapid Bootstrapping algorithm (Stamatakis et al., 2008).

For the Bayesian analyses, the best-fit evolutionary models for 12S, and each codon position of *CYT B* and the 12 nuclear loci were determined using the BIC implemented in jModeltest v2.1.3 (Darriba et al., 2012); these are given in Supplementary Table 3. We employed relaxed uncorrelated lognormal clock models (Drummond et al., 2006) and used approximate continuous-time Markov chains as the scale parameter prior (Ferreira and Suchard, 2008). The combined mitochondrial loci and each nuclear gene were given independent clock models. We used a random starting tree, birth–death tree prior and the program's default prior distributions of model parameters. Each analysis was run for 100 million generations and sampled every 10,000 generations. Trace v1.5 (Rambaut and Drummond, 2009) was used to ensure the Markov chains had reached stationary states by examining the effective sample size (ESS) values (i.e., ESS > 200).

To access the contribution of each gene to the concatenated phylogeny, we estimated branch support and partitioned branch support (PBS) (Baker and DeSalle, 1997) of the combined mitochondrial sequences and each nuclear loci using 1000 bootstrap replicates implemented in TreeRot v3 (Sorenson and Franzosa, 2007) and PAUP4.0b10 (Swofford, 2002).

For relationships not strongly supported by concatenated gene tree and/or species tree analyses (see below), we collapsed those branches one at a time. These alternative phylogenetic scenarios were tested using CONSEL v0.2 (Shimodaira and Hasegawa, 2001) and PAUP4.0b10 by calculating the *p*-value in the AU (Shimodaira, 2002) and KH tests (Kishino and Hasegawa, 1989).

2.2.2. Species delimitation

We first calculated the Kimura-2-parameter (K2P) distance of the *CYT B* gene between all potential Talpini lineages (i.e., between recognized species, and between specimens within species collected from geographically separated regions) using MEGA5. We then conducted species delimitation analyses using the "splits" v1.0-14 package (SPecies LImits by Threshold Statistics) for the R statistical environment. Splits delimits genetic clusters as putative species by defining the transitions between inter- and intra-specific diversification processes using a generalized mixed Yule-coalescent model (GMYC) (Pons et al., 2006). For this analysis, the time-calibrated concatenated gene tree (see Section 2.2.4) was used as the input tree, while the number of putative species were identified using a single threshold.

A second species delimitation analysis was conducted using the program BPP v2.1, which adopts the biological species concept and recognizes populations without recent gene flow as potential species (Yang and Rannala, 2010). This software provides the most accurate delimitation (Camargo et al., 2012), with the correct species model being inferred with high posterior probabilities when using 10–50 loci, even if only one sequence is sampled from each population (Zhang et al., 2011). The BPP analyses were performed using both a combined mitochondrial-nuclear dataset and with nuclear data alone. The best tree topology recovered by RAxML was used as guide tree for the BPP species delimitation analysis. According to the RAxML analyses, distinct lineages were found within *Euroscaptor longirostris*, *Mogera wogura* and *Parascaptor leucura*, implying that cryptic species may exist. Thus, these three species were treated as two potential taxa each. The lineage including

Author's personal copy

E. longirostris from the type locality (Baoxing, China) was considered as *E. longirostris*, while a second lineage represented by a single specimen from Vietnam was designated *Euroscaptor* sp. The type locality of *P. leucura* is in India (Hutterer, 2005). Thus, we treated the lineage represented by samples from western Yunnan (Parale_1, Parale_2 and Parale_3) as *P. leucura* and defined specimens exclusively from northeast Yunnan as *Parascaptor* sp. Specimens of *Mogera wogura* from Japan and China were recognized as *M. w. wogura* and *M. w. robusta*, respectively. Hence, 15 terminal branches were used in the guide tree. Two reversible jump Markov chain Monte Carlo (rjMCMC) algorithms for species delimitation (algorithms 0 and 1) were used. When using algorithm 0, finetune (ε) = 2, 10 or 20 were used; when using algorithm 1, finetune (α, m) = (1,0.5), (1.5,1) or (2,2) were used as suggested in the Software manual. Further, "locusrate = 1" specifying the random-rates model or "heredity = 1" which allows θ to vary among loci were both used, though not at the same time. Therefore, the analyses were repeated 12 times for each dataset. A gamma prior G (1, 100) was used on the population size parameters (θ_s). Similarly, the age of the root in the species tree (τ_0) was assigned the gamma prior G (2, 1000). Each rjMCMC iteration was run for 100,000 generations and sampled each 100 generations following a pre-burnin of 10,000 generations as determined by Tracer v1.5.

2.2.3. Species tree reconstruction

Species trees were calculated using the *BEAST model (Heled and Drummond, 2010) in BEAST v1.7.4. Based on the results of the K2P and species delimitation analyses (see Section 3.2), 14 lineages (including the outgroup) were treated as species in the *BEAST analyses. We used the same priors as in the gene tree reconstruction (see Section 2.2.1). All Bayesian analyses were repeated at least twice to confirm they converged on the same posterior distribution.

Concatenated gene and species trees can be found in TreeBASE (http://www.trebase.org; study ID:S14627).

2.2.4. Divergence-time analysis

The divergence time at each node was estimated simultaneously with Bayesian gene tree and species tree estimations using BEAST. Three calibration points were defined based on available fossil records (Fortelius, 2008): (i) the most ancient fossils of Talpini (*Geotrypus minor*; Ziegler, 2012) are from early Oligocene strata (MP21) at about 33.9–32.6 Ma (Islamoglu et al., 2010). We set up the prior at the root of the tree using a lognormal distribution (offset = 32.6), and allowed the oldest 95% credible interval (CI) to include the latest Paleocene (mean = 2.15 Ma, standard deviation [SD] = 1.0 Ma); (ii) the earliest record of Talpini in East Asia is dated between 20.5 and 16.4 Ma (Fortelius, 2008), so we treated the calibration as a lognormal distribution and set the earliest sample age to 16.4 Ma, and permitted the oldest 95% CI to encompass 20.5 Ma (offset = 16.4 Ma, mean = 1.3 Ma, SD = 1.0 Ma); (iii) the fossil records of Talpini in East Asia are relatively very poor compared with those of Europe, with some fossil taxa found in China moreover being reported under the generic name of *Talpa* either due to out-of-date taxonomy or misidentification. Nonetheless, a series of Chinese and Mongolian fossils (*Scaptochirus, Mogera* and "*Talpa*") are have been found in strata from the latest Miocene to the Pleistocene (Qiu and Storch, 2005), so we set up a calibration for the most recent common ancestor of the Asian taxa using a uniform distribution (lower boundary = 4.3 Ma) (Flynn and Wu, 1994).

3. Results

3.1. Sequence characteristics

We obtained ~9468 bp of sequence for most specimens (Supplementary Table 4) partitioned into 1980 bp of mitochondrial sequence (*CYT B* [1140 bp], 12S rRNA [840 bp]) and 7488 bp of nuclear sequence (*ADORA3* [336 bp], *ATP7A* [676 bp], *APP* [350 bp], *BCHE* [972 bp], *BDNF* [519 bp], *BMI1* [319 bp], *BRCA1* [816 bp], *CREM* [386 bp], *PLCB4* [290 bp], *RAG1* [1010 bp], *RAG2* [694 bp] and *TTN* [1120 bp]); no sequences were obtained from ~5% (16 out of 322) of the loci. All new sequences were deposited in GenBank under accession numbers: HG737870 to HG738128. No frame shift mutations or premature stop codons were observed in the coding regions of each locus.

3.2. Species delimitation

Calculated K2P distances of *CYT B* between *Mogera wogura wogura* and *M. w. robusta* (0.056), and between *Euroscaptor longirostris* and *E.* sp. (0.081), was below the range between recognized Talpini species (0.089–0.183), while that between *Parascaptor leucura* and *P.* sp. (0.129) was within this range (Supplementary Table 5). However, results of the splits analysis suggested that clades with divergence times earlier than 1.06 Ma may represent genetically distinct species (Supplementary Fig. 2b). Thus, a total number of 15 ML entities were recognized as potential species including *Euroscaptor* sp,. *M. w. robusta* and *Parascaptor* sp. (Supplementary Fig. 2a). In each BPP analysis, all parameters had high ESS values ($\geqslant$200). Even though different priors were used, the results were highly congruent (Supplementary Table 6). *P. leucura* and *P.* sp. were strongly supported as separate species (PP = 1.0) by both the nuclear and combined mitochondrial-nuclear datasets (Table 2). The combined 14 gene dataset also strongly supported *E. longirostris/E. sp.* and *M. wogura wogura/M. w. robusta* as independent species pairs (PP = 0.96–0.99). Conversely, the posteriors supporting *M. wogura* as separate species by nuclear-gene analyses alone were substantially lower (PP $\leqslant$ 0.76). Consequently, we recognized both *E. longirostris* and *P. leucura* as two potential species each, but conservatively accepted specimens of *M. wogura* to comprise a single species (see Section 4.3).

3.3. Concatenated gene and species trees

The concatenated ML and Bayesian analyses recovered the identical topology, and thus only the Bayesian tree is shown (Fig. 2a). Most of the nodes were strongly supported (i.e., bootstrap values

Table 2
Results of the species delimitation analyses implemented in BPP v2.1 (Yang and Rannala, 2010) using all 14 genes versus the 12 nuclear genes alone. Posterior probabilities supporting *E. longirostris* and *E.* sp., *M. w. wogura* and *M. w. robusta*, and *P. leucura* and *P.* sp. as potential distinct species using algorithm 0 and algorithm 1 are given. Posterior probabilities represent mean values; the result of each independent analysis is given in Supplementary Table 6.

	Combined mitochondrial-nuclear dataset (14 loci)		Nuclear dataset only (12 loci)	
Scenario	Algorithm 0	Algorithm 1	Algorithm 0	Algorithm 1
(*E. longirostris, E.* sp.)	0.96	0.96	0.94	0.95
(*M. w. wogura, M. w. robusta*)	0.98	0.99	0.73	0.76
(*P. leucura, P.* sp)	1.00	1.00	1.00	1.00

Author's personal copy

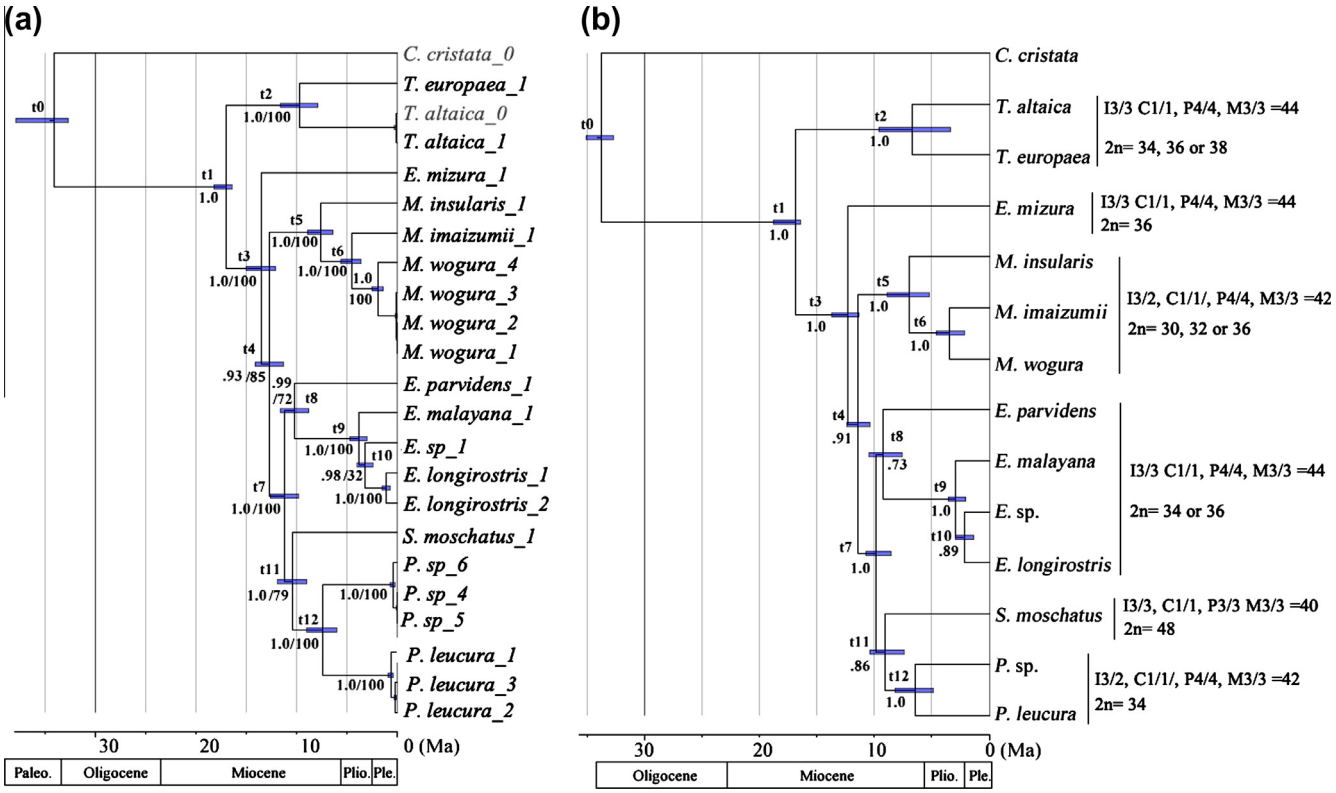

Fig. 2. Chronogram of the tribe Talpini based on partitioned Bayesian analyses using (a) concatenated sequences and (b) the *BEAST model. Numbers below the nodes indicate Bayesian posterior probabilities while branch lengths represent time. Horizontal node bars indicate the 95% confidence interval for each clade age. Dental formula and chromosome numbers for each genus are taken from He et al. (2012) and Shinohara et al. (2008).

Table 3
Results of the AU and KH tests. Node numbers are presented in Fig. 2a.

Phylogenetic scenario	Tree length	AU test	KH test
The ML/Bayesian tree	2642	0.999	0.974
Collapse node t4	2652	0.008	0.026
Collapse node t8	2658	<0.001	0.003
Collapse node t10	2654	0.003	0.016
Collapse node t11	2653	0.003	0.012

[BS] ⩾ 70 and Posterior probabilities [PP] ⩾ 0.95; Hillis and Bull, 1993; Huelsenbeck and Rannala, 2004) with few exceptions (e.g., t4 and t10 in Fig. 2a). The endemic Asian genera were strongly supported as monophyletic (PP = 1.0, BS = 100), with *Euroscaptor mizura* placed at the base of this clade, though this position was not strongly supported (PP = 0.93, BS = 85). The remaining *Euroscaptor* species were supported as monophyletic (PP = 0.99, BS = 72) and sister to *Parascaptor + Scaptochirus* (PP = 1.0, BS = 100). Thus, even though the phylogenetic position of *E. mizura* was not stable, *Euroscaptor* was still strongly supported as a paraphyletic group (Fig. 2a). Sister-relationships of *Parascaptor* and *Scaptochirus* were also strongly supported (PP = 1.0, BS = 79), but with a very short interior branch. The crown clades of non-*mizura Euroscaptor* and *E. longirostris*/*E.* sp. also exhibited short branches (Fig. 2a). The *BEAST analyses recovered the same topology as the concatenated gene tree (Fig. 2b). However, the basal position of *E. mizura* (t4), the sister-relationships between *E. parvidens* and other non-*mizura Euroscaptor* species (t8), between *E. longirostris* and *E.* sp. (t10), as well as between *Scaptochirus* and *Parascaptor* (t11) were not robustly supported (PP = 0.91, 0.73, 0.89 and 0.86, respectively).

We thus collapsed the branches t4, t8, t10 and t11 of the concatenated gene tree one at a time to test alternative phylogenetic scenarios. The resulting tree lengths increased by 10–16 steps, with each of these four alternative phylogenies being significantly rejected by both the AU and KH tests ($P < 0.03$; Table 3). Further, the branch support and most PBS values at each of these four nodes were near 0 ($-1 < - < 1$; Fig. 3). The sister-relationship between *E. parvidens* and other non-*mizura Euroscaptor* taxon was only supported by *ATP7A* (PBS = 1.3), but rejected by mitochondrial genes (PBS = −2). The mitochondrial partition also rejected the sister-relationship between *Parascaptor* and *Scaptochirus* (PBS = −1.2).

3.4. Molecular divergence time estimates

Our concatenated gene and species tree reconstructions revealed highly concordant divergence time estimates (Fig. 2 and Supplementary Table 7). Here we only focus on results based on calibrations of the species tree, because this approach provides a more biologically realistic framework (McCormack et al., 2011). Our analysis suggest that *Talpa* diverged from the Asian clades in the late Miocene around 16.88 Ma (95% CI = 18.75–16.42 Ma), with the most common recent ancestor of the extant Asian taxa being about 12.32 Ma (13.69–11.31 Ma). The genera endemic to Asia rapidly radiated from 12.32 to 9.06 Ma (13.69–7.42 Ma). Apart from the split between *E. parvidens* and the other non-*mizura Euroscaptor* species (9.22 Ma, 95% CI = 10.44–7.6 Ma), intra-generic divergence events primarily occurred in the latest Miocene (6.72–6.47 Ma; 95% CI = 9.63–3.35 Ma) and from the middle Pliocene to the early Pleistocene (4.63–1.34 Ma).

4. Discussion

4.1. Concatenated gene tree versus species tree

Our concatenated gene tree and species tree analyses generated identical topologies, though the posterior probabilities on the

Author's personal copy

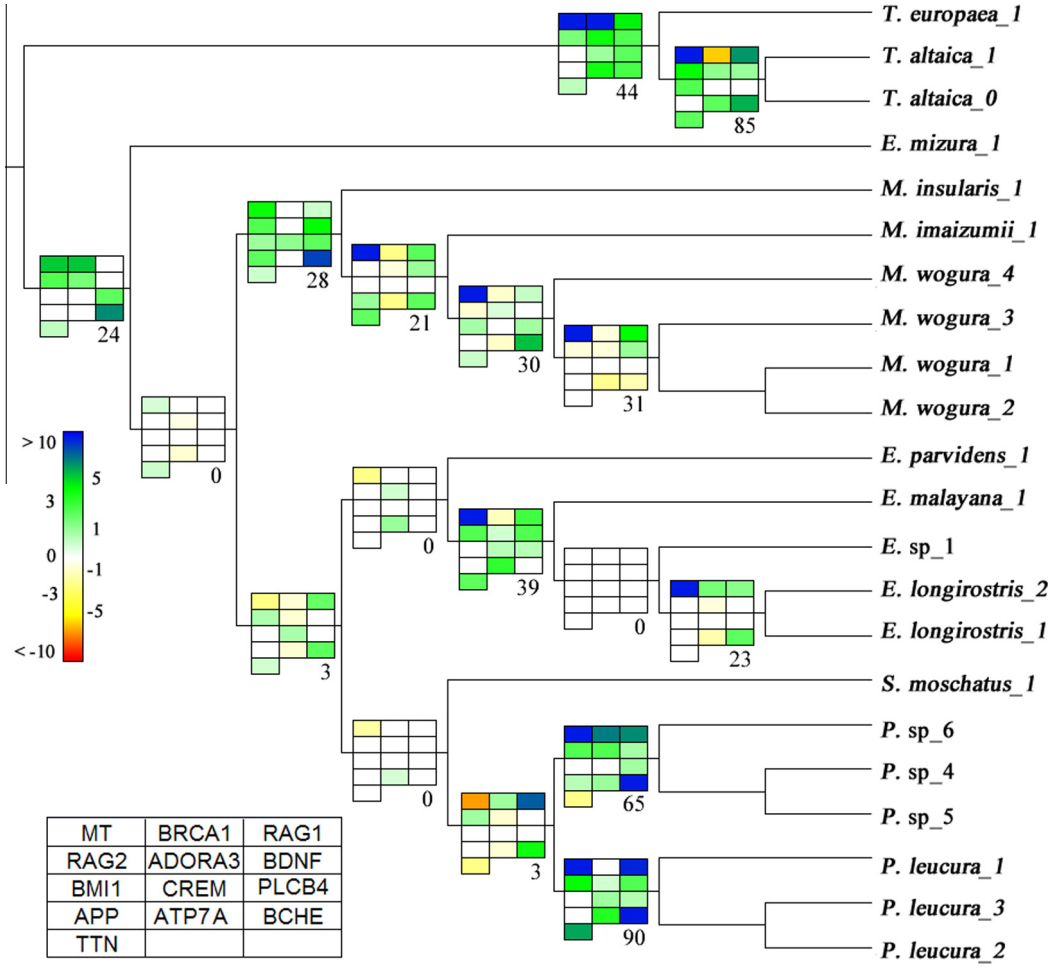

Fig. 3. Molecular phylogeny of the tribe Talpini. Numbers below each node represent branch support values. Boxes at internodes show partitioned branch support values for the combined mitochondrial (MT) dataset and each nuclear gene (see Section 2.2.1 for details).

species tree were lower at some nodes (Fig. 2). However, whether posterior probabilities on the species trees are expected to exhibit similar behaviors as the gene tree still needs to be explored (Edwards et al., 2007). Nonetheless, poorly supported clades are characterized by extremely short branch lengths and the branch supports and most PBS indices supporting these relationships are also very low (Fig. 3). These results suggest that the relatively low posterior probabilities at these nodes are not due to conflicting phylogenetic signals, but are more likely attributed to rapid diversification events. Under this scenario, only a small number of phylogenetically informative DNA substitutions are expected to have evolved and remain preserved in extant lineages (e.g., He et al., 2010; Xiong et al., 2009). Nonetheless, support for our presented species tree is bolstered by the observation that both the AU and KH tests significantly rejected all alternative phylogenetic hypotheses (Table 3).

4.2. Phylogeny and timing of the Talpini radiations

This is the first molecular study to include representatives of the genus *Parascaptor* and to employ fine taxon sampling from the other Asian taxa. Because the phylogeny is well supported, it can be used as a backbone to assess unresolved and conflicting phylogenetic hypotheses. For example, with regards to the monophyly of the Asian genera, our results are congruent with several previous studies (Crumpton and Thompson, 2012; Motokawa, 2004; Shinohara et al., 2008), but conflict with results obtained from a comprehensive 157 character morphological data matrix (Sanchez-Villagra et al., 2006) which supported *Parascaptor* as the sister taxon to all other Talpini genera (Fig. 1a). This discordance may due to extreme morphological adaptation and convergence to fossorial habitats within the tribe, as recently illustrated for the (polyphyletic) morphologically derived higher level relationships among placental mammals adapted to similar niches (Springer et al., 2013).

Although previous studies have observed paraphyly within the genus *Euroscaptor* (Crumpton and Thompson, 2012; Shinohara et al., 2008; Zemlemerova et al., 2013), we provide the first strong statistical evidence supporting *E. mizura* as a distinct evolutionary lineage (Fig. 2). The deep split between *E. mizura* and the other Asian taxa is concordant with an ancient (i.e., late Miocene; 13.69–11.31 Ma) colonization event in Japan. This date is substantially earlier than that recently proposed (5.2 Ma) on the basis of *CYT B* sequences (Kirihara et al., 2013), but overlaps with a dramatic drop in global sea level (and hence opening of a land bridge across the Korean Strait) at the start of the late Miocene (Haq et al., 1987). Notably, flying squirrels (*Petaurista leucogenys*) are also proposed to have colonized Japan during this period (12.51 Ma; Li et al., 2013), which also corresponds to the spread and subsequent rapid diversification of Southeast Asian tree-squirrels (Mercer and Roth, 2003).

A second key finding is the sister-taxon relationship between *Scaptochirus* and *Parascaptor*, which is not supported by morphological analyses (Motokawa, 2004; Sanchez-Villagra et al., 2006).

Author's personal copy

Interestingly, however, Stroganov (1948) grouped these species together owing to a striking correspondence of their auditory ossicles. Mason (2006) noted additional similarities in the auditory region of the skull of these genera and further suggested that their phylogenetic relationships should be re-examined. Based on the results of the present study, the most parsimonious explanation for the conspicuous morphological similarity of the middle ear apparatus in these sister lineages is that it represents a synapomorphic trait.

Our molecular divergence estimation analyses suggested a rapid radiation of the Asian genera in the late Miocene (13.69–7.42 Ma), and more specifically between 13.69–10.37 Ma and 10.42–7.42 Ma (Fig. 2; Supplementary Table 7). These periods coincide with enhanced global aridity and marked cooling (Miller and Fairbanks, 1983; Zachos et al., 2001). In East Asia, this climate change was bolstered by the uplift of the Himalayan Mountains and a strengthening of seasonal monsoons (An et al., 2001). Although the Himalayas had reached a relatively tectonically stable phase by about 13 Ma (Song et al., 2001), the mountains presumably achieved sufficient elevation to block the penetration of moisture which resulted in rapid regional cooling (Dettman et al., 2003). Subsequent accelerated uplift of the Himalayan–Tibetan plateau (Molnar et al., 1993) coupled with strengthening of the monsoons beginning about 9–8 Ma (An et al., 2001; Qiang et al., 2001; Song et al., 2007) have also been well documented. Thus, we hypothesize that climate change and ensuing vegetational turnover (Cerling et al., 1997; Zhang et al., 2009) may have triggered the early diversification of talpine moles in Asia. With this scenario as our working hypothesis, the Asian talpines most likely colonized Japan, eastern China and southwestern China in the late Miocene and then became isolated by both physical (mountain building) and climate-induced (unsuitable habitats) vicariant events over a relatively short period.

4.3. Cryptic species and taxonomic implications

The current study has several important taxonomic implications for the tribe Talpini. Because *E. mizura* represents a distinct lineage, and because the type species of *Euroscaptor* is *E. klossi* which is the sister-taxon of *E. malayana* (Shinohara et al., in prep.), we recommend that generic status should be given to *E. mizura*. Notably, our analyses suggest *E. parvidens* may also represent a genetically distinct lineage, with the timing of its divergence coinciding with the *Parascaptor*/*Scaptochirus* split (Fig. 2). Interestingly, while *Parascaptor* and *Scaptochirus* exhibit markedly different external and internal morphologies (e.g., body size, dental formula and fur pigmentation) (Nowak, 1999) and karyotypes (Fig. 2b), *E. parvidens* is not easily distinguished from the other *Euroscaptor* species, apart from its slender skull and smaller molars (Kawada et al., 2008b; Miller, 1940). In this regard, it is unfortunate that we were only able to examine a single *E. parvidens* specimen as Zemlemerova et al. (2013) recently presented evidence that individuals collected from separate mountain ranges in central and southern Vietnam are demarcated from one another by a high level of mitochondrial divergence, and hence may represent distinct lineages. This result, together with our finding of an ancient (late Miocene) divergence between *E. parvidens* and the other non-*mizura* *Euroscaptor* species, calls for a re-assessment of the taxonomic status of *E. parvidens*, with comprehensive molecular and morphological diagnoses warranted to resolve this issue.

Despite the fact that only three of the species included in this study were collected from multiple localities (Table 1), we found that at least two of them (*Parascaptor leucura* and *Euroscaptor longirostris*) appear to contain cryptic species. Although sample size is an important consideration in species delimitation analyses, computer simulations have demonstrated that the posterior probability of the correct model rapidly increases with the inclusion of more loci, such that as little as 10 loci (though up to 50 in cases of recent divergence) are required for BPP to infer true models of species delimitation when only one specimen is sampled from each population (Yang and Rannala, 2010; Zhang et al., 2011). Our dataset (2 mitochondrial and 12 nuclear loci) is within this range, with both the BPP and GMYC species delimitation analyses leading to similar results. Further, the K2P genetic distances between *Euroscaptor* sp., *Parascaptor* sp. and their sister lineages (0.081 and 0.129, respectively) were similar or higher than the average genetic distance between sister species of rodents and bats (0.081; Bradley and Baker, 2001).

Specimens of *M. w. wogura* (Japan) and *M. w. robusta* (Russia) have different karyotypes (Kawada et al., 2001), and are recognized as independent species by some researchers (e.g., Ohdachi et al., 2009). Their phylogenetic status has accordingly been the subject of recent debate, with both subspecies being strongly supported as monophyletic by nuclear genes (Kirihara et al., 2013), but not by mitochondrial markers (Kirihara et al., 2013; Koh et al., 2012; Zemlemerova et al., 2013). Similarly, our combined mitochondrial-nuclear species delimitation analysis supported Japanese and Russian specimens as distinct species, while this demarcation was not upheld in the K2P or nuclear gene analysis (Table 2). It is possible this latter result is an artifact, as three loci (*BRCA1*, *RAG2* and *APP*) were not obtained from the single Japanese specimen. However, our PBS analyses revealed that only two (*RAG1* and *BDNF*) out of the nine nuclear genes supported the monophyly of *M. w. robusta* (Fig. 3), indicative of close sequence similarity between the two subspecies (Kirihara et al., 2013). Finer taxon sampling with additional sequence data of both subspecies are thus required to better assess their taxonomic status.

Genetic support for cryptic diversity within the currently monotypic genus *Parascaptor* is consistent with observations of Sanchez-Villagra et al. (2006) who noted intraspecific morphological variability with *P. leucura* and stated that these characters represent "potential areas of interest in revisions of this species". More recent evidence indicates that the two Chinese *Parascaptor* phylogroups included in this study not only exhibit morphological differences with one another, but are moreover anatomically distinguishable from specimens collected in northeastern India (He et al., 2013). We treated the specimens from western Yunnan, China as *P. leucura* only because their distributions are closer to the type locality in Assam, India (Supplementary Fig. 1). Thus, it is plausible that specimens from both lineages included in this study represent undescribed species.

With respect to the *Euroscaptor longirostris*, two specimens were included from its type locality (Baoxing [=Moupin], China). Thus, despite possessing morphologically similar skulls (He, unpublished observations), the genetically distinct sample from northern Vietnam (*E.* sp.) appears to represent a separate species. This finding is consistent with recent observations of three distinct mitochondrial lines among *E. longirostris* specimens collected in southwestern China, northern Vietnam and northwestern Vietnam (Zemlemerova et al., 2013). It is unlikely these Vietnamese specimens belongs to the sympatrically distributed species *E. subanura* (Kawada et al., 2012), as this recently named lowland mole shares external morphology and presumably a close phylogenetic affinity with *E. parvidens* (Kawada et al., 2012). Consequently, the taxonomic status of these genetically distinct Vietnamese lineages also needs to be addressed.

In conclusion, despite our small sample sizes for *Euroscaptor* sp. ($n = 1$) and *Parascaptor* sp. ($n = 3$), our results argue that these specimens may represent cryptic species. This finding, together with the recent elevation/description of three additional Southeast Asian species, *E. malayana*, *E. subanura* and *Mogera kanoana* (Kawada et al., 2012, 2008a, 2007) and limited sampling efforts

Author's personal copy

in the mountains of Nepal, northeastern India and southwestern China suggests that species diversity in the tribe Talpini is still underestimated.

Acknowledgments

We appreciate the constructive comments and suggestions from two anonymous reviewers. We also thank Dr. Shin-ichiro Kawada for providing tissue samples of several *Euroscaptor* and *Mogera* specimens, Drs. Nguyen Truong Son and Dang Ngoc Can for assisting AS in the field survey in Vietnam, and Dr. Mieczyław Wolsan for kindly providing helpful suggestions on an earlier draft of this manuscript. This study was supported by the National Natural Science Foundation of China (Grant 31301869 to KH), by the Ministry of Education, Culture, Sports, Science and Technology, Japan (Grant 15770060 to AS), by Natural Sciences and Engineering Research Council (NSERC) Discovery and Accelerator Supplement Grants to KLC, by the Fund of State Key Laboratory of Genetics Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences (Grant GREKF11-03 to KH), and by the National Basic Research Program of China (973 Program) (2007CB411600 to XLJ).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.10.002.

References

An, Z., Kutzbach, J.E., Prell, W.L., Porter, S.C., 2001. Evolution of Asian monsoons and phased uplift of the Himalaya-Tibetan plateau since Late Miocene times. Nature 411, 62–66.

Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian drosophilids. Syst. Biol. 46, 654–673.

Bradley, R.D., Baker, R.J., 2001. A test of the genetic species concept: Cytochrome-*b* sequences and mammals. J. Mammal. 82, 960–973.

Camargo, A., Morando, M., Avila, L.J., Sites, J.W., 2012. Species delimitation with abc and other coalescent-based methods: a test of accuracy with simulations and an empirical example with lizards of the *Liolaemus Darwinii* complex (Squamata: Liolaemidae). Evolution 66, 2834–2849.

Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V., Ehleringer, J.R., 1997. Global vegetation change through the Miocene/Pliocene boundary. Nature 389, 153–158.

Colangelo, P., Bannikova, A.A., Krystufek, B., Lebedev, V.S., Annesi, F., Capanna, E., Loy, A., 2010. Molecular systematics and evolutionary biogeography of the genus *Talpa* (Soricomorpha: Talpidae). Mol. Phylogenet. Evol. 55, 372–380.

Corbet, G.B., 1978. The Mammals of the Palaearctic Region: a Taxonomic Review, London.

Corbet, G.B., Hill, J.E., 1992. The Mammals of the Indomalayan Region: a Systematic Review. Natural History Museum, London & Oxford University Press, Oxford, New York etc.

Crumpton, N., Thompson, R., 2012. The holes of moles: osteological correlates of the trigeminal nerve in Talpidae. J. Mamm. Evol., 1–13.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Meth. 9, 772–772.

Dettman, D.L., Fang, X., Garzione, C.N., Li, J., 2003. Uplift-driven climate change at 12 Ma: a long δ18O record from the NE margin of the Tibetan plateau. Earth Planet. Sci. Lett. 214, 267–277.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, 699–710.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Dubey, S., Salamin, N., Ohdachi, S.D., Barrière, P., Vogel, P., 2007. Molecular phylogenetics of shrews (Mammalia: Soricidae) reveal timing of transcontinental colonizations. Mol. Phylogenet. Evol. 44, 126–137.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Edwards, S.V., Liu, L., Pearl, D.K., 2007. High-resolution species trees without concatenation. Proc. Natl. Acad. Sci. USA 104, 5936–5941.

Ferreira, M.A.R., Suchard, M.A., 2008. Bayesian analysis of elapsed times in continuous-time Markov chains. Can. J. Stat. – Revue Can. Stat. 36, 355–368.

Flynn, L.J., Wu, W., 1994. Two new shrews from the Pliocene of Yushe Basin, Shanxi Province, China. Vertebrat. PalAsiatic. 17, 246–265.

Fortelius, M., 2008. Neogene of the Old World Database of Fossil Mammals (NOW). University of Helsinki. <http://www. helsinki. fi/science/now>.

Fortelius, M., Eronen, J., Jernvall, J., Liu, L., Pushkina, D., Rinne, J., Tesakov, A., Vislobokova, I., Zhang, Z., Zhou, L., 2002. Fossil mammals resolve regional patterns of Eurasian climate change over 20 million years. Evol. Ecol. Res. 4, 1005–1016.

Haq, B.U., Hardenbol, J., Vail, P.R., 1987. Chronology of fluctuating sea levels since the Triassic. Science 235, 1156–1167.

He, K., Li, Y.J., Brandley, M.C., Lin, L.K., Wang, Y.X., Zhang, Y.P., Jiang, X.L., 2010. A multi-locus phylogeny of Nectogalini shrews and influences of the paleoclimate on speciation and evolution. Mol. Phylogenet. Evol. 56, 734–746.

He, K., Wang, J.-H., Su, W.-T., Li, Q., Nie, W.-H., Jiang, X.-L., 2012. Karyotype of the gansu mole (*Scapanulus oweni*): further evidence for karyotypic stability in talpid. Mamm. Study 37, 341–348.

He, K., Bai, M., Wan, T., Li, Q., Wang, Y., Jiang, X., 2013. Shape variation in mandibles of *Parascaptor leucura* (Mammalia, Talpidae) based on geometric morphometrics: implication for geographic diversification. Acta Theriol. Sin. 33, 7–17.

Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570–580.

Hillis, D.M., Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42, 182–192.

Huelsenbeck, J., Rannala, B., 2004. Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. Syst. Biol. 53, 904–913.

Hutterer, R., 2005. Order Soricomorpha. In: Wilson, D.E., Reeder, D.M., (Eds.), Mammal Species of the World: A Taxonomic and Geographic Reference. John Hopkins University Press, Baltimore, pp. 220–311.

Islamoglu, Y., Harzhauser, M., Gross, M., Jimenez-Moreno, G., Coric, S., Kroh, A., Rogl, F., van der Made, J., 2010. From tethys to eastern paratethys: oligocene depositional environments, paleoecology and paleobiogeography of the Thrace Basin (NW Turkey). Int. J. Earth Sci. 99, 183–200.

Kawada, S., 2005. The historical notes and taxonomic problems of East Asian moles, Euroscaptor, Parascaptor and Scaptochirus, of continental Asia (Insectivora, Talpidae), Mamm. Study 30, S5–S11.

Kawada, S., Harada, M., Obara, Y., Kobayashi, S., Koyasu, K., Oda, S., 2001. Karyosystematic analysis of Japanese talpine moles in the genera *Euroscaptor* and *Mogera* (Insectivora, Talpidae). Zool. Sci. 18, 1003–1010.

Kawada, S., Shinohara, A., Kobayashi, S., Harada, M., Oda, S., Lin, L.K., 2007. Revision of the mole genus *Mogera* (Mammalia: Lipotyphla: Talpidae) from Taiwan. Syst. Biodivers. 5, 223–240.

Kawada, S., Yasuda, M., Shinohara, A., Lim, B.L., 2008a. Redescription of the Malaysian mole as to be a true species, Euroscaptor malayana (Insectivora, Talpidae). Mem. Natl. Mus. Nat. Sci. 45, 65–74.

Kawada, S., Son, N.T., Can, D.N., 2008b. Karyological diversity of talpids from Vietnam (Insectivora, Talpidae). In: Can, D.N., Endo, H., Son, N.T., Oshida, T., Canh, L.X., Phuong, D.H., Lunde, D.P., Kawada, S., Hayashida, A., Sasaki, M. (Eds.), Checklist of Wild Mammal Species of Vietnam. Institute of Ecology and Biological Resources, Hanoi, pp. 384–389.

Kawada, S., Son, N.T., Ngoc Can, D., 2012. A new species of mole of the genus Euroscaptor (Soricomorpha, Talpidae) from northern Vietnam. J. Mammal. 93, 839–850.

Kirihara, T., Shinohara, A., Tsuchiya, K., Harada, M., Kryukov, A.P., Suzuki, H., 2013. Spatial and temporal aspects of occurrence of *Mogera* species in the Japanese islands inferred from mitochondrial and nuclear gene sequences. Zool. Sci. 30, 267–281.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum-likelihood estimate of the evolutionary tree topologies from DNA-sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29, 170–179.

Koh, H.S., Jang, K.H., Han, E.D., Jo, J.E., Jeong, S.K., Ham, E.J., Lee, J.H., Kim, K.S., In, S.T., Kweon, G.H., 2012. Lack of genetic divergence between *Mogera wogura coreana* from Korea and *M. w. robusta* from Northeastern China and adjacent Russia (Soricomorpha: Mammalia), reexamined from 12S rRNA and cytochrome *b* sequences. Anim. Cells Syst. 16, 408–414.

Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29, 1695–1701.

Li, S., He, K., Yu, F.-H., Yang, Q.-S., 2013. Molecular phylogeny and biogeography of *Petaurista* inferred from the cytochrome *b* gene, with implications for the taxonomic status of *P. caniceps*, *P. marica* and *P. sybilla*. PLoS ONE 8, e70461.

Loy, A., Capula, M., Palombi, A., Capanna, E., 2001. Genetic and morphometric evidence of introgression between two species of moles (Insectivora: *Talpa europaea* and *Talpa romana*) in central Italy. J. Zool. 254, 229–238.

Luo, A., Qiao, H.J., Zhang, Y.Z., Shi, W.F., Ho, S.Y.W., Xu, W.J., Zhang, A.B., Zhu, C.D., 2010. Performance of criteria for selecting evolutionary models in phylogenetics: a comprehensive study based on simulated datasets. BMC Evol. Biol. 10, 242.

Mason, M.J., 2006. Evolution of the middle ear apparatus in talpid moles. J. Morphol. 267, 678–695.

McCormack, J.E., Heled, J., Delaney, K.S., Peterson, A.T., Knowles, L.L., 2011. Calibrating divergence times on species trees versus gene trees: implications for speciation history of Aphelocoma jays. Evolution 65, 184–202.

McKenna, M.C., Bell, S.K., 1997. Classification of Mammals: Above the Species Level. Columbia University Press, New York.

Mercer, J.M., Roth, V.L., 2003. The effects of Cenozoic global change on squirrel phylogeny. Science 299, 1568–1572.

Author's personal copy

Meredith, R.W., Janecka, J.E., Gatesy, J., Ryder, O.A., Fisher, C.A., Teeling, E.C., Goodbla, A., Eizirik, E., Simao, T.L., Stadler, T., Rabosky, D.L., Honeycutt, R.L., Flynn, J.J., Ingram, C.M., Steiner, C., Williams, T.L., Robinson, T.J., Burk-Herrick, A., Westerman, M., Ayoub, N.A., Springer, M.S., Murphy, W.J., 2011. Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. Science 334, 521–524.

Miller, G.S., 1940. A new mole from Annam. J. Mammal 21, 203–204.

Miller, K.G., Fairbanks, R.G., 1983. Evidence for Oligocene-Middle Miocene abyssal circulation changes in the western North Atlantic. Nature 306, 250–253.

Miller, M.A., Pfeiffer, W., Schwartz, T., 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. IEEE, 1–8.

Molnar, P., England, P., Martinod, J., 1993. Mantle dynamics, uplift of the tibetan plateau, and the indian monsoon. Rev. Geophys. 31, 357–396.

Motokawa, M., 2004. Phylogenetic relationships within the family Talpidae (Mammalia: Insectivora). J. Zool. (Lond.) 263, 147–157.

Murphy, W.J., Eizirik, E., Johnson, W.E., Zhang, Y.P., Ryder, O.A., O'Brien, S.J., 2001. Molecular phylogenetics and the origins of placental mammals. Nature 409, 614–618.

Myers, N., Mittermeier, R., Mittermeier, C., da Fonseca, G., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.

Nowak, R.M., 1999. Walker's Mammals of the World. Johns Hopkins University Press, Baltimore.

Ohdachi, S.D., Ishibashi, Y., Iwasa, M.A., Saitoh, T., 2009. The Wild Mammals of Japan. Shoukadoh, Tokyo.

Pons, J., Barraclough, T.G., Gomez-Zurita, J., Cardoso, A., Duran, D.P., Hazell, S., Kamoun, S., Sumlin, W.D., Vogler, A.P., 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Syst. Biol. 55, 595–609.

Qiang, X.K., Li, Z.X., Powell, C.M., Zheng, H.B., 2001. Magnetostratigraphic record of the Late Miocene onset of the East Asian monsoon, and Pliocene uplift of northern Tibet. Earth Planet. Sci. Lett. 187, 83–93.

Qiu, Z., Storch, G., 2005. The fossil record of the Eurasian Neogene insectivores (Erinaceomorpha, Soricomorpha, Mammalia), Part I.: China. Scripta Geol. Spec. Issue 5, 37–50.

Rambaut, A., Drummond, A., 2009. Tracer v1.5 <http://tree.bio.ed.ac.uk/software/tracer/>.

Sambrook, J., Russell, D., 2001. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press.

Sanchez-Villagra, M.R., Horovitz, I., Motokawa, M., 2006. A comprehensive morphological analysis of talpid moles (Mammalia) phylogenetic relationships. Cladistics 22, 59–88.

Schwarz, G., 1978. Estimating the dimension of a model. The annals of statistics 6, 461–464.

Shimodaira, H., 2002. An Approximately Unbiased test of Phylogenetic Tree Selection. Syst. Biol. 51, 492–508.

Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17, 1246–1247.

Shinohara, A., Kawada, S., Harada, M., Koyasu, K., Oda, S.-I., Suzuki, H., 2008. Phylogenetic relationships of the short-faced mole, Scaptochirus moschatus (Mammalia: Eulipotyphla), among Eurasian fossorial moles, as inferred from mitochondrial and nuclear gene sequences. Mamm. Study 33, 77–82.

Song, C.H., Fang, X.M., Li, J.J., Gao, J.P., Zhao, Z.J., Fan, M.J., 2001. Tectonic uplift and sedimentary evolution of the Jiuxi Basin in the northern margin of the Tibetan Plateau since 13 Ma BP. Sci. China Series D – Earth Sci. 44, 192–202.

Song, Y.G., Fang, X.M., Torii, M., Ishikawa, N., Li, J.J., An, Z.S., 2007. Late Neogene rock magnetic record of climatic variation from Chinese eolian sediments related to uplift of the Tibetan Plateau. J. Asian Earth Sci. 30, 324–332.

Sorenson, M., Franzosa, E., 2007. TreeRot. Boston University, Boston, MA.

Springer, M.S., Douzery, E., 1996. Secondary structure and patterns of evolution among mammalian mitochondrial 12S rRNA molecules. J. Mol. Evol. 43, 357–373.

Springer, M.S., Meredith, R.W., Teeling, E.C., Murphy, W.J., 2013. Technical comment on "The placental mammal ancestor and the post-K-Pg radiation of placentals". Science 341, 613–613.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol. 57, 758–771.

Stroganov, A., 1948. Systematics of moles (Talpidae). Trudy Zoologicheskogo Instituta AN SSSR [Proceedings of Zoological Institute, Academy of Sciences, USSR], pp. 286–405.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). version.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Teeling, E.C., Scally, M., Kao, D.J., Romagnoli, M.L., Springer, M.S., Stanhope, M.J., 2000. Molecular evidence regarding the origin of echolocation and flight in bats. Nature 403, 188–192.

Xiong, Y., Brandley, M.C., Xu, S., Zhou, K., Yang, G., 2009. Seven new dolphin mitochondrial genomes and a time-calibrated phylogeny of whales. BMC Evol. Biol. 9, 20.

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proc. Nat. Acad. Sci. 107, 9264.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292, 686–693.

Zemlemerova, E.D., Bannikova, A.A., Abramov, A.V., Lebedev, V.S., Rozhnov, V.V., 2013. New data on molecular phylogeny of the East Asian moles. Doklady Biol. Sci. 451, 257–260.

Zhang, C., Wang, Y., Deng, T., Wang, X., Biasatti, D., Xu, Y., Li, Q., 2009. C4 expansion in the central Inner Mongolia during the latest Miocene and early Pliocene. Earth Planet. Sci Lett. 287, 311–319.

Zhang, C., Zhang, D.X., Zhu, T.Q., Yang, Z.H., 2011. Evaluation of a Bayesian coalescent method of species delimitation. Syst. Biol. 60, 747–761.

Ziegler, R., 2012. Moles (Talpidae, Mammalia) from Early Oligocene karstic fissure fillings in South Germany. Geobios 45, 501–513.