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An enigmatic pygmy dormouse: molecular and morphological evidence for the species taxonomic status of *Typhlomys chapensis* (Rodentia: Platacanthomyidae)

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Abstract

Background: The taxonomic position of enigmatic pygmy dormouse *Typhlomys* (Rodentia: Platacanthomyidae) from Vietnam is reconsidered based on both morphology and sequence data.

Results: The analysis of mitochondrial and nuclear genes has shown that the pygmy dormouse from Lao Cai Province of northern Vietnam belongs to a distinct phylogenetic lineage of *Typhlomys*. The DNA analysis has demonstrated a strong genetic difference (0.245 to 0.252 for the cytochrome oxidase gene (COI), 0.079 to 0.082 for interphotoreceptor retinoid-binding protein gene (IRBP), and 0.028 for the growth hormone receptor gene (GHR) between this lineage and the sample from South China. Multivariate analysis of cranial and dental data, as well as of some external characters, has also separated the Vietnamese population from the pygmy dormouse from Fujian in southern China, the type locality of *Typhlomys cinereus* (Bull Soc Philomath Paris 12:8–10, 1877).

Conclusions: Both genetic and morphological data confirm that there is a second species, *Typhlomys chapensis* (Field Mus Nat Hist Zool Ser 18:193–339, 1932), in the heretofore monotypic genus *Typhlomys*.

Keywords: Mitochondrial DNA; Nuclear DNA; Morphology; Systematics; *Typhlomys chapensis*

Background

The enigmatic family Platacanthomyidae includes morphologically unique small rodents sporadically distributed in highlands of Southeast Asia (Musser and Carleton 2005). Evolutionary relationships of the platacanthomyids had been uncertain until a molecular phylogenetic study found the group to be the earliest extant lineage to split within the superfamily Muroidea (Jansa et al. 2009). This smallest murid family is currently composed of only two monotypic genera, *Platacanthomys* and *Typhlomys*. The spiny tree dormouse *Platacanthomys lasiurus* Blyth, 1859 has a restricted distribution in mountainous regions of southwestern India (Corbet and Hill 1992; Jayson and Jayaharia 2009). The pygmy dormouse, or the soft-furred tree dormouse, *Typhlomys cinereus* (Milne-Edwards 1877) is known from southern China (Wang et al. 1996; Smith 2008), with an outlying population at high elevations of Hoang Lien Mountains in northern Vietnam (Can et al. 2008; Abramov et al. 2012).

Little is known about the natural history of the pygmy dormouse because it has rarely been observed alive by scientists. To date, this species has been recorded only from the high mountain forests of southeastern China and northwestern Vietnam. These rodents closely resemble the dormice having long hairy tail and prominent ears. Their very small, reduced eyes, which resemble those of moles or shrews, gave them their generic name *Typhlomys* meaning a 'blind mouse'. Such eye morphology seems to suggest the burrowing lifestyle, whereas long semi-prehensile tail, long vibrissae, and large ears are evidence that it is definitely an arboreal animal.

The species composition of *Typhlomys* is still unclear because of the scarcity of museum materials available for

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study. A few taxonomic forms have been recognized on the basis of differences in body size and fur coloration (Wang et al. 1996; Musser and Carleton 2005). The Chinese pygmy dormouse *Typhlomys cinereus* was described from Fokien (=Fujian) in southern China (Milne-Edwards 1877). According to the taxonomic review of Wang et al. (1996), the nominotypical *Typhlomys cinereus cinereus* is distributed in northern Fujian and Zhejiang, southern Anhui, China. Three other Chinese subspecies have restricted ranges: *Typhlomys cinereus daloushanensis* Wang et Li, 1996 is known from southern Sichuan, Shaanxi, Gansu, Hubei, and Guizhou; *Typhlomys cinereus guangxiensis* Wang et Chen, 1996 is distributed in southwestern Guangxi; and *Typhlomys cinereus jingdongensis* Wu et Wang, 1984 was found in Yunnan. The Vietnamese population was described by Osgood (1932) as a separate species, *T. chapensis*, which is now considered a subspecies of *T. cinereus* (Corbet and Hill 1992; Wang et al. 1996; Musser and Carleton 2005).

Several specimens of *T. cinereus* were collected in the Hoang Lien Mountains, northwestern Vietnam during the mammalogical surveys carried out by the Joint Vietnam-Russian Tropical Research and Technological Centre. In the present study, sequences of mtDNA and nDNA genes of the pygmy dormouse from northern Vietnam have been analyzed and compared with those of Chinese *Typhlomys* for the first time. The taxonomic position of *Typhlomys* from Vietnam is thus reconsidered based on both morphology and sequence data.

Methods

Field works were conducted in 2009 to 2012 on the northern slope of the Fan Si Pan mountain area near Tram Ton Station, approximately 6 km west of Sapa (22°21′ N, 103°46′ E) in Lao Cai Province, Vietnam. Cage live traps and pitfall traps were used to collect small mammals. In total, thirteen specimens of *T. cinereus* were collected. Most of the animals were trapped by live cage traps set on the branches and ground in the montane tropical forest with bamboo underbrush; also, some animals were trapped by pitfall traps. Standard external body measurements (head and body length, tail length, hind foot length, and ear length) were taken in the field. Tissue samples were preserved in 96% ethanol. The specimens (skulls and skins) are kept in the Zoological Institute, Russian Academy of Sciences, Saint-Petersburg, Russia (ZIN).

The skulls and skins were compared with specimens kept in the collections of the Natural History Museum, London, UK (BMNH). For each adult skull, a series of 14 craniodental variables was taken: greatest length of skull (GL), condylobasal length (CBL), basal length (BL), palatal length (PL), interorbital breadth (IB), braincase breadth (BB), braincase height (BH), zygomatic width (ZW), diastema length (DL), nasal length (NL), upper molar row length (UML), lower molar row length (LML), breadth across upper molars (BUM), and length of foramina incisive (LFI). The variables were measured with digital calipers, to the nearest 0.01 mm. In total, 23 skulls of pygmy dormice from Vietnam (Sapa, $n = 15$) and South China (Fujian, $n = 8$) were studied (see the 'Appendix' section). For comparison, we used the external measurements available on museum tags, apparently representing measurements obtained in the field by original collectors.

Principal components analysis (PCA) and canonical discriminant function analysis (DFA) were used to evaluate distinctiveness among these samples. A one-way analysis of variance (ANOVA) was performed to test the differences among groups on all cranial variables. The software program Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA) was used for all analytical procedures.

Total DNA from 96% ethanol-preserved muscle tissue was extracted using a routine phenol/chloroform/proteinase K protocol (Kocher et al. 1989; Sambrook et al. 1989). The DNA was further purified by twofold ethanol precipitation or using a DNA Purification Kit (Fermentas, Thermo Fisher Scientific Inc., Pittsburgh, PA, USA). Four genes which proved to be useful for the phylogenetic analysis of the Asiatic murids (Suzuki et al. 2000, 2003; Michaux et al. 2002; Jansa et al. 2006) were targeted. These genes included the complete cytochrome *b* (cyt *b*) gene (1,143 bp), a portion (up to 1,610 bp) of the first exon of interphotoreceptor retinoid-binding protein (IRBP), and a portion (815 bp) of exon 10 of growth hormone receptor (GHR) which were amplified for further analysis. We also analyzed the 5′-proximal 680 bp portion of subunit I of the cytochrome oxidase gene (COI), which is generally used for species diagnoses and for DNA barcoding for a number of mammals. The cyt *b* was amplified using the L14723 and H15915 primers (Irwin et al. 1991). The COI gene was amplified using the universal conservative primers BatL 5310 and R6036R (Kocher et al. 1989; Irwin et al. 1991). The following universal PCR protocol was used to amplify both of the mtDNA fragments: initial denaturation for 1 min and 30 s at 95°C, denaturation for 30 s at 95°C, annealing for 1 min at 52°C, and elongation for 30 s at 72°C, followed by terminal elongation for 2 min at 72°C. The PCR reaction was performed in a 30- to 50-μl volume that contained 2.5 to 3 μl 10× standard PCR buffer (Fermentas), 50 mM of each dNTP, 2 mM $MgCl_2$, 10 to 12 pmole of each primer, 1 U of Taq DNA polymerase (Fermentas), and 0.5 μl (20 to 50 ng) of total DNA template per tube. The reaction was performed using a Tercik (DNK-Tekhnologia, Protvino, Moscow Province, Russia) thermocycler. The IRBP gene (1,000 to 1,610 bp) was amplified using the IRBP125f, IRBP1435r, IRBP1125r, and IRBP1801r primers (Suzuki et al. 2000), according to the method of Stanhope et al. (1992). Nested PCR

technique was applied for GHR gene following the method of Jansa et al. (2009). An approximately 1.0 kb of exon 10 from the GHR gene was amplified using primers GHRF1 and GHRendAlt. This polymerase chain reaction product was reamplified using nested primer GHRF1 paired with GHR750R and GHRF50 paired with GHRendAlt. The PCR products were purified using a DNA Purification Kit (Fermentas). The double-stranded DNA products were directly sequenced in both directions using Applied Biosystems 3130 Genetic Analyzers and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Thermo Fisher Scientific Inc.). All of the sequences that were obtained were deposited in GenBank (KC209546-KC209557; KC209570-KC209577; KJ949607-KJ949615).

As a comparative material, we analyzed all the IRBP and GHR sequences from different Muridae and some other rodent groups used by Jansa et al. (2009) including the sequences for *T. cinereus daloushanensis* collected from Guizhou Province, China (voucher deposited at Royal Museum of Ontario, Canada; ROM 118593). The GenBank accession number GQ272606 is for IRBP, and GQ272603 for GHR genes (see Jansa et al. 2009). We also included into the dataset an original COI sequence (JF444274) descended from the same specimen which was presented by Eger et al. (unpublished) as a direct submission to GenBank in 2011 (released in 2012).

There are no data on cyt *b* gene currently available for *T. cinereus* in the GenBank database; thus, the cyt *b* sequences presented here (KC209548 to KC209557) can be regarded as priority genetic vouchers for this group.

In total, ten animals were genotyped (ten for cyt *b*, eight for COI gene, two for IRBP, and nine for GHR genes, see the 'Appendix' section). The sequences were aligned using BioEdit (Ibis Biosciences, Carlsbad, CA, USA) (Hall 1999) and Clustal W (incorporated into BioEdit and MEGA 5.05) software and were verified manually. Both the basic sequence parameter calculations (i.e., variable sites, parsimony informative sites, base composition biases, nucleotide frequencies, and nucleotide substitution tables) and the best-fitting gene evolution models as well as inter- and intrapopulation divergence evaluations were performed using the MEGA 5.05 software (Tamura et al. 2011). The most frequently used algorithms, such as maximal parsimony (MP) and maximal likelihood (ML), were applied for the phylogenetic reconstructions and tree constructions using the MEGA 5.05 software. Bayesian analyses were also performed using MRBAYES v.3.1 software (Huelsenbeck and Ronquist 2001).

For trees construction, a number of nucleotide evolution models were tested by MEGA 5.05 models module. As a result, the GTR + G + I substitution model was used for the IRBP gene, and the Kimura 2-parameter + G + I model was proved to the best for GHR gene evolution. The gamma shape parameters for the concatenated dataset were evaluated and calculated from a general dataset. The robustness of the tree was assessed using the bootstrap procedure with 1,000 replications. All of the trees were constructed and visualized directly with MEGA 5.05 or with TreeView 1.6.6 software (Page 1996). For the Bayesian analyses, four independent runs of 1,000,000 generations each were performed under the GTR + G + I substitution model. We used a flat Dirichlet prior for the relative nucleotide frequencies and for the relative rate parameters, a discrete uniform prior for the topologies, and an exponential distribution for the gamma shape parameter and all branch lengths. A burn-in period of 100,000 generations was determined graphically using TRACER v.1.4 (Rambaud and Drummond 2007) to ensure convergence and to be certain that the runs were not trapped on local optima.

The divergence times between lineages were estimated on the basis of the mean net intergroup distance (taking into account the correction for ancestral mtDNA polymorphism) between the lineages. One fossil-based calibration point was used. For segregation time evaluation, an average time of most investigated Muridae genera splits the *Apodemus*/*Micromys*/*Mus*/*Rattus* divergence events (12 million years; which is equal to $d = 0.090$ for IRBP gene, Suzuki et al. 2004).

Results

DNA analysis

Even at the step of preliminary sequence alignment, the samples from the Vietnamese population were found to be substantially different from the Chinese *T. cinereus*, both for mtDNA and for nDNA genes. As compared with the sequences from Guizhou Province (GQ272606 for IRBP and GQ272603 for GHR genes) discussed by Jansa et al. (2009) and the COI sequences (JF444274) presented by Eger et al. (unpublished), not only tremendous genetic distances (0.245 to 0.252 for COI, 0.079 to 0.082 for IRBP, and 0.028 for GHR genes) but also the considerable structural rearrangements of the genes were discovered for some genes (Figure 1). For example, one triplet and another one double-triplet deletion have been revealed in the homological 630-bp part of the COI gene in the original samples from Sapa, and another three triplet insertions can be found in the homological 1,260-bp part of the IRBP gene. No structural rearrangements have been observed in the GHR gene. Such the extra ordinary level of variation obviously overcomes the level of genetic intraspecific variability known in mammalian species, even if geographically distant and completely isolated subspecies are concerned. This fact brings up the question about reliability of the species assignment and yet has drawn our attention to the accuracy of the undertaken data analysis in order to exclude any methodological artifacts during the sample preparation.

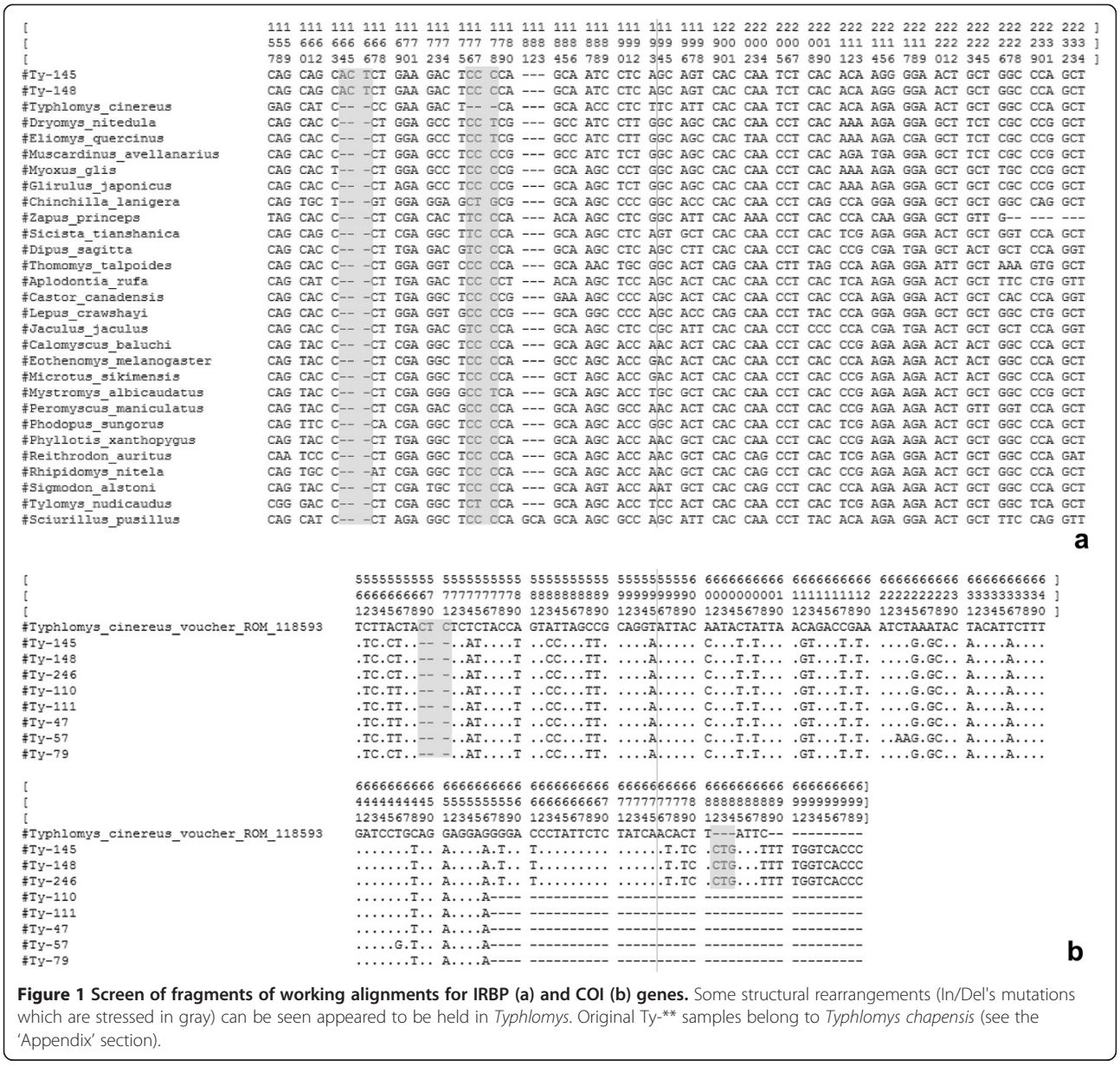

Figure 1 Screen of fragments of working alignments for IRBP (a) and COI (b) genes. Some structural rearrangements (In/Del's mutations which are stressed in gray) can be seen appeared to be held in *Typhlomys*. Original Ty-** samples belong to *Typhlomys chapensis* (see the 'Appendix' section).

We have checked out all the COI, IRBP, and GHR gene sequences obtained for cross-contaminations with a special emphasis for the possible numt pseudogene occurrence for COI gene sequences (Triant and DeWoody 2007). No traces of contamination events, no occurrence of additional stop codons, no reading frame shift or reliable transition/transversion or position bias as compared with normal mammalian mtDNA sequences have been detected. These facts, together with the concerted character of mitochondrial COI gene and nuclear IRBP and GHR gene variability, allow us to conclude that our data are very special and resulted in valid genetic vouchers rather than in artificial products of laboratory contamination or methodological artifacts.

The final argument to demonstrate the reliability of our samples has been the phylogenetic analysis, which has been performed in full integrity of the IRBP and GHR gene sequence data for the rodent lineages used in Jansa et al. (2009) including both data for Vietnamese and Chinese *Typhlomys.* The consensus phylogenetic trees (ML, BY, T3P, and K2P algorithms) are presented in Figure 2. It can be seen that in spite of considerable genetic distances, the obtained tree topology and the level of nodes bootstraps/posterior probabilities are in full agreement with the data presented by Jansa et al. (2009). So, the validity of the studied samples is obvious. Nevertheless, the Vietnamese samples construct an independent, very divergent but yet highly reliable sister clade with the Chinese sample. The

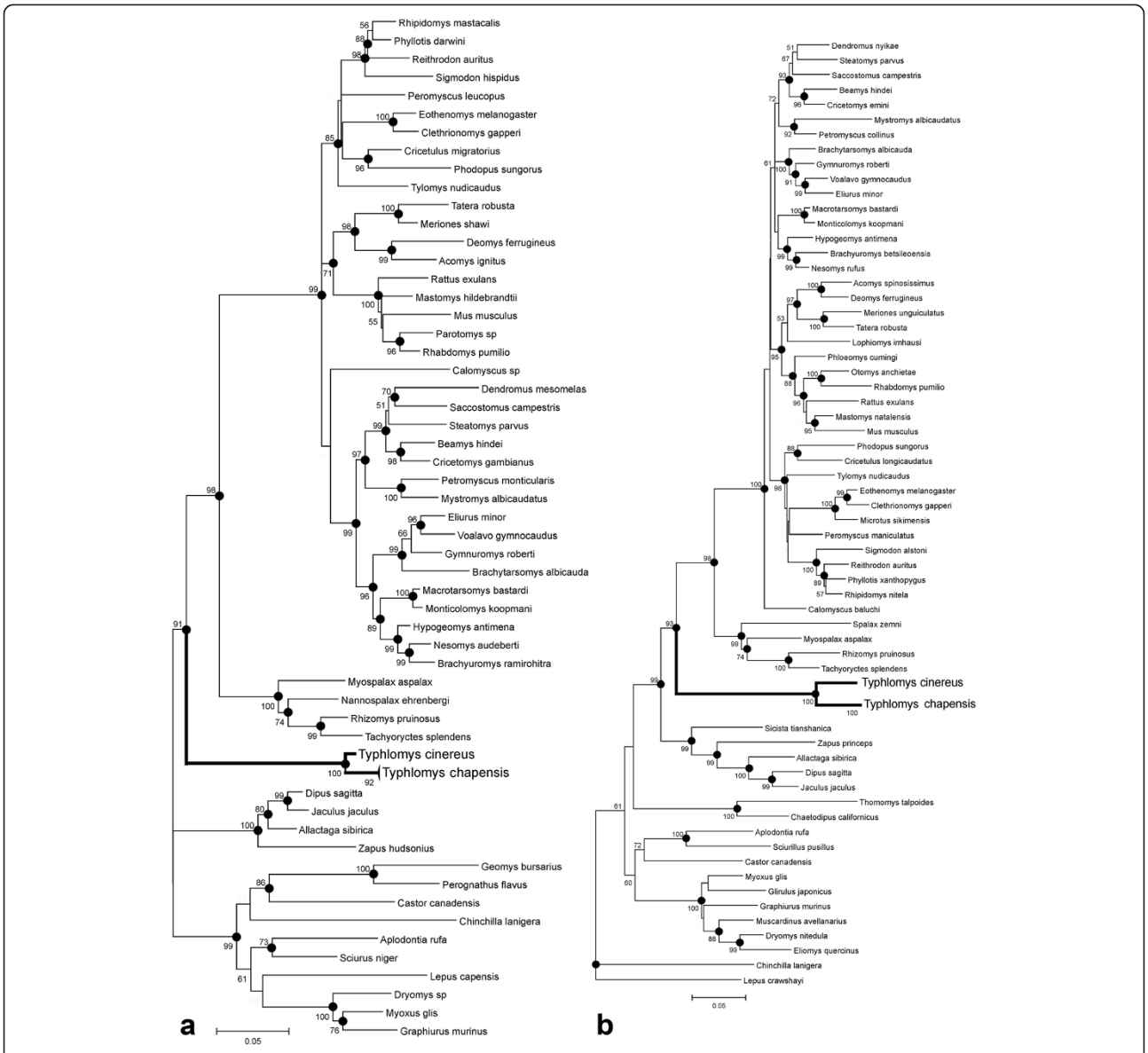

Figure 2 The phylogenetic tree resulting from maximum-likelihood analysis. (a) The interphotoreceptor retinoid-binding protein gene (IRBP) dataset under its best-fit model (GTR + G + I). **(b)** Growth hormone receptor gene (GHR) dataset under its best-fit model (K2P + G + I). Nodal support from a maximum-likelihood bootstrap analysis is indicated over the nodes (values less than 50 are not shown). For the Bayesian analysis, black circles indicate posterior probability values that exceed 0.8.

average divergence time estimated on the basis of the IRBP gene sequences is no less then 8.7 million years. An analysis of intergroup diversity performed based on the same dataset has shown that the level of *Typhlomys* lineages divergence is as high as or even more elevated than the level that proved to be characteristic for the majority of Muridae genera (0.065-0.090 for IRBP, Suzuki et al. 2004).

Morphology

A summary of the descriptive statistics of morphometric variables is given in Table 1. In a principal components analysis drawing on 14 craniodental measurements, the Vietnamese and Chinese specimens are grouped together, and these groups are essentially discrete (Figure 3). The two groups diverge along the first principal component, reflecting particular differences in an overall cranial size. Discriminant function analysis that draws on the same variables has provided another means of illuminating these and other morphometric distinctions (Figure 4, Table 2). The discrimination between two groups has been most strongly based upon the first canonical axis (CAN 1). The variables that greatly contributed to the

Table 1 Skull measurements of *Typhlomys*

Characters	Lao Cai Province, Vietnam		Fujian Province, China	
	Males ($n = 4$)	Females ($n = 11$)	Males ($n = 4$)	Females ($n = 4$)
GL	24.55, 0.97	24.53, 1.11	22.28, 0.53	22.50, 0.38
	23.52 to 25.87	22.77 to 25.9	21.65 to 22.82	22.00 to 22.80
CBL	22.15, 1.32	22.05, 1.19	20.07, 0.37	20.56, 0.44
	20.76 to 23.94	20.39 to 23.65	19.76 to 20.50	19.95 to 20.97
BL	20.28, 1.34	20.13, 1.164	18.56, 0.71	18.97, 0.31
	18.85 to 22.06	18.55 to 22.00	17.68 to 19.16	18.50 to 19.15
PL	6.47, 0.09	6.51, 0.35	5.58, 0.15	5.58, 0.13
	6.35 to 6.58	5.80 to 6.80	5.46 to 5.80	5.43 to 5.73
IB	5.53, 0.36	5.48, 0.27	5.13, 0.08	5.01, 0.17
	5.06 to 5.87	5.17 to 6.06	5.06 to 5.22	4.84 to 5.19
BB	11.43, 0.40	11.08, 0.50	10.30, 0.59	9.99, 0.28
	11.10 to 12.01	10.20 to 11.86	9.76 to 10.90	9.60 to 10.23
BH	7.92, 0.68	8.23, 0.54	6.89, 0.48	7.13, 0.29
	7.32 to 8.72	7.37 to 9.05	6.30 to 7.31	6.72 to 7.37
ZW	12.88, 0.75	13.04, 0.79	11.73, 0.46	11.81, 0.41
	12.10 to 13.87	11.38 to 14.08	11.22 to 12.26	11.45 to 12.40
DL	6.85, 0.33	6.98, 0.39	5.85, 0.47	6.20, 0.28
	6.41 to 7.17	6.20 to 7.54	5.45 to 6.45	5.94 to 6.60
NL	7.21, 0.27	7.43, 0.34	6.83, 0.46	6.73, 0.38
	6.89 to 7.55	6.97 to 7.90	6.15 to 7.13	6.31 to 7.20
UML	3.96, 0.27	3.85, 0.19	3.63, 0.23	3.46, 0.11
	3.57 to 4.14	3.55 to 4.09	3.39 to 3.86	3.40 to 3.62
LML	4.16, 0.26	4.14, 0.23	3.74, 0.11	3.76, 0.19
	3.79 to 4.36	3.80 to 4.51	3.65 to 3.89	3.55 to 3.98
BUM	5.66, 0.29	5.56, 0.27	5.04, 0.08	5.01, 0.09
	5.24 to 5.90	5.24 to 5.90	4.94 to 5.12	4.88 to 5.11
LFI	2.11, 0.08	2.04, 0.28	1.87, 0.11	1.89, 0.09
	2.00 to 2.19	1.75 to 2.47	1.73 to 2.00	1.80 to 2.02

Mean, standard deviation, and min-max values of skull measurements (in millimeters) of adult *Typhlomys* specimens originated from Vietnam and China.

first axis and based on standardized canonical coefficients have been the greatest length of skull, the basal length, braincase height, and breadth across upper molars. Both populations display no remarkable sexual dimorphism (Table 1).

The skull of Vietnamese *Typhlomys* is relatively large, with the markedly enlarged braincase (see Table 1 and Figure 5). These cranial distinctions are complemented by some external distinctions. Means and extremes of measurements (in millimeters) of Vietnamese pygmy dormice from 5 males are head and body length, 83.0 (79 to 86); tail length, 121.7 (110 to 135); hind foot length, 21.8 (20 to 23); and ear length, 17.8 (17 to 19), and from 17 females are head and body length, 80.5 (70 to 98); tail length, 116.9 (100 to 134); hind foot length, 22.2 (21 to 24); and ear length, 17.7 (14 to 19). Chinese pygmy dormice *T. cinereus cinereus* are obviously smaller. According data from Wang et al. (1996), external measurements for 15 adults are head and body length, 77.1 (70 to 89); tail length, 99.9 (92 to 111); hind foot length, 19.5 (18.5 to 20); and ear length, 14.0 (11 to 16). The pelage coloration of the Vietnamese specimens is also different from that of Chinese counterparts. The dorsal pelage of the Sapa specimens is uniformly blackish gray (Abramov et al. 2012: Figure four); the ventral surface is almost of the same coloration contrary to the mouse-gray dorsal pelage with grayish white underside in the specimens from Fujian. The

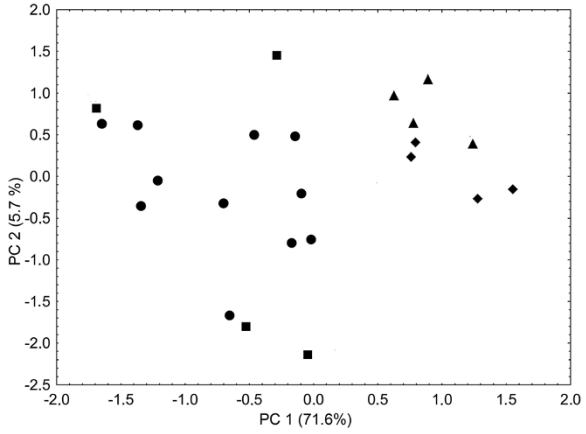

Figure 3 Results of the principal components analysis. Ungrouped morphometric separation of *Typhlomys* specimens; the data were drawn from 14 cranial measurements. Symbols: circles = Vietnam, females; squares = Vietnam, males; triangles = China, females; diamonds = China, males.

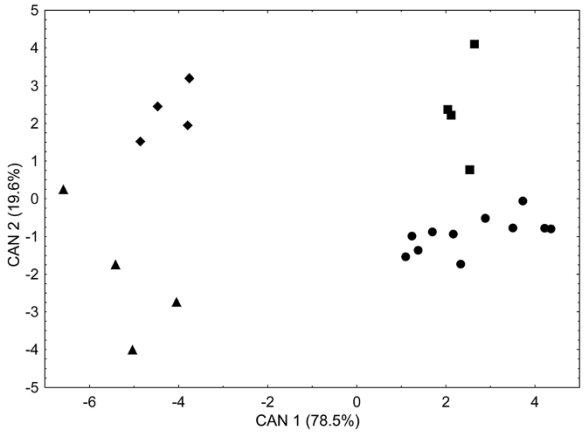

Figure 4 Results of the discriminant function analysis. Grouped morphometric separation drawn from the same specimens and measurements for *Typhlomys* from Vietnam and China. The meanings of the symbols are the same as those in Figure 3.

Table 2 Results of multivariate analyses

Characters	PCA		DFA			ANOVA			
						Males		Females	
	PC 1	PC 2	CAN 1	CAN 2	CAN 3	*F*	*p*	*F*	*p*
GL	−0.985	−0.014	0.312	−0.056	0.263	16.79	0.006	12.25	0.004
CBL	−0.977	0.159	0.228	−0.068	0.342	9.15	0.023	5.72	0.032
BL	−0.959	0.225	0.180	−0.046	0.311	5.12	0.064	3.71	0.076
PL	−0.888	−0.204	0.467	−0.066	0.167	96.65	0.000	25.93	0.000
IB	−0.744	−0.418	0.232	0.066	0.037	4.72	0.072	10.59	0.006
BB	−0.852	−0.106	0.297	0.155	0.278	10.06	0.019	16.29	0.001
BH	−0.548	0.114	0.295	−0.163	0.045	6.17	0.047	14.84	0.002
ZW	−0.938	0.098	0.240	−0.067	0.056	6.78	0.040	8.43	0.012
DL	−0.920	−0.015	0.318	−0.192	0.344	11.88	0.014	12.61	0.004
NL	−0.760	0.035	0.227	−0.069	−0.294	1.98	0.209	11.66	0.005
UML	−0.742	−0.397	0.234	0.156	0.075	3.56	0.108	14.94	0.002
LML	−0.831	−0.348	0.252	−0.024	0.199	8.81	0.025	8.93	0.010
BUM	−0.886	−0.419	0.334	0.042	0.320	16.95	0.006	15.94	0.002
LFI	−0.690	−0.125	0.113	0.028	0.245	11.99	0.013	1.12	0.309
Cumulative variance (%)	71.6	77.3	78.5	98.1	100.0				

Factor loadings and cumulative variance for the principal components in the principal components analysis illustrated in Figure 2 and canonical correlations and cumulative variance for the canonical variates in the discriminant function analysis illustrated in Figure 3.

upper surface of the hind feet in Vietnamese animals is dark colored, whereas it is whitish in Chinese ones.

Discussion

The phylogenetic analysis of mitochondrial and nuclear genes has shown a significant divergence between Vietnamese and Chinese pygmy dormice. It is obvious that the two *Typhlomys* clades are to be regarded as species level lineages. Moreover, the level of their divergence is more consistent with the generic level for many of rodents (Jansa et al. 2006; see also Figure 2). The morphological analysis has also revealed significant

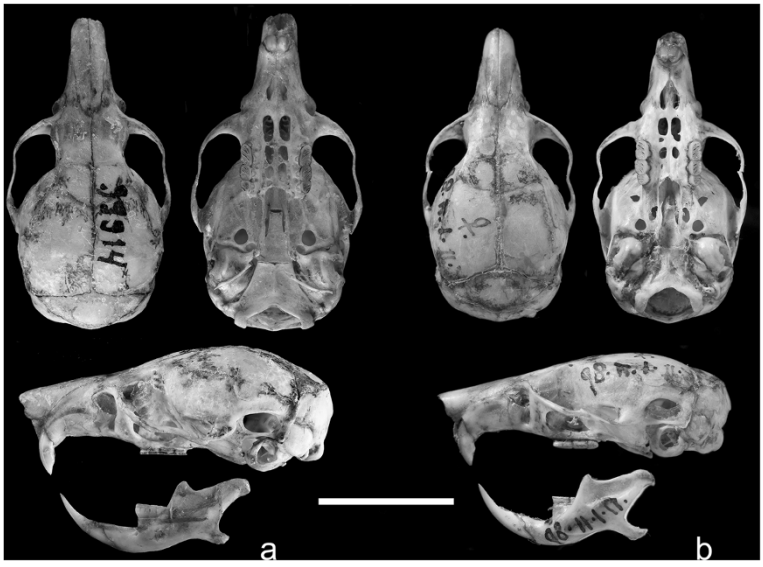

Figure 5 Dorsal, ventral, and lateral views of the cranium, and lateral view of mandible. *Typhlomys chapensis*, Vietnam, Sapa, ZIN 99914 **(a)** and *Typhlomys cinereus*, China, Fujian, BMNH 98.11.1.11 **(b)**. Scale bar = 1 cm. Credit the images: **a** – Alexei V. Abramov, **b** - © The Trustees of the Natural History Museum, London.

differences in the cranial and external characters between the populations from Vietnam and China. Together, these data suggest that a reassessment of the taxonomy of *Typhlomys* is required. The latest viewpoint that recognizes the monotypic *Typhlomys cinereus* with five subspecies (Corbet and Hill 1992; Wang et al. 1996; Musser and Carleton 2005) does not reflect the actual variation.

Conclusions

Our data have confirmed the earlier assumptions of Osgood (1932) and Smith (2008) about a species rank for the Vietnamese *T. chapensis*. According to the morphometric analysis of Wang et al. (1996), the populations of *chapensis* and *guangxiensis* are phenetically most alike, clustering apart from the other three taxa (*cinereus*, *daloushanensis*, and *jingdongensis*). Due to the lack of morphological and genetic data from southern China, where *guangxiensis* occurs, we have had no possibility to re-assess the taxonomic status of the latter form. Based on the orography of this region alone, one can assume that the *guangxiensis* from southwestern Guangxi is most likely to belong to *T. chapensis* rather than to *T. cinereus*.

Given the complex geography of southern China and especially the influence of many isolated mountain ranges, it is possible that multiple *Typhlomys* taxa, perhaps of a species rank, exist. In order to resolve this issue, a thorough geographic sampling that should include samples from China representing a wide geographic coverage is required. In addition, an inclusion of unrepresented subspecies of *Typhlomys*, including the samples from type localities, is essential for determining the priority of available names in case taxa are to be elevated to species.

Appendix

Specimens included in the study

The following are acronyms prefacing specimen numbers: BMNH, The Natural History Museum, London, UK and ZIN, Zoological Institute, Russian Academy of Sciences, Saint-Petersburg, Russia.

The specimens included in the morphological study are the following:

Vietnam, Lao Cai Province, Sapa District: ZIN 99914, ZIN 99915, ZIN 99916, ZIN 100882, ZIN 100883, ZIN 100884, ZIN 100885, BMNH 33.41.380, BMNH 33.41.381, BMNH 33.41.382, BMNH 33.41.383, BMNH 33.41.384, BMNH 33.41.385, BMNH 33.41.387, and BMNH 33.41.388.

China, Fujian Province: BMNH 96.1.2.27, BMNH 88.11.107, BMNH 88.11.108, BMNH 88.11.109, BMNH 88.11.110, BMNH 88.11.111, BMNH 88.11.118, BMNH 98.11.1.10, and BMNH 98.11.1.11.

The following are the specimens from Vietnam, Lao Cai Province, Sapa District, that are included in the molecular analyses:

ZIN 99914, genetic voucher Ty-145, GenBank number (IRBP) KC209546, GenBank number (cyt *b*) KC209551, GenBank number (COI) KC209573, and GenBank number (GHR) KJ949612;
ZIN 99916, genetic voucher Ty-148, GenBank number (IRBP) KC209547, GenBank number (cyt *b*) KC209552, GenBank number (COI) KC209574, and GenBank number (GHR) KJ949613;
ZIN 100411, genetic voucher Ty-411, GenBank number (cyt *b*) KC209555 and GenBank number (GHR) KJ949615;
ZIN 100882, genetic voucher Ty-246, GenBank number (cyt *b*) KC209553, GenBank number (COI) KC209575, and GenBank number (GHR) KJ949614;
ZIN 100883, genetic voucher Ty-247, and GenBank number (cyt *b*) KC209554;
ZIN 101563, genetic voucher Ty-47, GenBank number (cyt *b*) KC209548, GenBank number (COI) KC209570, and GenBank number (GHR) KJ949607;
ZIN 101564, genetic voucher Ty-57, GenBank number (cyt *b*) KC209549, GenBank number (COI) KC209571, and GenBank number (GHR) KJ949608;
ZIN 101565, genetic voucher Ty-79, GenBank number (cyt *b*) KC209550, GenBank number (COI) KC209572, and GenBank number (GHR) KJ949609;
ZIN 101566, genetic voucher Ty-110, GenBank number (cyt *b*) KC209556, GenBank number (COI) KC209576, and GenBank number (GHR) KJ949610;
ZIN 101567, genetic voucher Ty-111, GenBank number (cyt *b*) KC209557, GenBank number (COI) KC209577, and GenBank number (GHR) KJ949611.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AVA and WR conceived and coordinated the study. AEB gathered and analyzed the DNA sequences. AVA performed the morphological and taxonomical assessments. All authors read and approved the final manuscript.

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