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Genetic and morphologic diversity of the moles (Talpomorpha, Talpidae, *Mogera*) from the continental Far East

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Abstract

Taxonomy of the East Asian moles of the genus *Mogera* is still controversial. Based on the sequence data of 12 nuclear genes and one mitochondrial gene, we examine genetic variation in the *Mogera wogura* species complex and demonstrate that *M. robusta*, from the continental Far East, and *M. wogura*, from the Japanese Islands, are not conspecific. Our data do not support the existence of two or more species of *Mogera* in the Russian Far East. We suggest that the form "*coreana*" from the Korean Peninsula should be treated as a subspecies of *M. robusta*. Our morphological analysis shows that *M. r. coreana* differs from typical *M. robusta*, from Primorye, primarily in its smaller size. We show that there is strong morphological variability among continental moles, which may be associated with ecological and geographical factors. The time since the split between *M. wogura* s. str. and *M. robusta* dates back to the Middle Pleistocene (0.30–1.0 Myr), while *M. r. coreana* separated from *M. r. robusta* in the Upper Pleistocene (0.04–0.18 Myr). The results of the mismatch analysis indicate recent demographic expansion in populations of moles from Primorye.

KEYWORDS
continental Far East, cranial variation, molecular dating, moles, nuclear genes

1 | INTRODUCTION

The genus *Mogera* Pomel, 1848 (Talpomorpha, Talpidae), includes 5–7 species of subterranean moles which are widely distributed in insular and continental parts of East Asia (Abe, 1995; Hutterer, 2005; Kawada et al., 2001, 2007; Motokawa & Abe, 1996; Zemlemerova, Bannikova, Abramov, Lebedev, & Rozhnov, 2013). One of the most intriguing problems of *Mogera* taxonomy is the number of species that are distributed in the continental Far East. It is still unclear whether all moles from the continental Far East and the southern part of the

Japanese archipelago should be classified as a single polymorphic species Japanese mole, *M. wogura* Temminck, 1842 or if it would be better to split the latter into several distinct species. First, the taxonomic status of the Ussuri mole, *Mogera robusta* Nehring, 1891 should be clarified. It has been shown that the Ussuri mole can be distinguished from the *M. wogura* sensu stricto by its large body size (the source of its Latin name), morphology of the auditory ossicles and certain dental characters (Stroganov, 1948), pelage color, glans penis shape, and preanal glands (Okhotina, 1965, 1966). Based on this, some researchers recognize *M. robusta* as a distinct species (Corbet, 1978; Ohdachi, Ishibashi, Iwasa, & Saitoh, 2009; Okhotina, 1966; Stroganov, 1948); however, others treated it as a subspecies of *M. wogura* (Abe, 1995; He, Shinohara, Jiang, & Campbell, 2014; Hutterer, 2005).

Second, if *M. robusta* is accepted as a full species, then the status of the smaller moles occurring in Korea, adjacent regions of China and Primorye (Russian Far East), should be addressed. Some researchers (Stroganov, 1948) believe that they should be regarded as a continental form of *M. wogura*, which was described as a distinct subspecies *M. w. coreana* Thomas, 1907. Moreover, the smaller form was reported to occur in sympatry with *M. robusta* in the Korean Peninsula and NE China (Stroganov, 1957; Yudin, 1971, 1989) but not co-occur with it in southern Primorye (Khasan district) (Okhotina, 1966). Some ecological differences between the two putative species were described (Okhotina, 1966). In contrast, Corbet (1978) treated *coreana* as a distinct subspecies of *M. robusta*. Finally, it cannot be ruled out that smaller continental moles belong to a separate species distinct from both *M. robusta* and *M. wogura*. Thus, the classification of *Mogera* from the continental Far East is still controversial.

Tsuchiya et al. (2000) using partial (402 bp) cytochrome b (*cytb*) sequences demonstrated a complex pattern of variation within the *M. wogura* sensu lato but revealed no substantial divergence among the samples from South Korea and Primorye, with the exception of a single sequence from the Ussuri Nature Reserve (NR). To re-examine the taxonomic status of *M. w. coreana*, Koh et al. (2012) compared the partial *12S rRNA* and complete *cytb* gene sequences of mtDNA of a few specimens from South Korea and Primorye, but again, no differentiation was found. Nuclear data (*A2ab*, *Bmp4*, *Tcf25*, *vWf* genes) and again *cytb* did not reveal any essential differences between samples from Primorye and the Korean Peninsula either (Kirihara et al., 2013).

Accurate karyological data from continental samples of *Mogera* from Primorye are absent. Kawada et al. (2001) noted that moles from South Korea (listed as *M. wogura*) differed considerably in terms of chromosome constitution from the Japanese population of *M. wogura* (2*n* = 36, NF = 58 and 2*n* = 36, NF = 52, accordingly). Based on personal communication with A. Kryukov, the authors noted that the Korean karyotype is similar to that from Primorye (Kawada et al., 2001, p. 1009). Next, in a paper (Kawada et al., 2007) describing karyotypes of other species, the authors cited their own earlier article (Kawada et al., 2001) but claimed that these samples were not from Korea, as stated before, but were from Russia.

[Correction added on 8 May 2019, after first online publication: The personal communication reference mentioned for the Korean karyotype has been updated to "personal communication with A. Kryukov" in this version.]

The aim of our study was to analyze genetic and morphological variation in *Mogera* from the continental Far East in order to determine the number of species distributed there and to elucidate the taxonomic status and content of *M. robusta*. To accomplish this task, we sequenced fragments of twelve nuclear genes and the complete mitochondrial cytochrome *b* gene in an extended sample of moles including museum samples from Primorye listed by Okhotina (1966) as *M. wogura*. Additionally, karyological analysis and a multivariate analysis of cranial characters have been used to examine the pattern of variation among the continental populations.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

The original material for the molecular phylogenetic study consists of 92 specimens of *Mogera robusta* from Primorye and 13 specimens of the *"coreana"* form. Voucher specimens are deposited in the Zoological Institute of the Russian Academy of Sciences (ZIN), Zoological Museum of Lomonosov Moscow State University (ZMMU), Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences (FSCEATB), and Conservation Genome Resource Bank for Korean Wildlife (CGRB), Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, South Korea. All studied *Mogera* specimens were collected during expeditions carried out by FSCEATB in 1994-2013, CGRB in 2004–2009, and ZIN in 2012-2016. A list of the analyzed specimens, including their collection localities and museum catalog numbers, is provided in Figure 1, Table 1, and Supporting Information Table S1.

Additionally, 45 sequences of *M. wogura*, 58 sequences of *M. robusta*, 12 sequences of *M. insularis*, one sequence of *M. tokudae*, 13 sequences of *M. imaizumii*, two sequences of *T. europaea*, one sequence of *E. parvidens*, and 11 sequences of *E. mizura* were downloaded from GenBank (Supporting Information Table S2) and used in the phylogenetic analyses.

2.2 | DNA extraction, PCR amplification, and sequencing

Genomic DNA from ethanol-preserved tissues was extracted using a standard protocol of proteinase K digestion, phenol–chloroform deproteinization, and isopropanol precipitation (Sambrook, Fritsch, & Maniatis, 1989). To test for the existence of *M. wogura* on the continent, as reported by Okhotina (1966), we also extracted DNA from dried skins of three specimens identified by Okhotina as *Mogera wogura* and stored in the ZIN collection and one "*coreana*" specimen from North Korea stored in the ZMMU collection. The DNA was purified directly using the Qiagen QIAquick PCR purification kit.

We sequenced the complete mitochondrial *cytochrome b* gene and fragments of twelve nuclear genes: *apolipoprotein B* (*Apo*B), *α-2b adrenergic receptor* (*A2ab*), *adrenoceptor beta 2* (*ADRB2*), exon 11 of the *breast cancer type 1 susceptibility protein* (*BRCA1*), exon 11 of the *breast cancer type 2 susceptibility protein* (*BRCA2*), *butyrylcholinesterase* (*BCHE*), *dentin matrix acidic phosphoprotein 1* (*DMP1*), *enamelin* (*ENAM*), exon 10 of the

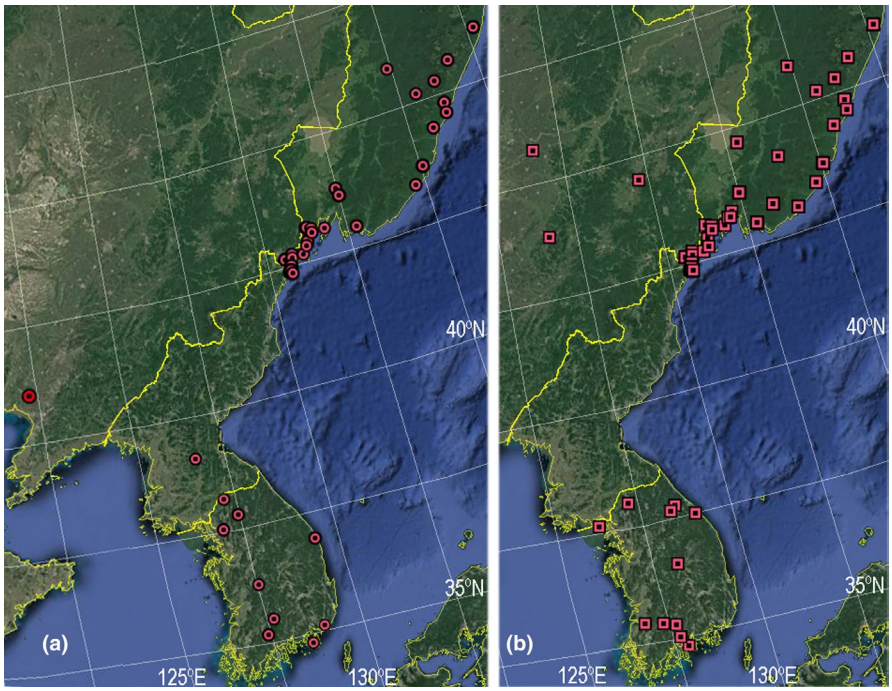

FIGURE 1 Map of sampling localities for specimens of *Mogera robusta* used in this study (a) molecular data; (b) morphological data). Localities are listed in Table 1

growth hormone receptor (*GHR*), *recombination activating protein* gene 1 (*RAG1*), *titin* (*TTN*), *von Willebrand factor* (*vWF*). Total numbers of analyzed sequences for each markers are as follows: *cytb*—145, *RAG1*—32, *BRCA1*—31, *BRCA2*—32, *ApoB*—32, *A2ab*—27, *ADRB2*—32, *BCHE*—32, *ENAM*—31, *GHR*—31, *TTN*—32, *vWF*—32, and *DMP1*—32. The primers for amplification and sequencing are provided in the Supporting Information Table S3.

The PCR protocol for all genes included an initial denaturation at 94°C for 3 min; then 30 cycles of 94°C for 30 s, 52–65°C (depending on the primer pair) for 1 min, and 72°C for 1 min; and a final extension of 72°C for 6 min.

DNA extracted from the old museum specimens was highly degraded; thus, only short fragments of *cytb* were obtained using the combination of primers L772a/H1070, F835/H1070 (for ZIN 89272) (Supporting Information Table S3). To avoid contamination, the extraction and amplification of the DNA from the museum specimens were carried out in the Laboratory of Historical DNA, Zoological Museum, Lomonosov Moscow State University, where no previous work on Talpidae tissues had been performed.

PCR products were visualized using a 1.5% agarose gel and then purified using ammonium–ethanol precipitation. Approximately, 10–30 ng of the purified PCR product was used for sequencing with each primer by the auto-sequencing system ABI 3100-Avant using the ABI PRISM®BigDyeTM Terminator v. 3.1 (Applied Biosystems, Foster City, CA, USA). The sequences obtained in this study can be accessed via GenBank (accession numbers: MK168808-MK169210, Supporting Information Table S2).

2.3 | Karyological analysis

We analyzed the karyotypes of nine moles from Primorye: two specimens from the Sikhote-Alin NR (##727, 728 in Table 1 and Supporting Information Table S4), three from Ussuri NR and its vicinity (##604, 903, 904), three from Kedrovaya Pad NR (##503, 608, 609), and one from the south part of Khasan district (#605). Chromosome preparations were made from bone marrow cells with a direct routine air-drying method and stained conventionally with Giemsa solution or Q-H-banding (Yoshida, Ikeuchi, & Sasaki, 1975). The arrangements by size and morphology in karyograms were prepared after taking photos with an Axioscope 40 microscope.

2.4 | Phylogenetic analysis

2.4.1 | Alignment, partitioning, and base composition

All of the sequences were aligned by eye using BioEdit version 7.0.9.0 (Hall, 1999). We phased each nuclear gene, for all individuals using the default settings in the program Phase in DnaSP (Rozas, Sanchez-DelBarrio, Messeguer, & Rozas, 2003) and used the resulting haplotypes for constructing the species tree.

Phylogenetic reconstructions were performed with the following data sets: (a) an extended sample of taxa for *cytb*; (b) all nuclear genes combined; (c) nuclear sequences combined in a species-tree estimation. The program PartitionFinder (Lanfear, Calcott, Ho, & Guindon, 2012) was used to determine the best partitioning strategy for nuclear concatenation among five a priori candidate schemes: (a) partitioning by gene; (b) partitioning by codon position; (c) partitioning by gene and codon position (three subsets per gene); (d) as in scheme c but with the 1st and 2nd codon positions combined (two subsets per gene); and (e) no partitioning. The *cytb* data set was always partitioned into three codon positions.

Pairwise genetic distances of the *cytb* gene sequences have been estimated using the Kimura-2 parameter model (Kimura, 1980) implemented in MEGA7 (Kumar, Stecher, & Tamura, 2016).

TABLE 1 Characterization of the material of *Mogera robusta*

Genetic vouchers	Morphological material	Coll.No.	Haplotypes	Country	Region	Locality	Latitude	Longitude
607	[a]	FSCEATB 607	H1	Russia	Primorye, Khasan District	Sukhanovka	42.72	131.12
819	[a]	FSCEATB 819	H1	Russia	Primorye, Khasan District	Karasik River	42.56	130.70
706	[a]	FSCEATB 706	H1	Russia	Primorye, Khasan District	Karasik River	42.57	130.67
818	[a]	FSCEATB 818	H1	Russia	Primorye, Khasan District	Karasik River	42.56	130.71
270	[a]	ZIN 99913	H1	Russia	Primorye, Khasan District	Lotos Lake	42.46	130.64
AVA 16-126	[a]	ZIN 104250	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-127	[a]	ZIN 104251	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-130		ZIN 104252	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-131	[a]	ZIN 104253	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-132	[a]	ZIN 104254	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-133	[a]	ZIN 104255	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-134	[a]	ZIN 104256	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-135	[a]	ZIN 104257	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-136	[a]	ZIN 104258	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-137		ZIN 104259	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-138	[a]	ZIN 104260	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-139	[a]	ZIN 104261	H1	Russia	Primorye, Khasan District	Lotos Lake	42.47	130.66
AVA 16-140	[a]	ZIN 104262	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-142		ZIN 104263	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-144	[a]	ZIN 104264	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-146	[a]	ZIN 104265	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-147	[a]	ZIN 104266	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-148	[a]	ZIN 104267	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-149	[a]	ZIN 104268	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-150	[a]	ZIN 104269	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62

(Continues)

TABLE 1 (Continued)

Genetic vouchers	Morphological material	Coll.No.	Haplotypes	Country	Region	Locality	Latitude	Longitude
AVA 16-151	[a]	ZIN 104270	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-152	[a]	ZIN 104271	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-153	[a]	ZIN 104272	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-154	[a]	ZIN 104273	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-155	[a]	ZIN 104274	H1	Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
AVA 16-177	[a]	ZIN 104278	H1	Russia	Primorye, Khasan District	Tesnaya River	42.71	130.57
AVA 16-180	[a]	ZIN 104279	H1	Russia	Primorye, Khasan District	Tesnaya River	42.71	130.57
AVA 16-182	[a]	ZIN 104280	H1	Russia	Primorye, Khasan District	Tesnaya River	42.71	130.57
605	[a]	FSCEATB 605	H10	Russia	Primorye, Khasan District	South of Khasan settl.	42.40	130.69
707		FSCEATB 707	H10	Russia	Primorye, Khasan District	South of Khasan settl.	42.40	130.69
2117	[a]	FSCEATB 2117	H10	Russia	Primorye, Khasan District	South of Khasan settl.	42.39	130.67
AVA 16-114	[a]	ZIN 104249	H11	Russia	Primorye, Khasan District	Bamburovo	42.95	131.32
306		ZIN 99912	H11	Russia	Primorye, Khasan District	Poima River	42.87	131.27
884	[a]	FSCEATB 884	H12	Russia	Primorye, Krasnoarmeisk District	Vostretsovo	45.90	134.92
AVA 14-241	[a]	ZIN 102796	H13	Russia	Primorye, Dalnegorsk District	Serzhantovo	44.41	135.71
727	[a]	FSCEATB 727	H14	Russia	Primorye	Sikhote–Alin Nature Reserve	45.33	136.15
252	[a]	ZIN 99911	H15	Russia	Primorye, Khasan District	Barabash	43.19	131.52
AVA 16-161	[a]	ZIN 104276	H16	Russia	Primorye, Khasan District	Tsukanovo	42.78	130.80
AK002HS1170			H17	Russia	Primorye, Khasan District	Kedrovaya Pad Nature Reserve	43.11	131.51
AVA 14-210	[a]	ZIN 102785	H18	Russia	Primorye, Olga District	Margaritovo	43.40	134.75
17/1990	[a]	ZMMU S-176640	H19	Russia	Primorye	Ussuri Nature Reserve	43.68	132.55
AVA 12-146	[a]	ZIN 101363	H2	Russia	Primorye, Ternei District	15 km south of Plastun	44.63	136.21
AVA 12-147	[a]	ZIN 101364	H2	Russia	Primorye, Ternei District	15 km south of Plastun	44.63	136.21
AVA 12-148	[a]	ZIN 101365	H2	Russia	Primorye, Ternei District	15 km south of Plastun	44.63	136.21

(Continues)

TABLE 1 (Continued)

Genetic vouchers	Morphological material	Coll.No.	Haplotypes	Country	Region	Locality	Latitude	Longitude
AVA 14-213	[a]	ZIN 102788	H2	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-214	[a]	ZIN 102789	H2	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-262	[a]	ZIN 102801	H2	Russia	Primorye, Ternei District	Zapadnaya Kema River	45.66	136.71
AVA 14-263	[a]	ZIN 102802	H2	Russia	Primorye, Ternei District	Zapadnaya Kema River	45.66	136.71
AVA 14-269	[a]	ZIN 102803	H2	Russia	Primorye, Ternei District	13 km NW of Maksimovka	46.14	137.74
AVA 14-280	[a]	ZIN 102804	H2	Russia	Primorye, Ternei District	13 km NW of Maksimovka	46.14	137.74
AVA 14-286	[a]	ZIN 102805	H2	Russia	Primorye, Ternei District	13 km NW of Maksimovka	46.14	137.74
AVA 14-293	[a]	ZIN 102806	H2	Russia	Primorye, Ternei District	13 km NW of Maksimovka	46.14	137.74
AVA 14-296	[a]	ZIN 102807	H2	Russia	Primorye, Ternei District	13 km NW of Maksimovka	46.14	137.74
AVA 14-212	[a]	ZIN 102787	H3	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-215	[a]	ZIN 102790	H3	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-226	[a]	ZIN 102791	H3	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-227	[a]	ZIN 102792	H3	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-228	[a]	ZIN 102793	H3	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-229	[a]	ZIN 102794	H3	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 14-230	[a]	ZIN 102795	H3	Russia	Primorye, Olga District	Vasilkovka River	43.73	135.10
AVA 16-112	[a]	ZIN 104247	H4	Russia	Primorye, Khasan District	Bamburovo	42.95	131.32
AVA 16-113	[a]	ZIN 104248	H4	Russia	Primorye, Khasan District	Bamburovo	42.95	131.32
606	[a]	FSCEATB 606	H4	Russia	Primorye, Khasan District	Kraskino	42.71	130.78
AVA 16-176	[a]	ZIN 104277	H4	Russia	Primorye, Khasan District	Tesnaya River	42.71	130.57
609	[a]	FSCEATB 609	H4	Russia	Primorye, Khasan District	Kedrovaya Pad Nature Reserve	43.12	131.51
3/2011			H4	Russia	Primorye, Khasan District	Barabashevka River	43.23	131.40
1/2011			H5	Russia	Primorye, Khasan District	Barabashevka River	43.23	131.36
AVA 16-158	[a]	ZIN 104275	H5	Russia	Primorye, Khasan District	Tsukanovo	42.78	130.80
4/2011			H5	Russia	Primorye, Khasan District	Barabashevka River	43.23	131.40

(Continues)

TABLE 1 (Continued)

Genetic vouchers	Morphological material	Coll.No.	Haplotypes	Country	Region	Locality	Latitude	Longitude
C2011			H5	Russia	Primorye, Khasan District	Barabashevka River	43.23	131.40
9/1990	[a]	ZMMU S - 176638	H6	Russia	Primorye	Ussuri Nature Reserve	43.68	132.55
604	[a]	FSCEATB 604	H6	Russia	Primorye	Ussuri Nature Reserve	43.68	132.55
AK821	[a]	FSCEATB 821	H6	Russia	Primorye	Ussuri Nature Reserve	43.68	132.55
218		ZIN 99909	H6	Russia	Primorye, Mikhailovka District	Otradnoe	43.84	132.50
16/1990	[a]	ZMMU S - 176639	H7	Russia	Primorye	Ussuri Nature Reserve	43.68	132.55
AVA 12-198	[a]	ZIN 101366	H7	Russia	Primorye, Partizansk District	Novolitovsk	42.96	132.80
411	[a]	FSCEATB 411	H7	Russia	Primorye	Vicinity of Vladivostok	43.12	131.90
219		ZIN 99910	H7	Russia	Primorye, Mikhailovka District	Otradnoe	43.84	132.50
820	[a]	FSCEATB 820	H8	Russia	Primorye	Ussuri Nature Reserve	43.68	132.55
728	[a]	FSCEATB 728	H8	Russia	Primorye	Sikhote–Alin Nature Reserve	45.33	136.15
AVA 14-255	[a]	ZIN 102799	H8	Russia	Primorye, Ternei District	12 km north of Plastun	44.84	136.23
AVA 14-256	[a]	ZIN 102800	H8	Russia	Primorye, Ternei District	12 km north of Plastun	44.84	136.23
AVA 12-128	[a]	ZIN 101362	H9	Russia	Primorye, Krasnoarmeisk District	Iman River	45.21	135.52
AVA 14-243	[a]	ZIN 102797	H9	Russia	Primorye, Dalnegorsk District	Serzhantovo	44.41	135.71
AVA 14-211		ZIN 102786	H9	Russia	Primorye, Olga District	Margaritovo	43.40	134.75
2/2011				Russia	Primorye, Khasan District	Barabashevka River	43.23	131.40
		ZIN 89271		Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
		ZIN 89272		Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
		ZIN 89273		Russia	Primorye, Khasan District	Khasan Lake	42.44	130.62
6342		CGRB 6342	H1	South Korea		Gapyeong-gun, Gyeonggi-do	37.83	127.51
2227		CGRB 2227	H1	South Korea		Gurye-gun, Jeollanam-do	35.21	127.46
8244		CGRB 8244	H21	South Korea		Uljin-gun, Gyeongsangbuk-do	37.00	129.40
2294		CGRB 2294	H22	South Korea		Cheorwon-gun, Gangwon-do	38.21	127.22
8563		CGRB 8563	H23	South Korea		Gurye-gun, Jeollanam-do	35.21	127.46

(Continues)

TABLE 1 (Continued)

Genetic vouchers	Morphological material	Coll.No.	Haplotypes	Country	Region	Locality	Latitude	Longitude
1176		CGRB 1176	H24	South Korea		Seoul	37.58	127.00
6345		CGRB 6345	H25	South Korea		Seoul	37.58	127.00
8243		CGRB 8243	H26	South Korea		Uljin-gun, Gyeongsangbuk-do	37.00	129.40
771		CGRB 771	H27	South Korea		Hamyang-gun, Gyeongsangnam-do	35.52	127.73
6344		CGRB 6344	H28	South Korea		Gapyeong-gun, Gyeonggi-do	37.83	127.51
10068		CGRB 10068	H29	South Korea		Geoje-si, Gyeongsangnam-do	34.85	128.58
7740		CGRB 7740		South Korea		Okcheon-gun, Chungcheongbuk-do	36.30	127.57
		ZMMU S-55231		North Korea		Iotoku (= South Pyongan Province, Yangdok)	39.17	126.83
HS373			H30	South Korea		Pusan	35.18	129.08
LN111103			H1	China		Liaoning	41.10	122.30
LN111102			H1	China		Liaoning	41.10	122.30
LN111104			H20	China		Liaoning	41.10	122.30

Notes. Zoological Institute of the Russian Academy of Sciences (ZIN), Zoological Museum of Lomonosov Moscow State University (ZMMU), Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences (FSCEATB), Conservation Genome Resource Bank for Korean Wildlife (CGRB), Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, South Korea.
Bold: Samples from Genbank.
[a]Morphological material.

2.4.2 | Phylogenetic tree reconstruction

Phylogenetic trees were generated using maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood analysis was performed in Treefinder (October 2008 version) (Jobb, 2008). The appropriate models of sequence evolution were selected for each partition, employing the method implemented in Treefinder and using BIC as the criterion. A tree search was conducted with the following options: parameter optimization simultaneous with tree search, optimized partition rates, proportional branch lengths for all partitions, and maximum search depth. Bootstrap support (1,000 pseudoreplicates) was estimated using model parameters and rate values optimized for the ML topology.

2.4.3 | Species-tree reconstruction and divergence-time estimation

A Bayesian tree reconstruction was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Models with either two or six rate matrix parameters were selected for each partition based on the results of the model selection in Treefinder. Each analysis included two independent runs of four chains (one cold plus three heated following the default settings). The chain length was set at 20 million generations with sampling every 5,000 generations. With these settings, the effective sample size exceeded 200 for all estimated parameters. Tracer 1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) was used to check for convergence and determine the necessary burn-in fraction, which was set to 2 million generations.

To construct the species tree from data on twelve potentially discordant independent nuclear loci, we have employed a Bayesian coalescent framework as implemented in *BEAST (Heled & Drummond, 2010). The units of the analyses correspond to species or well-supported intraspecific groups as inferred in *cytb* analysis. To test whether populations from the Korean Peninsula and Primorye can be treated as separate entities, we performed a species delimitation analysis in BPP 2.1 (Rannala & Yang, 2003; Yang & Rannala, 2010) using default options.

Based on the results of the molecular clock tests, we used separate strict clock models for each nuclear gene. The tree was calibrated using the age of the split between *Euroscaptor* and *Mogera* (10.0 ± 1.05 Myr) as estimated in He et al. (2014) and Bannikova et al. (2015). We used the same partitioning scheme and models as in the ML analysis. Yule prior for the species-tree shape and the

piecewise constant population size model were assumed. To improve convergence, we used informative priors for clock rate parameters, which were modeled using gamma distributions with mean and variance set equal to those of the corresponding posterior distributions produced in a preliminary run in BEAST version 1.8.4 (Drummond, Suchard, Xie, & Rambaut, 2012) performed on nuclear concatenation. In total, we conducted two runs of 2500 million generations each in BEAST version 1.8.4 (Drummond et al., 2012). Parameter convergence was assessed in Tracer 1.6 (Rambaut et al., 2014).

2.4.4 | Population analysis

Median-joining networks among haplotypes of continental moles (Primorye, Korea and China) were reconstructed in Network 5.0.0.1 (Bandelt, Forster, & Rohl, 1999). The gene and nucleotide diversities and mismatch analysis were calculated in Arlequin 3.5.1.2 (Excoffier & Lischer, 2010).

To determinate groups of samples, the allele frequencies of twelve nuclear genes were analyzed with STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). The *K* values were set from 1 to 12; 200,000 iterations were performed with the Monte Carlo method for Markov chains (MCMC) after 200,000 burn-in period; an admixed model without correlation of alleles was used in the analysis. Analysis of the number of clusters was made with the online program STRUCTURE HARVESTER (Earl & von Holdt, 2012) as described by Evanno, Regnaut, and Goudet (2005).

2.5 | Morphological analysis

We examined 352 skulls from the collections of ZIN, ZMMU, FSCEATB, Far Eastern Federal University, Russia (FEFU), and Hokkaido University (HU) (Supporting Information Table S4). Only adult (1–2 years old) specimens were studied. The age was determined using the degree of dental abrasion according to Okhotina (1966). Seven cranial variables were measured following Kawada et al. (2007) using a digital calliper with an accuracy of 0.01 mm: greatest length of the skull (GLS), palatal length from the anterior tip of the 1st incisor to the posterior lip of the palate (PL), length of the upper tooth row (I^1–M^3), distance between the upper canine and 3rd molar (C–M^3), rostral breadth at the canines (RB), distance between the lower 1st premolar and 3rd molar (Pm_1-M_3), breadth across upper second molars (BAM). Principal component analysis (PCA) was used to evaluate a cranial variation. The software program Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA) was used for all analytical procedures.

3 | RESULTS

3.1 | Alignment and partitioning in the DNA analysis

The final alignment of *cytb* includes 1,140 bp for 139 specimens including the five outgroups (*M. insularis*, *M. imaizumii*, *M. tokudae*, *Talpa*, and *Euroscaptor*). In the combined analysis of 12 nuclear genes, the data set contains 31 specimens, including three outgroups (*M. insularis*, *M. imaizumii*, and *M. tokudae*) and the final alignment consists of 8,393 nucleotide positions including 600 bp of *ApoB*, 684 bp of *A2ab*, 780 bp of *ADRB2*, 569 bp of *BRCA1*, 768 bp of *BRCA2*, 939 bp of *BCHE*, 975 bp of *DMP1*, 612 bp of *ENAM*, 818 bp of *GHR*, 482 bp of *RAG1*, 411 bp of *TTN*, and 755 bp of *vWF*. The optimum partitioning scheme for the nuclear genes identified by Partition Finder under the BIC criterion was found to be by partitioning by codon position (scheme b). The best-fit substitution models employed for each of the subsets are provided in Supporting Information Table S5.

3.2 | Phylogenetic analysis of the *cytb* data

In Figure 2, the ML tree is shown with the values of the Bayesian posterior probabilities in BI and the bootstrap support in ML analyses shown above the branches. Four supported groups were found on the mitochondrial phylogenetic tree: three Japanese island groups (Kinki-Tokai, Chugoku-Shikoku, and Kyushu) and one continental group (Primorye, China, and South Korea). The last group has a high level of support in all analyses. The genetic distance (K2P) between groups from Kinki-Tokai and from Primorye, China, and South Korea is approximately 6% (Table 2). The genetic distance (K2P) between continental specimens is approximately 0.1%–0.2%.

The median network was constructed in order to provide insights on the intralineage relationships in the continental group (Figure 3). The haplotypes H1 and H2 are the most common and differ from

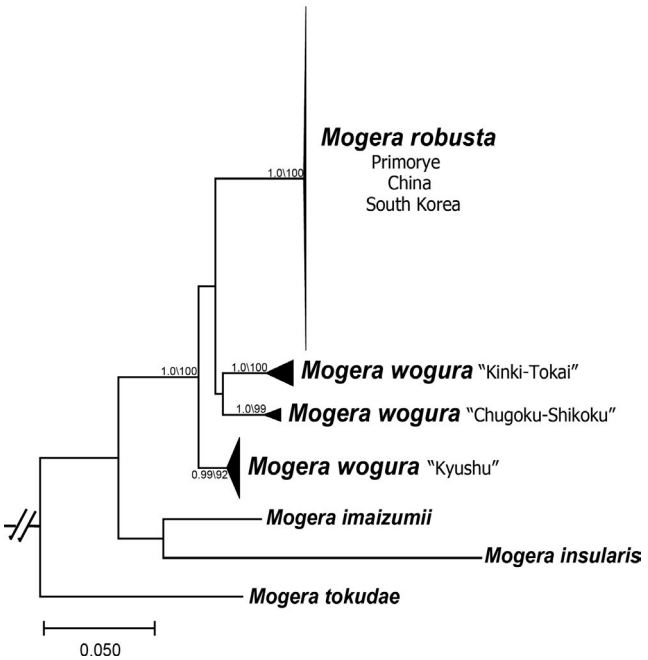

FIGURE 2 The ML phylogeny of the moles as inferred from the complete *cytb* gene sequence. Values above the branches correspond to Bayesian posterior probabilities (BPP) in MrBayes and bootstrap support (1,000 pseudoreplicates) in ML analyses, respectively. The three insular lineages of *Mogera wogura* are designated according to Kirihara et al. (2013). The out-group (representatives of the genera *Euroscaptor* and *Talpa*) is not shown

TABLE 2 Interspecific diversity of *cytb* in moles: under the diagonal—K2P distance between groups; over the diagonal—p-distance between groups (%)

	Primorye, China, South Korea	Kinki-Tokai	Kyushu	Chugoku-Shikoku
Primorye, China, South Korea		5.6 ± 0.5	5.0 ± 0.6	5.2 ± 0.4
Kinki-Tokai	5.9 ± 0.7		4.9 ± 0.5	4.5 ± 0.6
Kyushu	5.3 ± 0.6	5.1 ± 0.6		4.1 ± 0.5
Chugoku-Shikoku	5.5 ± 0.7	4.7 ± 0.6	4.3 ± 0.6	

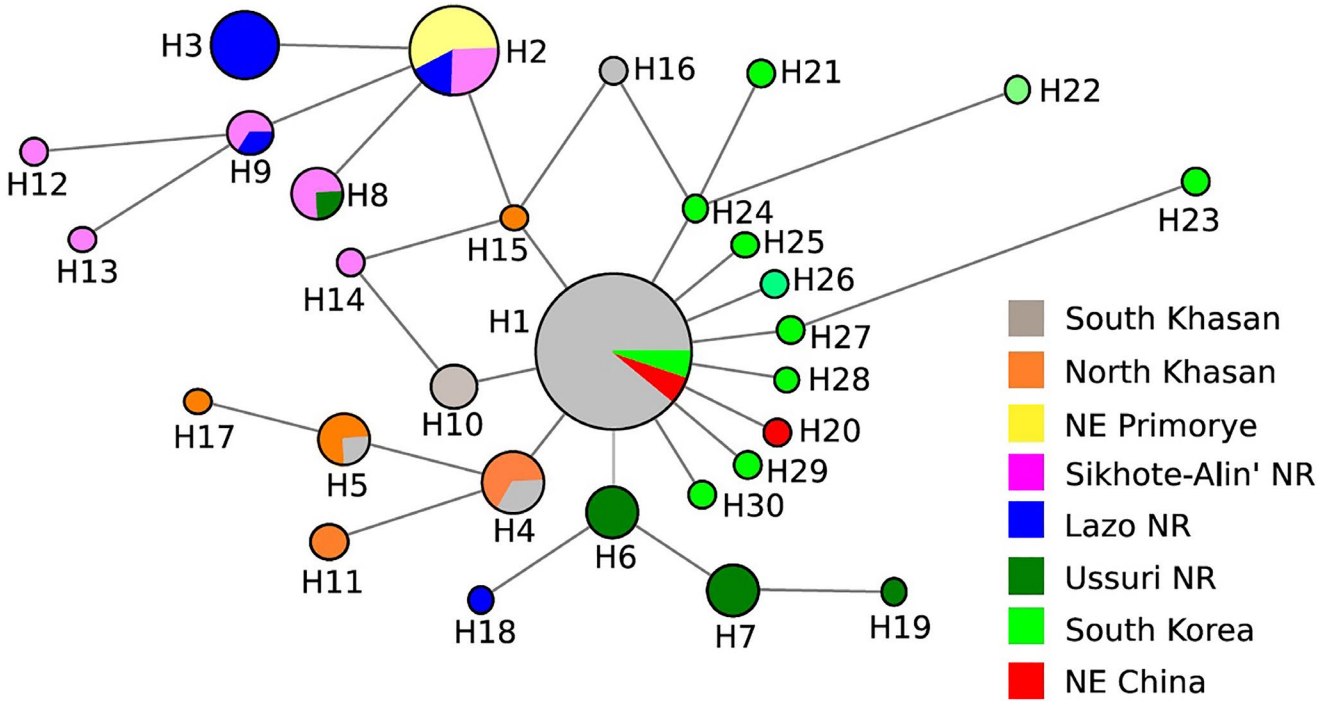

FIGURE 3 Network of the cytb haplotypes from the continental lineage. Circles represent haplotypes and with size proportional tothe number of individuals sharing the same haplotype. Circles are colored according to the geographical origin of the haplotypes (Table 1)

each other at two nucleotide positions. Haplotype H1 was shared by 37 specimens, 33 of which were from South Khasan, two from South Korea, and two from China. Haplotype H2 was shared by 12 specimens, seven of which were from NE Primorye, three from Sikhote–Alin NR, and two from Lazo NR. Other haplotypes from South Korea and China are connected to H1 by 1–3 mutations. All remaining haplotypes from South Korea (H21–H30) show intragroup diversity. It is worth mentioning that specimens from South and North Khasan have no common haplotypes with specimens from another part of Primorye.

Based on the network, two geographical groups of haplotypes from Primorye can be recognized. The first consists of six haplotypes (H2, H3, H8, H9, H12, and H13) and is distributed in the northern part of the studied area, and the second includes all remaining haplotypes (H1, H4, H5, H6, H7, H10, H11, H14, H15, H17, H18, and H19). The gene diversity was high in both groups (0.7460 ± 0.0571 and 0.7618 ± 0.0514), and the nucleotide diversity was less so (0.000956 ± 0.000721 and 0.001339 ± 0.000900). The results of the mismatch analysis (Supporting Information Figure S1) indicate recent demographic expansion in populations of moles from Primorye.

The specimen from Ussuri NR (#AB037647) that early formed the "continent-2" group in the study of Tsuchiya et al. (2000) was reanalyzed (extracted and amplified) in the present work. The new sequence of 1,140 bp (#604 in Table 1) grouped with the others continental specimens (haplotype H6) and did not form a separate branch.

Three specimens (##2227, 6345 and 8563) from South Korea originally produced heterogeneous amplicons, suggesting the presence of two *cytb*-like sequences. However, after re-extraction and re-amplification, the additional sequence disappeared, while the remaining product was found to be similar to typical continental haplotypes. We determined the structure of the additional amplicon by comparing heterogeneous and homogeneous sequences. Then, we constructed the mitochondrial phylogenetic tree using both types of sequences from these specimens (Supporting Information Figure S2). All of the additional sequences grouped with the sequence #AB037647, thus belonging to "continent-2" group of Tsuchiya et al. (2000). The K2P-distance between the two continental groups is ~6.5%, which is comparable to that between continental and insular lineages (Table 2). We believe that the "continent-2" lineage is in fact a nuclear pseudogene.

To confirm this suggestion, we amplified another mitochondrial gene *COI* in a sample including specimens ##2227, 6345, 8563, eight sequences from South Korea, and nine sequences from Primorye. We found no substantial variation among the sequences from South Korea and Primorye, thus suggesting existence of a single mitochondrial lineage on the continent (Supporting Information Figure S3).

Museum specimens from the continental area that were labeled as "*M. wogura*" (sensu Okhotina) and one specimen from Korea were not included in the phylogenetic analysis because of the short length of the sequenced fragments (S-55231, ZIN 89271, ZIN 89272, and ZIN 89273). For these samples, only 247 bp were sequenced (MK169187–MK169190) with different combinations of primers. The obtained fragments were similar (K2P-distance of approximately 0.1%) to the corresponding parts of the complete *cytb* sequence of *M. robusta* from Russian Far East, comprising all diagnostic substitutions that distinguish the continental population (#218) from the insular ones (#HG737876) (Supporting Information Figure S4).

3.3 | Phylogenetic analysis of the nuclear data set

The result of combined analysis is presented in Figure 4. On the ML tree inferred from the concatenated alignment of twelve nuclear genes, the continental group received a high level of support in all analyses; within this, three specimens from South Korea occupied the basal position.

As a result of the analysis in STRUCTURE HARVESTER, the optimum solution (the highest value of ΔK) corresponded to using three clusters (Supporting Information Figure S5).

However, the analysis of allele frequencies of twelve nuclear genes did not reveal any differences between samples from Primorye, China, and South Korea (Supporting Information Figure S6).

We constructed Median-joining networks (Supporting Information Figure S7) for each nuclear gene. We found unique alleles for *M. wogura* and a few unique alleles for some specimens of *M. robusta* and *"coreana."* Unique alleles for *M. wogura* were found for all genes, except *ApoB* and *BRCA1*.

Furthermore, the results of the BPP species delimitation analyses provided 100% support for a separation between *M. robusta* sensu stricto and *"coreana."*

3.4 | Molecular time estimates

Based on the results of the species-tree reconstruction with nuclear data using *BEAST, the age of the split between the insular *M. wogura* and continental *M. robusta* is estimated to be ~0.64 Myr (0.30–1.0 Myr), which corresponds to the Middle Pleistocene

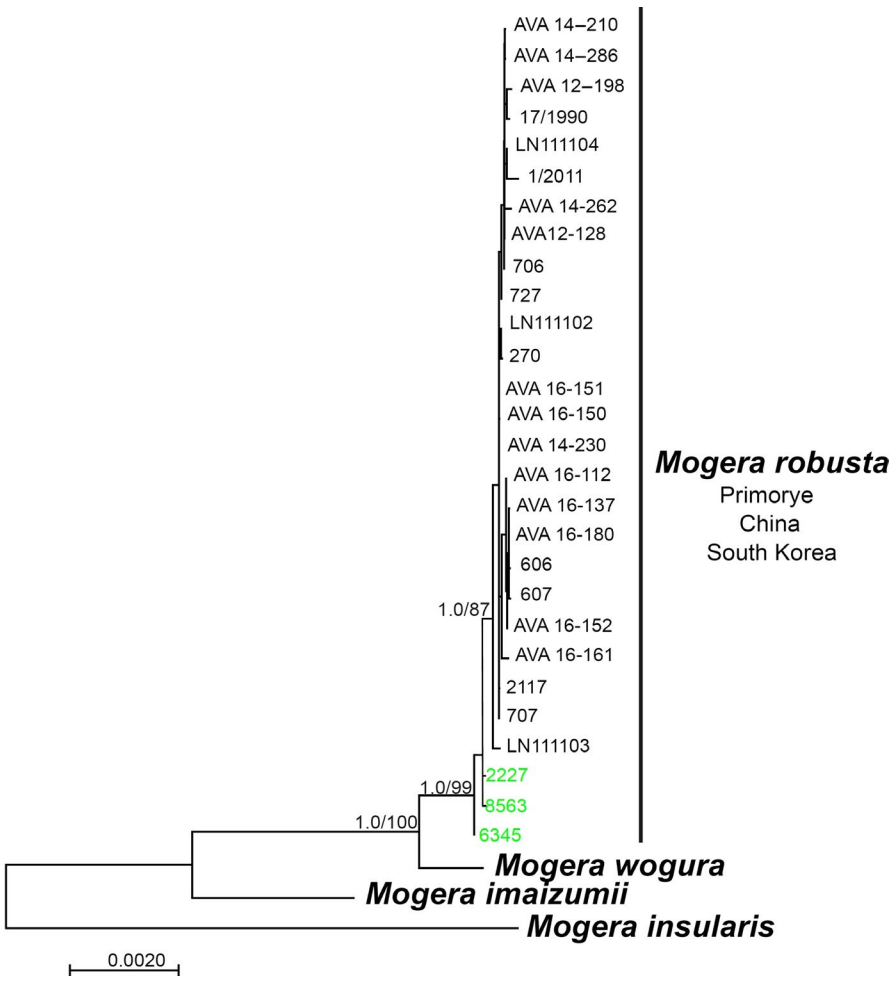

FIGURE 4 The ML phylogeny of the moles as inferred from a concatenated alignment of twelve nuclear genes. Values above the branches correspond to Bayesian posterior probabilities (BPP) in MrBayes and bootstrap support (1,000 pseudoreplicates) in ML analyses, correspondingly. Korean samples are colored in green

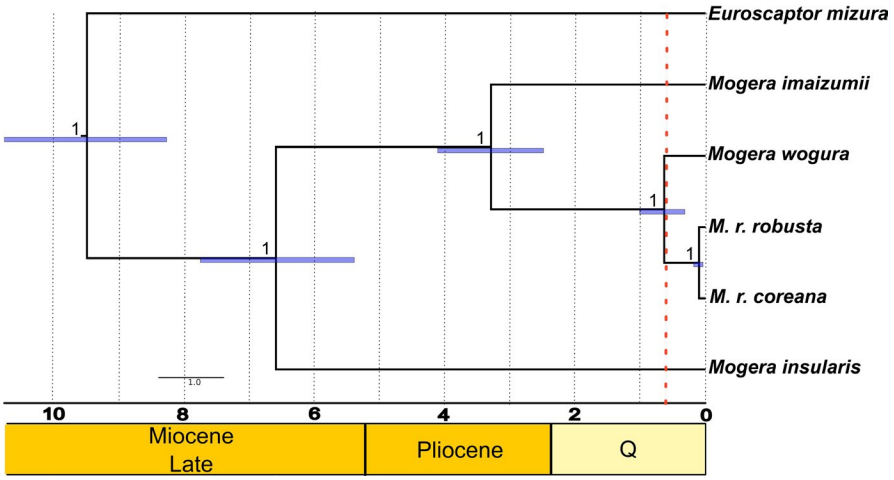

FIGURE 5 Timescale of major divergence events among *Mogera* using the Bayesian multispecies coalescent approach (*BEAST). The divergence times correspond to the mean posterior estimate of their age in Myr. The blue bars represent the 95% HPD interval. Numbers above the branches correspond to posterior probabilities for each node

(Figure 5). *Mogera robusta* split into two sublineages as early as the Upper Pleistocene, approximately 0.10 Myr (0.04–0.18 Myr).

3.5 | Karyological analysis

Here, we present, for the first time, a description of the karyotype of Ussuri mole. The diploid number (2*n*) in all karyotypes studied was 36, NFa = 58. The constitution of chromosomes was as follows: 5 M, 4 SM, 3 ST, and 5 A. The X-chromosome is submetacentric, and the Y-chromosome is small acrocentric (Figure 6). There is a secondary constriction on the first pair of submetacentric chromosomes. All studied karyotypes were the same, including that from the South Khasan specimen.

3.6 | Morphometric analysis

Results of the PCA of craniometric characters in the continental moles are shown in Figure 7. As inferred from the scatterplot, the specimens from South Khasan grouped with the specimens from Primorye and China and differ greatly from the specimens from South Korea. Such division into groups is observed both for males and females. The two groups diverge along the first principal component, reflecting, in particular, difference in overall cranial size. The Korean moles separated clearly from all other continental populations with smaller sizes. The GSL of males, for example, was 36.8 in Korea, 41.3 in the South Khasan, and averaged 42.8–44.8 for different localities in Primorye.

4 | DISCUSSION

4.1 | The taxonomic status of Ussuri mole

The Japanese mole *M. wogura* was described from Kyushu, Japanese Islands, by Temminck in 1842 and in the continental area, it has been listed from eastern China and Korea (Okhotina, 1966; Stroganov, 1948; Yudin, 1989), in Primorye—from the left bank of Tumen River to the Sukhanov mountain range (Okhotina, 1966; Yudin, 1971). Ussuri mole *M. robusta* was described from the vicinities of Vladivostok, Primorye, by Nehring in 1891, and it was reported from Primorye and north-eastern China (Okhotina, 1966; Stroganov, 1948) and the Korean Peninsula (Stroganov, 1957; Yudin, 1989).

There was not always information about which small specimens (continental or insular) were used in describing the morphological characters of moles from Primorye, listed as *M. wogura* by Russian authors. Thus, Stroganov (1948) analyzed insular *M. wogura* and continental *M. robusta*. Okhotina (1966) used only six small specimens of "*M. wogura*" from the Khasan area of Primorye. Yudin (1971, 1989) analyzed nine combined sample specimens (from Primorye, Korean Peninsula, and Japan). All of them pointed out the smaller sizes of "*M. wogura*." According to Stroganov (1948), the Ussuri mole has gray–brown pelage color, whereas Japanese mole has dark gray–brown pelage color with tawny neck and golden bands on the fore feet and belly (Okhotina, 1966). These species also differ in morphology of the auditory ossicles (Stroganov, 1957). Ussuri mole has a right angle between the *processus manubrium* and the *collum mallei*, while Japanese mole has a blunt angle; *apophysis orbicularis* is absent in Ussuri mole and developed in Japanese mole. Stroganov (1957), speaking about these differences, pointed out that they were small, the amplitude of the oscillations and their stability have not been elucidated and, perhaps, in more detailed research, it would be shown to be individual or age variation. Jones and Johnson (1960) also did not regard the slight differences between the ossicles of *robusta* and *wogura* as of specific worth. Ussuri mole has preanal glands and more massive and different external genitalia of males (Okhotina, 1966; Yudin, 1971, 1989), but the variability in these characteristics within "*M. wogura*" was not sufficiently studied.

Korean moles were described as a subspecies of *M. wogura*, *M. w. coreana*, from Kim-hoa, 65 miles north-east of Seoul (Thomas, 1907). Morphological differences in skull and body size between the moles from Korea and Primorye were previously reported (Jones & Johnson, 1960). Until now, craniometric analysis of *Mogera* has been reported on a very small amount of material, especially from Russia (Abe, 1995; Asahara, Kryukov, & Motokawa, 2012; Okhotina, 1966; Stroganov, 1957). Here, we present extended data set on this subject. Among seven characters studied, significant

FIGURE 6 Karyograms of *Mogera robusta* from Sikhote-Alin NR (Primorye) #728, conventional staining (a), *Mogera robusta* from Ussuri NR #904, Q-H-banding (b) and *M. wogura* from Korea (c) (Kawada et al., 2001)

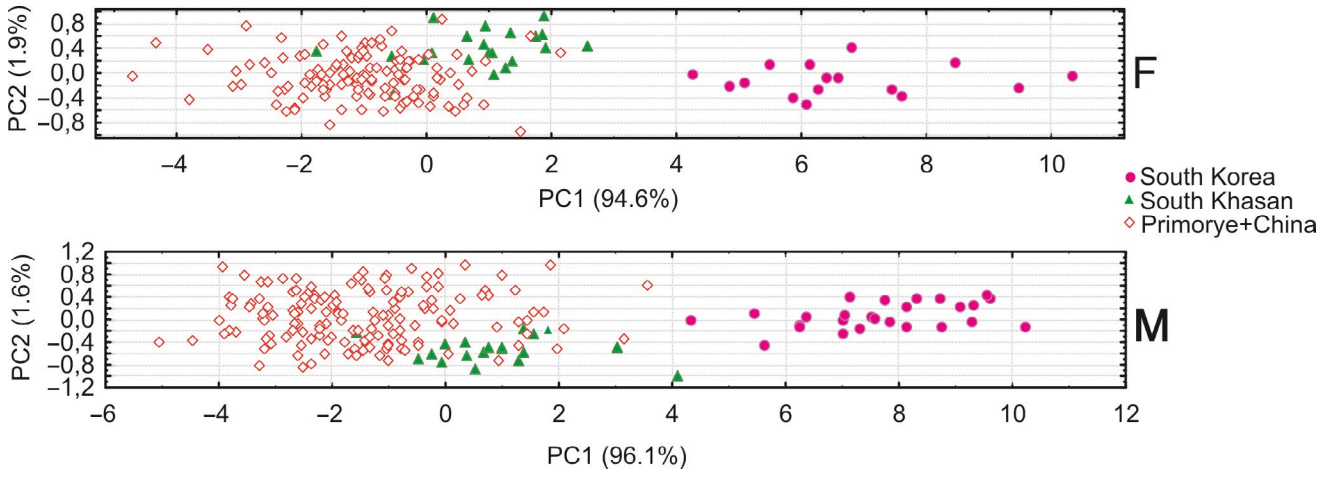

FIGURE 7 Ungrouped morphometric separation (principal components analysis) of moles from Primorye, China, and South Korea. F—females, M—males

differences were found in five of them, such as greatest length of the skull which is significantly larger in the Primorye population (Supporting Information Table S4). However, our comparative morphometric analysis revealed only small size differences, without hiatus, between specimens from the South Khasan area and other moles from Primorye, which were treated by Okhotina (1966) as the distinct species of *M. wogura* and *M. robusta*, accordingly (Figure 7). Due to a lack of material from North Korea, we were not able to verify the assumption of a cline with increasing size from south to north. However, in Primorye itself, we found no size variation in skulls from the North Khasan area in the extreme south to the Sikhote-Alin NR in the north. Abe (1967) showed that body size is neither effective nor useful as a single character in assessing the specific identity of moles. In addition to sexual dimorphism and age variation, the skull size has been shown to be dependent on soil hardness and mean minimum temperature in Japan (Abe, 1996).

The body size of *M. wogura* in western Japan varies from small in the south to large in the north (Ohdachi et al., 2009), which may be viewed as an analogy of the situation with continental moles. It has been previously pointed out (Abe, 1967) that mountain regions of Japan are populated by smaller moles compared to moles from lowlands. Therefore, we assume that there is a rather strong size variation within continental moles, which may be associated with both ecological and geographical factors.

The karyotypes of all moles from Primorye look the same and correspond completely with the description of the karyotype of Korean moles (Kawada et al., 2001; Tsuchiya, 1988). All of them contain 12 pairs of biarmed chromosomes and five pairs of acrocentric (one-armed) chromosomes. In contrast, the karyotype of *M. wogura* from western Japan has eight pairs of acrocentric chromosomes. Comparing both karyotypes after G-banding, it was suggested that three pericentric inversions took place in the 10th, 13th, and 16th pairs and a paracentric inversion in the 17th chromosome (Kawada et al., 2001). Thus, NFa in these species differs from 58 in Korea and Russia to 52 in west Japan. This character proved to be important criterion for recognition among many *Mogera* species. The secondary constriction on the first pair of submetacentric chromosome (Figure 6) was previously found in karyotypes of all other studied species of *Mogera* (Kawada et al., 2001; Tsuchiya, 1988). The only difference between the Ussuri and Korean moles is the size of Y-chromosome: It is dot-like in Korean moles, as well as in all *Mogera* moles previously studied (Kawada et al., 2001), but small and acrocentric in Ussuri moles; this, however, should be validated with further studies.

Our molecular analysis did not reveal any pronounced differences between samples from north-eastern China, South Korea, and Primorye, including the small-sized specimens from the South Khasan area. Both nuclear and mtDNA data are concordant with the previous molecular studies (Kirihara et al., 2013; Koh et al., 2012). Our additional analysis of *cytb* (Supporting Information Figure S4) showed that sequences of *M. robusta* specimens are very similar to the partial fragments obtained from the museum specimens of continental "*M. wogura*" from the Okhotina's collection.

The mitochondrial *cytb* gene sequences studied by Tsuchiya et al. (2000) revealed three clades in Japan (Honshu, Shikoku, and Kyushu) and two distinct continental clades from South Korea and Primorye. According to that study, the "continent-1" group included all specimens from South Korea and Primorye and the "continent-2" group included only one specimen from Ussuri NR (#AB037647). In the present study, we reanalyzed the specimen from Ussuri NR and demonstrated that "continent-2" group only consists of the specimen #AB037647 and additional signals from three specimens from South Korea. Thus, we conclude that "continent-2" described in Tsuchiya et al. (2000) does not represent a separate mtDNA lineage but might be in fact a pseudogene. The genetic distance (K2P) between continental specimens is approximately 0.1%–0.2%. Only BPP-analysis supports differentiation between samples from the Primorye and China and from South Korea. However, this could be due to the tendency of BPP to overestimate the number of putative species (Sukumaran & Knowles, 2017).

Additionally, we found no clear evidence for introgression of either nuclear or mitochondrial genes between the continent and insular specimens. We found unique alleles for *M. wogura* in all genes except *ApoB* and *BRCA1*. This can be explained by the young age of the split between *M. robusta* and *M. wogura*. According to our results, based on species tree, the time of the divergence between *M. wogura* s. str. and *M. robusta* dates back to the Middle Pleistocene (0.30–1.0 Myr). This is associated with the land-bridge formation at MIS 16 (0.65 Myr), which is indicated by the immigration of the proboscidean species *Stegodon orientalis* from the continent to Japanese Islands (Yoshikawa, Kawamura, & Taruno, 2007). Our results are not in agreement with previous molecular time estimates based on mitochondrial data (Kirihara et al., 2013). This can be explained by the discordance of the mitochondrial gene tree of Kirihara et al. (2013) and our multilocus species tree, which takes into account ancestral polymorphism (Degnan & Rosenberg, 2009). Additionally, it could be associated with the early saturation of third codon positions of mtDNA due to generally much-elevated rates of evolution of mtDNA compared to nuclear DNA (Meyer, 1994).

According to the Genetic Species Concept, the species threshold is determined based on the level of differentiation between known genetically isolated lineages (Baker & Bradley, 2006; Bradley & Baker, 2001). The essential mitochondrial genetic distance between *M. wogura* and *M. robusta* (~6%) corresponds to those between pairs of the sister species in other groups of the strictly fossorial moles such as *Talpa* or *Euroscaptor* (Bannikova et al., 2015; Nicolas, Martínez-Vargas, & Hugot, 2017; Tsuchiya et al., 2000; Zemlemerova, Bannikova, Lebedev, Rozhnov, & Abramov, 2016). The phylogeographic break might have occurred when the Korea Strait could block gene exchange between these two lineages. Our analysis supports treating insular and continental specimens as distinct allopatric lineages, associated with corresponding separate species *M. wogura* and *M. robusta*. Thus, we hypothesize that *M. wogura* is absent from the continent and there are only *M. robusta* Nehring, 1891 there.

The split between *M. robusta* from Primorye and "*coreana*" dates back to the Upper Pleistocene (0.04–0.18 Myr). As it is a relatively recent event that did not lead to any significant differentiation, we cannot validate the species status of the two sublineages within continental moles. As the name *robusta* (Nehring, 1891) has priority over *coreana* (Thomas, 1907), both should be named *M. robusta* Nehring, 1891, with two subspecies: *M. robusta robusta* Nehring, 1891 and *M. robusta coreana* Thomas, 1907.

4.2 | Phylogeographical structure of the moles from Primorye

The star-like pattern of the mtDNA network regarding primarily the affinity of Korean and South Khasan moles means close connection between them and a recent origin from the same ancestor. Then, it is worth mentioning the tendency for clustering among the haplotypes from North Primorye (H2) and delivered, Khasan district (H4), and

Ussuri NR (H6). The limited number of the samples studied prevents further interpretation of this observation.

According to our results, high haplotype and low nucleotide diversities were found in populations of moles from Primorye. This fact indicates that these populations recently originated from an ancestral population with a low effective population size (Avise, 2000). Thus, the populations from Primorye are young and currently undergoing expansion.

The intraspecific variation in moles from Primorye could be associated with the influence of Late Quaternary climatic oscillation on subterranean mammals. This fact is well established for another species of moles—*Talpa europaea* (Feuda et al., 2015). Traditionally, phylogeographical analyses have suggested a contraction of species ranges during the glacial phases and a subsequent expansion in the interglacial periods (Hewitt, 2000). During the Last Glacial, moles might have been isolated in the main Korean mountain range (the so-called "Baekdudaegan") (Chung et al., 2014) and in Primorye (Berman et al., 2005; Kolesnikov, 1969; Nazarenko, 1990), which resulted in limited gene flow and certain genetic differentiation.

Based on much higher genetic variability within *M. wogura* in Japanese islands compared to that on the continental forms, Koh et al. (2012) hypothesized dispersal of the former to the mainland giving rise to a new form there. We do not accept that interpretation due to well-known high variability of insular populations in general (K2P ~ 5%) (Kirihara et al., 2013) and complex history of multiple invasions to the Japanese archipelago from the radiation center somewhere in the continent (Tsuchiya et al., 2000; Shinohara et al., 2004; Suzuki, 2009; Kirihara et al., 2013, etc.).

5 | CONCLUSION

Our data show that Ussuri mole *M. robusta* and Japanese mole *M. wogura* should be treated as distinct species and do not support the presence of *M. wogura* on the continent: it inhabits the Japanese islands only. There is significant genetic difference between continental and Japanese moles at the species level as inferred from the analysis of twelve nuclear genes, one mitochondrial gene, and karyotypes. No genetic difference between moles from Primorye (Russian Far East) and Korean Peninsula was revealed by molecular and karyological data. Based on craniometric differences, the populations from Primorye and Korea should be treated as distinct subspecies, *M. r. robusta* and *M. r. coreana* accordingly. Additional sampling from the northern part of the Korean Peninsula and north-eastern China is needed to analyze ecogeographic patterns of morphological variation in continental *Mogera*.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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