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Taxonomic position of Chinese voles of the tribe Arvicolini and the description of 2 new species from Xizang, China

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China has 26 species in the tribe Arvicolini. The taxonomic status of these voles remains controversial despite much effort. Herein, we evaluate the taxonomic position of 22 species plus 2 unidentified taxa using mitochondrial DNA gene sequences (*cytb* + *CO1*). We also evaluate 18 species and 2 unidentified taxa using morphological data. Phylogenetic analyses of *cytb* resolve monophyly for the genera *Alexandromys*, *Lasiopodomys*, *Microtus*, *Neodon*, *Proedromys*, and *Volemys* with strong support. *Stenocranius* clusters with *Chionomys* but with very weak support. Analyses of concatenated *cytb* + *CO1* resolve the same genera with strong support, but the topology of the tree differs from that of *cytb* in that *Chionomys* roots at the base of the tree independent of *Stenocranius*, which forms the sister-group of *Lasiopodomys* in a more terminal position. The matrilineal genealogy excludes the type species *Arvicola amphibius* from the rest of the Arvicolini. This species forms the sister-group of *Ondatra* with high support. *Neodon* includes *N. irene*, *N. linzhiensis*, *N. fuscus*, *N. leucurus*, *N. sikimensis*, *Microtus clarkei*, and 2 unidentified specimens. *Alexandromys* includes the former species *Microtus oeconomus*, *M. kikuchii*, *M. limnophilus*, *M. fortis*, and *M. maximowiczii*. Finally, *Microtus* has the subgenera *Blanfordimys*, *Microtus*, *Mynomes*, *Pedomys*, *Pitymys*, and *Terricola*, which includes the Chinese species *M. agrestis*, *M. arvalis*, and *Blanfordimys juldaschi*. General mixed Yule-coalescent species delimitation modeling demarcates 6 currently recognized species and 2 new species of *Neodon*. A principal component analysis of the morphological data among 7 matrilines shows that all variables have positive loadings of high magnitude on the 1st component. Canonical discriminant analysis for *Neodon* (including *M. clarkei* and 2 unidentified species) correctly classifies 93.0% of specimens. Overall, our analyses support the recognition of *Alexandromys*, *Lasiopodomys*, *Microtus*, *Neodon*, *Proedromys*, and *Volemys* as genera. *Stenocranius* includes *Microtus gregalis*, and the genealogical position of *Stenocranius* remains uncertain. The status of *Arvicola* requires further study. We assign *M. clarkei* to *Neodon* and describe 2 new species of *Neodon*.

Key words: Arvicolini, China, Hengduan Mountains, *Neodon*, new species, *Proedromys*

Arvicolinae is a speciose and controversial group of cricetid rodents. Musser and Carleton (2005) recently revised the taxonomy of Chinese voles based on prior morphological, karyological, and a few molecular studies. Among the 15 genera and 49 species of voles in China (Musser and Carleton 2005; Smith and Xie 2009), 7 genera and 24 species belong to the tribe Arvicolini (Musser and Carleton 2005).

Many Chinese voles only occur in the mountains of West China where collecting is very difficult. Consequently, most previous studies were based primarily on morphology and old museum specimens (Miller 1896; Hinton 1923, 1926;

Lidicker 1968; Feng et al. 1986; Yang et al. 1992; Liu et al. 2007, 2010). Although molecular studies on European, American, and northern Asian voles abound (Conroy and Cook 1999, 2000; Martine et al. 2000; Jaarola et al. 2002, 2004; Galewski et al. 2006; Bužan et al. 2008; Robovsky et al. 2008; Bannikova et al. 2009, 2010; Martínková and Moravec 2012; Petrova et al. 2015), few molecular studies have evaluated Chinese species. Liu et al. (2007, 2012) described 2 new species of Chinese voles, and clarified the phylogenetic positions of the genus *Phaiomys* and the species *P. leucurus* and *Lasiopodomys fuscus* based on mitochondrial genes and morphology.

Many Chinese genera and species in Arvicolini remain in dispute. For example, Musser and Carleton (2005) re-recognized *Arvicola* as the type genus of Arvicolini and phylogenetic analyses of nuclear gene sequences agree with this arrangement (Galewski et al. 2006; Abramson et al. 2009b). Phylogenetic analyses of mitochondrial genes exclude *Arvicola* from the rest of the Arvicolini (Conroy and Cook 1999; Bužan et al. 2008; Bannikova et al. 2009). Musser and Carleton (2005) recognized *Stenocranius* as a subgenus of *Microtus*. However, Abramson et al. (2009a), based on nuclear genes, and Bannikova et al. (2010) and Martínková and Moravec (2012), based on both mitochondrial genes and nuclear genes, resolved *Stenocranius* as having a close relationship with *Lasiopodomys*. Abramson and Lissovsky (2012) included *Stenocranius* in *Lasiopodomys*. Petrova et al. (2015) agreed with this arrangement. Zagorodnyuk (1990) erected *Volemys* with *V. clarkei*, *V. kikuchii*, *V. millicens*, and *V. musseri*. Musser and Carleton (1993) recognized the genus, but subsequently (Musser and Carleton 2005) returned *V. clarkei* and *V. kikuchii* to *Microtus*. Chen et al. (2012) recovered *V. musseri* and *Proedromys bedfordi* as sister-taxa with high support based on analyses of nuclear *GHR* and *IRBP* and suggested the taxonomic status of *Volemys* needed additional study. Further, *P. liangshanensis* and *P. bedfordi* did not cluster together on their *GHR* and *IRBP* tree (Chen et al. 2012). Musser and Carleton (2005) recognized *Alexandromys* as a subgenus of *Microtus*. Conroy and Cook (2000), Galewski et al. (2006), and Bannikova et al. (2010) resolved *Alexandromys* as a monophyletic group based analyses of mtDNA and nuDNA sequences. Abramson and Lissovsky (2012) considered *Alexandromys* to be a genus.

In the last few years, we have accumulated a collection of voles from different regions of China. Herein, using both molecular and morphological data, we explore the phylogenetic relationships of the Chinese voles of the tribe Arvicolini and reassess their classification.

Materials and Methods

Ethics statement.—All samples were obtained following ASM guidelines and the laws and regulations of China for the implementation of the protection of terrestrial wild animals (State Council Decree 1992; Sikes et al. 2011). Collecting protocols were approved by the Ethics Committee of the Sichuan Academy of Forestry (no specific permit number). Voucher specimens were deposited in the Sichuan Academy of Forestry, Chengdu, China.

Samples and sequencing.—We sequenced 108 specimens of Arvicolini for the gene encoding mitochondrial cytochrome *b* (*cytb*). The sequences represented 14 species plus 2 unidentified species of *Neodon*. We also sequenced 134 specimens of Arvicolini for cytochrome c oxidase subunit 1 (*CO1*), which represented 17 species plus the 2 unidentified species of *Neodon*. For comparison, we retrieved 52 *cytb* and 6 *CO1* sequences of Arvicolini from GenBank, especially representatives of the subgenera of *Microtus*. One specimen of Myodini was sequenced for *cytb*. From GenBank, we downloaded 7 haplotypes of *cytb* and 6 haplotypes of *CO1* of myodines, 2 haplotypes of *cytb* and 1 haplotype of *CO1* of Lemmini, 1 haplotype of *cytb* of Ellobiusini, 2 haplotypes of *cytb* of Prometheomyini, and 1 haplotype each of *cytb* and *CO1* from Lagurini, Ondatrini, and Phenacomyini. The outgroup, based on previous work (Galewski et al. 2006), consisted of 1 sequence each of *cytb* and *CO1* from *Mesocricetus auratus*, *Rattus norvegicus*, and *Meriones meridianus*, all retrieved from GenBank. Sample localities were mapped in Fig. 1 and detailed information was listed in Supplementary Data SD1.

Laboratory protocols.—All tissue samples were maintained in 95% ethanol at −70°C prior to DNA extraction. Total genomic DNA was extracted from muscle tissues using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland). DNA amplification of *cytb* was performed for each individual with the following universal primers: L14724 (5′-CGAAGCTTGATATGAAAAACCATCGTT-3′—Pääbo and Wilson 1988) and H15915R (5′-GGAATTCATCTCTCCGGTTTACAAGA-3′—Irwin et al. 1991). We also used 2 internal primers: L15408 (5′-ATAGACAAA.ATCCCATTCCA-3′—Irwin et al. 1991) and H15149 (5′-AACTGCAGCCCCTCAGAATG-3′—Kocher et al. 1989). PCR amplification was performed in a reaction mixture of 25 µl containing 5 pM of each primer, 100 µM of each dNTP, 2.5 µl 10 × LA PCR Buffer, 1.25 U of TaKaRa LA Taq (TaKaRa Biotechnology Co., Ltd., Dalian, China), and 50–100 ng genomic DNA. The PCR amplification was performed at 95°C for 5 min, followed by 34 cycles of 30 s at 94°C, annealing for 50 s at 48–50°C, and a 1.5-min extension at 72°C, with a final extension at 72°C for 10 min. Primers COIF 5′-TTGCAATTCGATGTGATT-3′ and COIR 5′-ATGATGCTGGCTTGAAAC-3′ were used for *CO1* amplification. Amplification of *CO1* was carried out with a touchdown protocol. PCR included an initial denaturation step at 95°C for 3 min, followed by a touchdown program including 40 cycles at 95°C for 45 s, annealing for 1 min and 72°C for 1.5 min, where annealing temperature was decreased from 60°C to 50°C by 0.5°C per cycle in the 1st 20 cycles and maintained at 50°C for the last 20 cycles. A final extension of 72°C for 10 min was also included.

Amplified PCR products were checked on a 1% agarose gel and visualized with ethidium bromide staining to verify PCR quality. Purification of PCR products was conducted with a MiniBEST DNA Fragment Purification Kit v.3.0 (TaKaRa). Cycle sequencing was performed using the BigDye 3.1 Terminator cycle sequencing kit (Applied Biosystems,

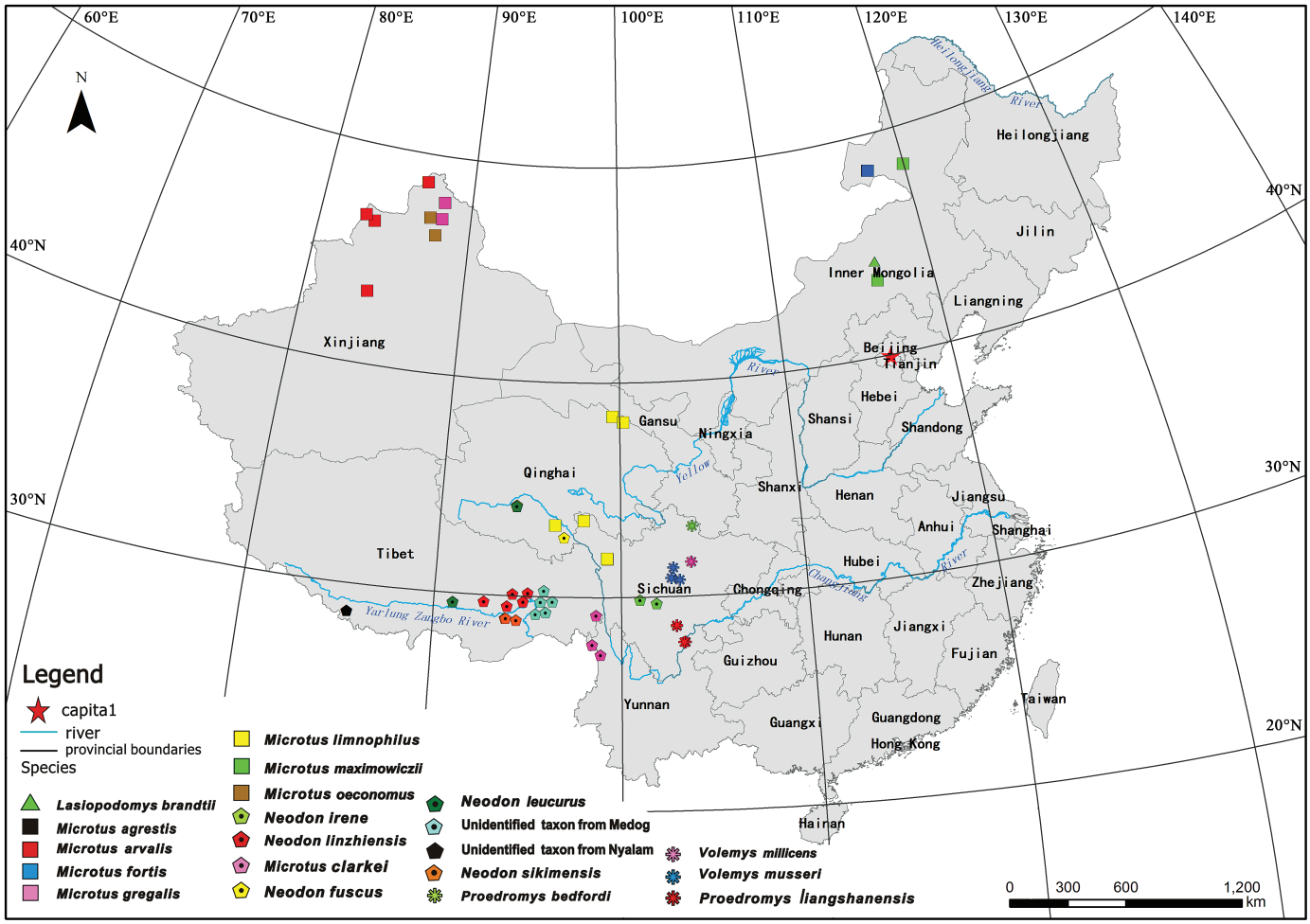

Fig. 1.—Geographic distribution of Chinese samples of the species of Arvicolini used in this study.

Foster City, California) according to the manufacturer's instructions, and nucleotide sequences were determined using an ABI PRISM 3730 sequencer (Applied Biosystems). The possibility of NUMTs was determined with the following methods: 1) translating the nucleotide sequences into amino acids to confirm expected start and stop codon positions, and the absence of premature stop codons and insertions or deletions (Luo et al. 2004); 2) constructing neighbor-joining phenograms to infer sequence orthology using MEGA5 (Tamura et al. 2011); and 3) submitting sequences for BLAST comparisons (Altschul et al. 1997).

Phylogenetic analyses.—All nucleotide sequences were edited using SeqMan from Lasergene in DNAStar v.6.0 (Madison, Wisconsin; http://www.dnastar.com) and aligned using ClustalX v.2.10 (Thompson et al. 1997) with default parameters followed by manual correction when required. Identical haplotypes were collapsed using DnaSP v.5.10.01 (Librado and Rozas 2009). Phylogenetic congruence was tested using the partition-homogeneity test (Farris et al. 1994) with 100 replicates as implemented in PAUP* v.4.0b10 (Swofford 2001). Further analyses were performed on the combined data because the results of the test indicated no significant conflict. Sequence variation and divergence were calculated using MEGA5. We chose the Kimura 2-parameter model (Kimura 1980) to summarize interspecific sequence divergence for *Neodon*, which used *cytb* sequences. MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) was used for Bayesian inference analyses. The phylogenetic analyses were conducted on the following 2 data sets: 1) *cytb* alone, and 2) concatenated *cytb* and *CO1*. Most taxa with *cytb* from GenBank did not have corresponding *CO1* sequences and insufficient taxon sampling has often been cited as a major source of error in phylogenetic analyses (Hillis et al. 2003 and references therein). Therefore, we did not use *CO1* data alone to construct the genealogy. If the taxa had *cytb* data only, the *CO1* data were treated as missing. We partitioned the sequence data by gene and codon position (Brandley et al. 2005) and estimated the best model of evolution for each partition by using PartitionFinder v.1.0 (Luo et al. 2010; Lanfear et al. 2012). For *cytb*, the best models for the 1st, 2nd, and 3rd codon positions were SYM+I+G, HKY+I+G, and GTR+I+G, respectively. Concatenated *cytb* and *CO1* had the same best models for the codon positions.

Species delimitation.—Two methods were used to delineate species boundaries: general mixed Yule-coalescent (GMYC) model (Pons et al. 2006; Cummings et al. 2008) and genealogical sorting index (GSI—Cummings et al. 2008). Both methods were appropriate for single-locus analyses (Cummings et al. 2008; Fujisawa and Barraclough 2013). Further, the GMYC

model used maximum-likelihood statistics to delimit species from single-locus ultrametric trees without the need for any prior definition of species. This method aimed to identify a time point with the highest likelihood in the tree where the branching rate shifts from speciation (Yule) to population coalescent process. Our GMYC analyses were performed for *cytb* under the single-threshold model, using the R package SPLITS (Monaghan et al. 2009).

GSI quantified the degree of exclusive ancestry of predefined groups on a tree. We used morphological data, the genealogy, and the results from the GMYC analysis to define groups. Each tip in the tree was assigned to a group representing a putative or hypothetical species. For both *CO1* and *cytb*, we calculated the *gsi* for 100 trees, which were randomly selected from the Bayesian posterior distribution of trees. These trees were then used to calculate an ensemble *gsi* statistic (gsi_T), which represented a summary of the index across the Bayesian posterior distributions (Weisrock et al. 2010). The significance of all *gsi* statistics was assessed using 1,000 randomized permutations. All analyses were performed using the web interface www.genealogicalsorting.org.

Morphology.—Six genera and 26 species of Arvicolini have been recorded from China (Musser and Carleton 2005; Liu et al. 2007, 2012). Our study used 176 specimens representing 5 genera, 18 species, and 2 unidentified forms of *Neodon* as follows: *Neodon leucurus*, *N. irene*, *N. fuscus* (topotype), *N. linzhiensis* (type specimen series), *N. sikimensis*, and unidentified specimens from Nyalam (Neilamu) and Mêdog (Motuo), *Microtus* (*Alexandromys*) *clarkei* (topotype), *M.* (*A.*) *fortis*, *M.* (*A.*) *limnophilus*, *M.* (*A.*) *maximowiczii*, *M.* (*A.*) *oeconomus*, *M.* (*Microtus*) *arvalis*, *M.* (*M.*) *agrestis*, *M.* (*Stenocranius*) *gregalis*, *Lasiopodomys brandtii*, *P. liangshanensis* (type specimen series), *P. bedfordi*, *V. musseri* (topotype), *V. millicens* (topotype; Supplementary Data SD2). The external, cranial, dental, and glans penis characters were examined. Abbreviations used in the morphological comparison followed Liu et al. (2007). External measurements were as follows: head and body length (HBL, from snout to the anus), tail length (TL), ear length (EL), and hind foot length excluding the claws (HFL). Skull measurements included the following: greatest length (SGL), skull basal length (SBL), condylobasal length (CBL), zygomatic breadth (ZB), mastoidal breadth (MB), least interorbital width (IOW), skull height (SH, from horizontal plane to the highest point when placing the skull horizontally with upper incisors and auditory bullae on ground), auditory bulla length (ABL), width across molars (M-M), length of maxillary toothrow (LMxT), length of the mandibular toothrow (LMbT), length of mandible (ML, length of projected mandible on a flat surface including lower incisor), and length of exposed parts of incisor on lower mandible (LEPILM, from the tip of incisor to the anterior-most margin of the mandible).

Statistical analyses of the morphological data were performed using SPSS v.13.0 for Windows. A principal component analysis (PCA) of 7 consistent mitochondrial lineages (*Neodon*, *Alexandromys*, *Lasiopodomys*, *Microtus*, *Proedromys, Volemys*, and *Stenocranius*) was used to evaluate variation in the 17 measurements taken from 176 specimens. A canonical discriminant analysis was also computed for 176 specimens (155 specimens of 18 known species and 21 specimens of the 2 unidentified species). For assessing the taxonomic status of the 2 unidentified species, PCA was performed for all species of Chinese *Neodon*. When the PCA failed to distinguish species or taxa, *t*-tests were used to determine statistical significance ($P < 0.05$).

Preparation of the glans penis followed Hooper (1958) and Lidicker (1968). Organs were measured and drawn to scale with the aid of a camera lucida. Bacular structures were assessed from translucent glans specimens. Measurements and abbreviations for the glans and bacula followed Hooper (1958), Yang and Fan (1988), and Yang et al. (1992), and included the following: length of glans (LG), diameter of glans (DG), total length of bony baculum (TLBB), proximal baculum length (PBL), width of proximal baculum base (WPBB), width of proximal baculum at the middle point (WPBM), height of proximal baculum base (HPBB), distal baculum length (DBL), width of distal baculum (WDB), and lateral baculum length (LBL).

Results

Matrilineal genealogy.—After alignment, 1,140 base positions were obtained from 108 samples, representing 54 different haplotypes for *cytb*. Sequences of *CO1* obtained from 134 samples of Arvicolini, representing 76 different haplotypes, consisted of 1,542 bp. Adding 53 *cytb* sequences from GenBank, we detected 536 (47%) variable sites in 107 *cytb* sequences, of which 496 (43.5%) were potentially parsimony-informative and 40 (3.5%) were singletons. The 2,682-bp segment of the concatenated data from 113 sequences contained 1,093 (40.8%) variable sites, of which 1,045 (39.0%) were potentially parsimony-informative and 48 (1.9%) were singletons. In the 113 concatenated sequences, *CO1* data were not available for 31 terminals including *Blanfordimys juldaschi*, *B. bucharensis*, *Chionomys nivalis*, and 11 species of Euromerican *Microtus*. No deletions, insertions, or premature stop codons were found, indicating that paralogous nuclear insertions likely had not been amplified. One hundred thirty newly obtained haplotypes of Arvicolini and 1 new haplotype of Myodini (*Alticola stoliczkaus*) were deposited in GenBank (accession numbers: KP190209–KP190337, KU577347–KU577349).

The interspecific K2P distances in *Neodon* (*cytb*) ranged from 10.1% to 13.0%. The lowest distance (10.1%) occurred between *N. sikimensis* and unidentified specimens from Mêdog, Xizang (Table 1).

In both data sets, analyses resolved 7 clades in Arvicolini and this tribe formed the sister-group of Lagurini (Figs. 2 and 3). The type species of Arvicolini, *Arvicola amphibius*, grouped with the Ondatrini with high support (PP = 1.0). Using *cytb* alone, all clades had high posterior probabilities, except for *Stenocranius* + *Chionomys* (PP = 0.51). *Lasiopodomys* (PP = 1.0) fell at the base of the Arvicolini. The remaining 6 lineages consisted of *Neodon* (PP = 1.0), *Alexandromys* (PP = 0.99), *Volemys* (PP = 1.0), *Microtus* (PP = 0.98,

Table 1.—Estimates of the mean distance (K2P) between clades in the genus of *Neodon*.

	N. irene	*N. linzhiensis*	*N. fuscus*	*N. leucurus*	*N. sikimensis*	Unidentified taxon from Mêdog	*Microtus clarkei*
N. irene							
N. linzhiensis	0.108						
N. fuscus	0.119	0.129					
N. leucurus	0.105	0.120	0.120				
N. sikimensis	0.105	0.112	0.130	0.107			
Unidentified taxon from Mêdog	0.109	0.116	0.130	0.120	0.101		
Microtus clarkei	0.119	0.123	0.120	0.114	0.110	0.106	
Unidentified taxon from Nyalam	0.103	0.118	0.123	0.113	0.102	0.117	0.111

including *Blanfordimys* and subgenera *Microtus*, *Mynomes*, *Pedomys*, *Pitymys*, and *Terricola*), *Proedromys* (PP = 0.96), and *Stenocranius* + *Chionomys* (Fig. 2). Strongly supported *Neodon* included *N. irene*, *N. linzhiensis*, *N. fuscus*, *N. leucurus*, *N. sikimensis*, *M. clarkei*, and the 2 unidentified samples. *Microtus clarkei* fell in the middle of this lineage and the unidentified samples from Nyalam had a sister-group relationship with other species of *Neodon* (Fig. 2). Strongly supported *Volemys* (PP = 1.0) contained *V. millicens* and *V. musseri* and highly supported *Proedromys* (PP = 0.96) included *P. liangshanensis* and *P. bedfordi* (Fig. 2).

Phylogenetic analyses of the concatenated data recovered *Chionomys* (PP = 0.99) at the base of the Arvicolini (Fig. 3). Six other clades consisted of *Neodon* (PP = 1.0), *Alexandromys* (PP = 1.0), *Volemys* (PP = 1.0), *Microtus* (PP = 0.95, including *Blanfordimys* and subgenera *Microtus*, *Mynomes*, *Pedomys*, *Pitymys*, and *Terricola*), *Proedromys* (PP = 0.98), and *Stenocranius* + *Lasiopodomys* (PP = 0.97). Although all lineages had the same species compositions as in the *cytb* tree, some relationships differed among genera or among species within genera (Fig. 3).

Species delimitation.—The single-locus GMYC analyses suggested the presence of multiple species. The GMYC model (multiple species) provided a highly significant better fit ($P < 0.001$) to the *cytb* trees than the null model with only coalescent branching rates (single species). The single-threshold model delimited 12 lineages of *Neodon*. The well-established species *N. fuscus*, *N. linzhiensis*, *M. clarkei*, and the unidentified specimens from Mêdog (Fig. 4) consisted of single species. *Neodon sikimensis* had 2 deeply divergent lineages, *N. irene* had 3, and *N. leucurus* had 2. The unidentified specimens from Nyalam were borderline for 2 species. The species delimitation analysis resolved *V. musseri*, *M. arvalis*, *M. gregalis*, *M. kikuchii*, and *M. oeconomus* as being comprised of more than a single taxon.

Genealogical sorting (gsi_T) for the Bayesian posterior distributions of *cytb* and *CO1* ranged from 0.99 to 1. The gsi_T for all groups rejected significantly the null hypothesis of mixed genealogical ancestry ($P < 0.005$). All well-established species and 2 putative new species of *Neodon* also received high gsi_T values (> 0.99), suggesting deep genealogical divergences.

Morphology.—A PCA evaluated morphological variation in 17 measurements (ABL, CBL, EL, HBL, HFL, IOW, LEPILM, LM, LMbT, LMxT, MB, M-M, SBL, SGL, SH, TL, and ZB) among *Alexandromys*, *Lasiopodomys*, *Microtus*, *Neodon*, *Proedromys*, *Stenocranius*, and *Volemys*. The 1st component separated individuals mainly on the basis of ML, ZB, SGL, SH, LEPILM, SBL, LMbT, CBL, LMxT, M-M, MB, HBL, and ABL (58.44% of variation), the 2nd component on the basis of EL, TL, and HFL (17.67% of variation), and the 3rd on the basis of IOW (8.23% of variation; Supplementary Data SD3). The trivariate scatter plot of specimen-scores on components 1, 2, and 3 mainly distinguished *Alexandromys*, *Proedromys*, *Volemys*, *Lasiopodomys*, and *Stenocranius* from each other. *Neodon* and *Microtus* (including *M. agrestis* and *M. arvalis*) were not distinguished clearly from each other (Fig. 5A). Regardless, the canonical discriminant analysis separated and correctly classified most of the 176 original specimens and the probability of correct discrimination was 97.4%. Fifty-three of 57 (93.0%) specimens of *Neodon* (including *M. clarkei*) were correctly separated and classified. Nineteen of 21 (90.5%) specimens of 2 unidentified taxa classified into *Neodon*.

PCA clustered all species of *Neodon* together, including *M. clarkei* and the 2 unidentified species. The trivariate scatter plot of specimen-scores on components 1, 2, and 3 distinguished 5 of the 8 taxa roughly; the 2 putative new species mingled with each other. *Microtus clarkei* blended slightly with *Neodon sikimensis* and the putative new species from Nyalam (Fig. 5B). However, the *t*-test obtained statistically significant differences in 7 morphometric characters (TL, EL, SBL, MB, IOW, M-M, and LEPILM) between the 2 putative new species (Supplementary Data SD4).

Comparisons of molars for the 2 unidentified species, the 5 species of *Neodon*, *M. clarkei*, and *V. millicens* were summarized in Table 2. Measurements and skull comparisons for the 2 unidentified species and *M. clarkei* and *V. millicens* were given in Table 3 and Fig. 6, and their glans penes were compared in Table 4 and Fig. 7. Considering the comparison of Liu et al. (2012), molar pattern and measurements and morphology of glans penes clearly distinguished the 7 species in *Neodon*, *M. clarkei*, and *V. millicens* from each other.

Discussion

Our analyses of *cytb* and *ctyb* + *CO1* resolve some relationships within the tribe Arvicolini. Using *cytb* alone, analyses obtain strong support for the genera *Alexandromys*, *Lasiopodomys*, *Microtus*, *Neodon*, *Proedromys*, and *Volemys*, but the clade *Chionomys* + *Stenocranius* receives very low support. Analyses of the concatenated data resolve 7 clades with very high support (PP ≥ 0.97):

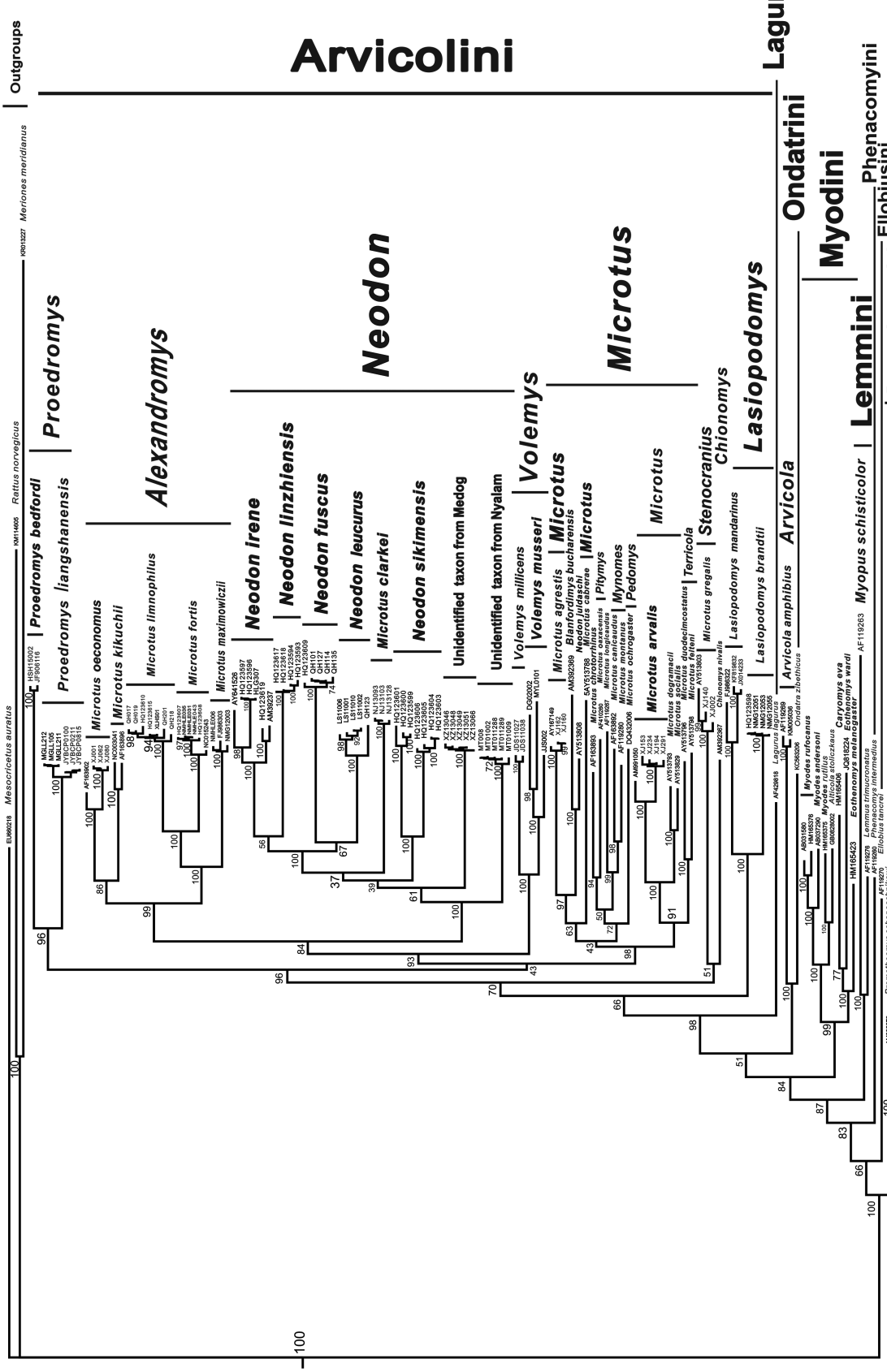

Fig. 2.—Fifty percent majority rule consensus tree from Bayesian inference analysis of *cytb* sequences. Numbers are Bayesian posterior probabilities, which are shown for critical nodes only.

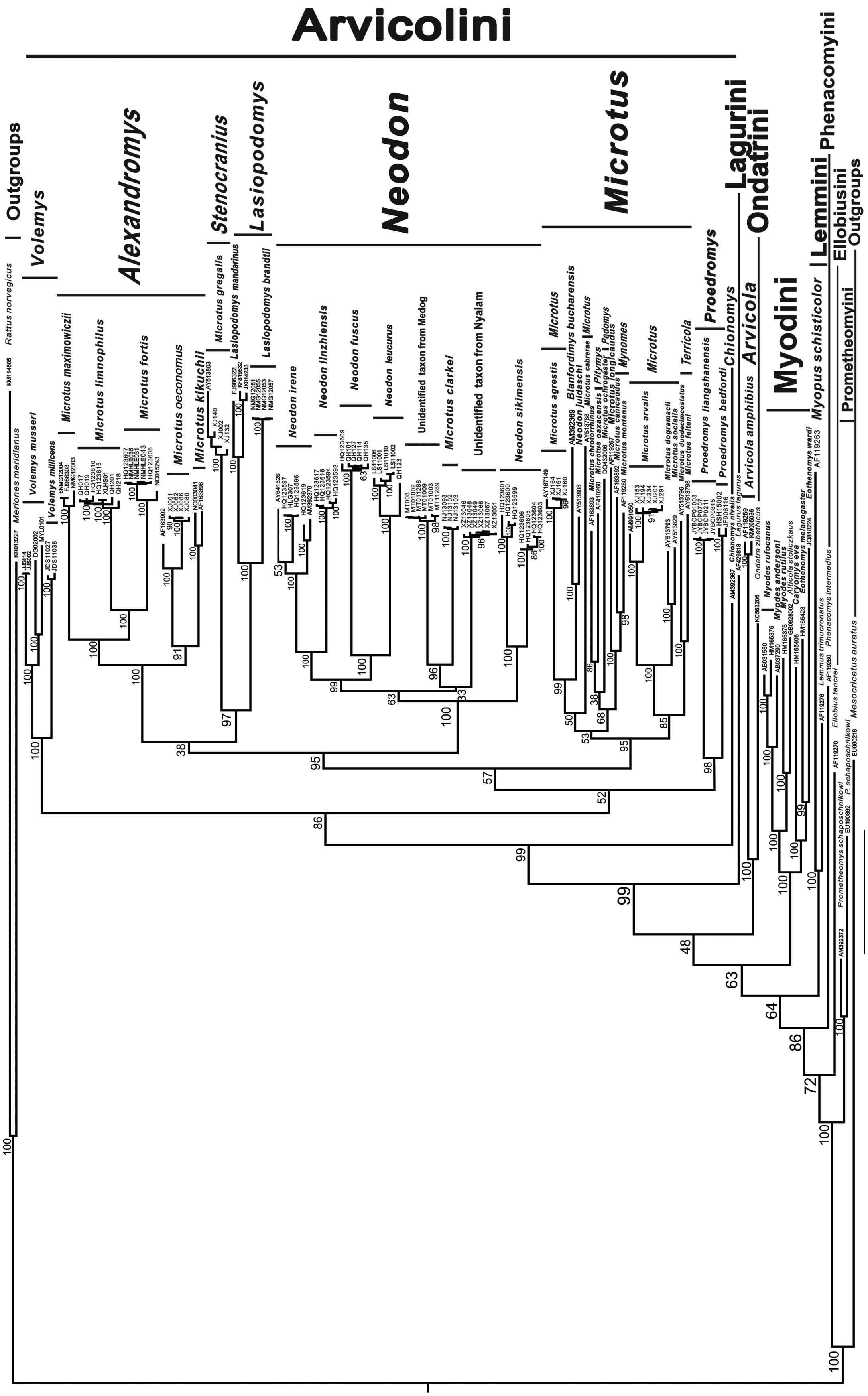

Fig. 3.—Fifty percent majority rule consensus tree from Bayesian inference analysis of concatenated *cytb* and *CO1*. Numbers are Bayesian posterior probabilities, which are shown for critical nodes only.

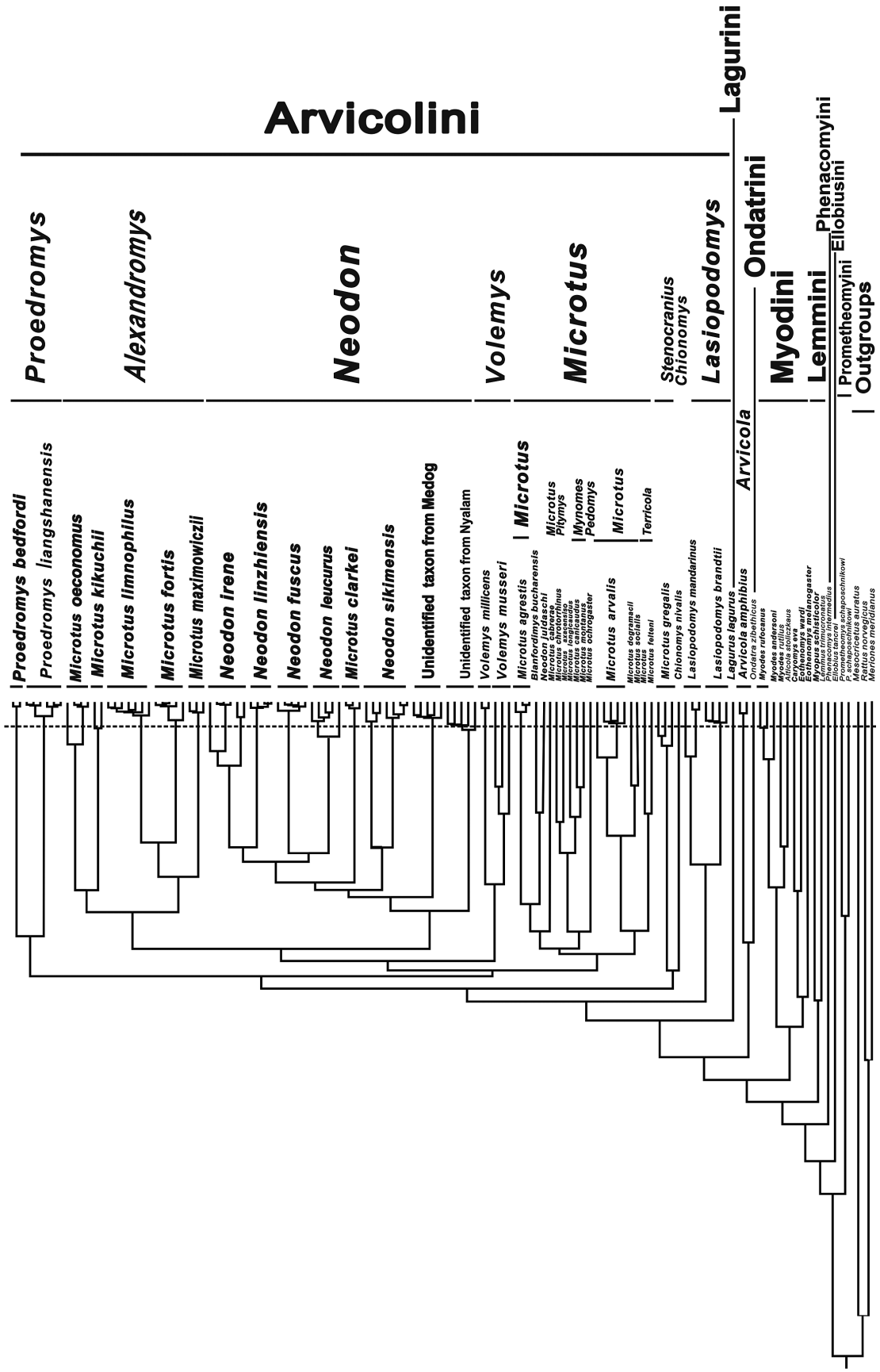

Fig. 4.—GMYC-based species delimitation based on *cyt b* trees. Thick vertical lines denote designated species and genera. Dashed lines indicate the maximum-likelihood transition point where the branching rates switch from interspecific to intraspecific events, as estimated by the GMYC model. GMYC = general mixed Yule-coalescent.

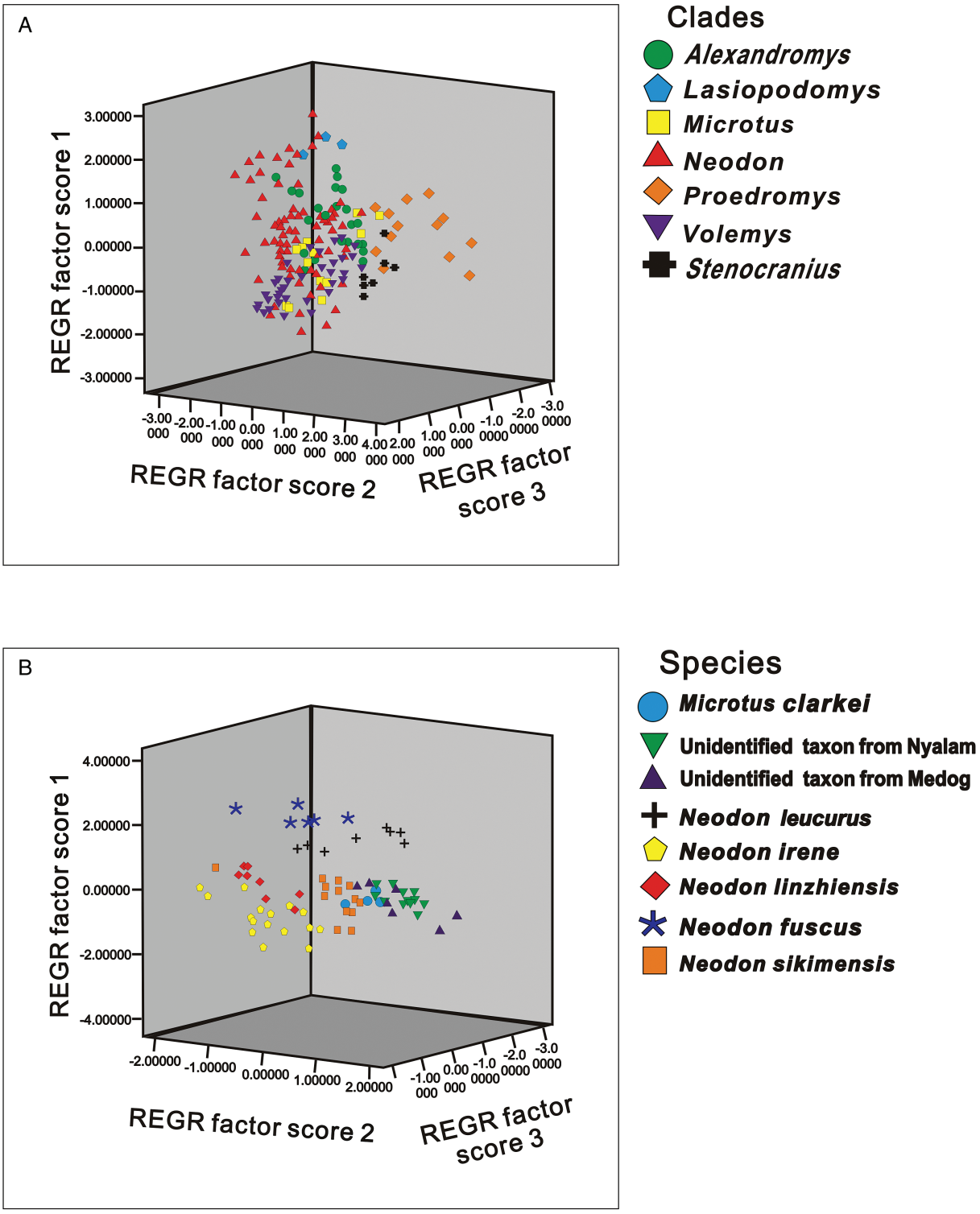

Fig. 5.—Principal component analysis (PCA) trivariate plots. A) PCA among 6 matrilines of Arvicolini; B) PCA among 8 aggregations of *Neodon*.

Alexandromys, *Chionomys*, *Lasiopodomys* + *Stenocranius*, *Microtus*, *Neodon*, *Proedromys*, and *Volemys*. Analyses of both data sets remove the type species (*A. amphibius*) of the tribe Arvicolini and resolve it as the sister-group of *Ondatra* with very strong support (PP = 1.0).

The phylogenetic relationships among *Alexandromys*, *Chionomys*, *Lasiopodomys*, *Microtus*, *Neodon*, *Proedromys*, *Stenocranius*, and *Volemys* remain ambiguous. Using nuclear *GHR*, Galewski et al. (2006) concluded that Arvicolini included the genera *Arvicola*, *Blanfordimys*, *Chionomys*, *Lasiopodomys*, *Microtus*, *Neodon*, and *Phaiomys*. Their analyses did not support the splitting of *Lasiopodomys*, *Neodon*, and *Phaiomys* from *Microtus*. In contrast, our analyses recover a monophyletic group containing *Alexandromys*, *Lasiopodomys*, *Microtus*,

Table 2.—Comparison of molar morphology between 2 new species and 5 species of the same genus and *Microtus clarkei*, *Volemys millicens*.

	M_1	M^1	M^2	M^3
Unidentified taxon from Mêdog	4 closed triangles; 6 inner and 5 outer angles	3 inner and 3 outer angles	3 inner and 3 outer angles	4 inner and 4 outer angles in 70% specimens; 4 inner and 3 outer angles in 30% specimens
Unidentified taxon from Nyalam	3 closed triangles; 6 inner and 5 outer angles	4 inner and 3 outer angles	3 inner and 3 outer angles	4 inner and 4 outer angles in 80% specimens; 4 inner and 3 outer angles in 20% specimens
Neodon sikimensis	3 closed triangles; 6 inner and 5 outer angles	3 inner and 3 outer angles	3 inner and 3 outer angles	4 inner and 3 outer angles
Neodon irene	3 closed triangles; 5 inner and 4 outer angels	3 inner and 3 outer angles	2 inner and 3 outer angles	3 inner and 3 outer angles
Neodon linzhiensis	5 closed triangles; 6 inner and 4 outer angles	3 inner and 3 outer angles	2 inner and 3 outer angles	50% specimens have 4 inner and 3 outer angles; the rest have 3 inner and 3 outer angles
Neodon fuscus	4 closed triangles; 5 inner and 4 outer angles	3 inner and 3 outer angles	2 inner and 3 outer angles	3 inner and 3 outer angles
Neodon leucurus	3 closed triangles; 5 inner and 3 outer angles	3 inner and 3 outer angles	2 inner and 3 outer angles	3 inner and 3 outer angles
Microtus clarkei	5 closed triangles; 6 inner and 4 outer angles	3 inner and 3 outer angles	3 inner and 3 outer angles	4 inner and 3 outer angles
Volemys millicens	4 closed triangles; 5 inner and 4 outer angles	3 inner and 3 outer angles	3 inner and 3 outer angles	4 inner and 3 outer angles

Table 3.—Comparison of measurements (in mm) between 2 new species, *Microtus clarkei*, and *Volemys millicens*. Mean values (in mm) are followed by ranges in parenthesis (w: g).

	New species from Mêdog		New species from Nyalam		*Microtus clarkei*	*Volemys millicens*
	Holotype	Other specimens	Holotype	Other specimens		
W	39.2	34.3 (26.3–41.4)	36	31.7 (26–36)	44.8 (30–52)	21.3 (20–24)
HBL	110	100.9 (89–110)	106	105.5 (98–115)	117.6 (103–130)	92.2 (85–102)
TL	44	48.0 (43–55)	46	43.6 (38–50)	66.3 (53–71)	46.1 (42–49)
HFL	20	19.6 (18–20)	19	18.3 (17–20)	21.3 (21–22)	17.4 (16–18)
EL	14	13.6 (12–15)	13	12.2 (11–13)	15.2 (15–16)	12.4 (12–13)
TL/HBL	40	47.6 (42.0–55.0)	43.4	41.3 (37.0–44.0)	56.4 (50.0–60.0)	50.0 (45.2–52.9)
SGL	26.81	26.65 (24.11–28.54)	27.08	26.12 (25.25–27.19)	29.50 (28.86–30.17)	24.22 (23.14–25.10)
SBL	25.39	25.11 (22.7–26.85)	25.32	24.02 (22.67–25.60)	27.77 (27.36–28.41)	22.44 (21.45–23.39)
CBL	26.79	26.49 (24.01–28.13)	26.62	26.22 (24.09–27.05)	28.68 (27.93–30.06)	23.34 (22.33–24.19)
ZB	15.87	15.45 (13.42–16.56)	15.75	14.87 (13.98–15.72)	16.51 (16.25–16.75)	13.13 (12.28–13.65)
IOW	3.75	4.01 (3.52–4.32)	3.42	3.67 (3.56–4.06)	4.13 (3.96–4.17)	4.15 (3.93–4.37)
MB	12.47	12.20 (11.37–12.61)	11.9	11.78 (11.29–12.25)	13.50 (12.5–13.75)	11.66 (11.21–11.92)
SH	10.46	9.97 (9.26–10.62)	10.07	9.77 (9.24–10.53)	10.43 (10.28–10.66)	8.39 (7.88–8.80)
ABL	6.8	6.72 (6.05–7.07)	6.75	6.65 (6.42–6.95)	8.17 (8.08–8.22)	6.77 (6.46–7.24)
LMxT	6.41	6.41 (5.95–7.07)	6.42	6.34 (6.18–6.48)	6.65 (6.50–6.70)	5.41 (5.13–5.82)
LMbT	6.24	6.32 (5.92–6.76)	6.51	6.38 (6.17–6.61)	6.65 (6.51–6.70)	5.25 (5.04–5.54)
M-M	5.58	5.56 (5.30–5.71)	5.58	5.39 (5.01–5.61)	5.47 (5.54–5.86)	4.73 (4.55–5.00)
ML	19.88	19.39 (17.45–21.11)	19.48	18.71 (17.33–19.48)	20.76 (19.76–21.40)	16.97 (16.04–17.66)
LEPILM	9.19	8.95 (7.31–9.75)	8.89	8.19 (7.52–8.89)	8.93 (8.27–9.48)	7.14 (6.66–7.56)

Neodon, *Proedromys*, and *Volemys*, but with the exclusion of *Arvicola*.

Mitochondrial genes are powerful markers for resolving relationships within recently evolved groups and nuclear genes are more suitable at deeper levels (Galewski et al. 2006). Therefore, we recognize *Alexandromys*, *Lasiopodomys*, *Microtus*, *Neodon*, *Proedromys*, and *Volemys* as valid genera of Arvicolini. This conclusion agrees with Musser and Carleton (2005) that *Lasiopodomys*, *Microtus*, *Neodon*, *Proedromys*, and *Volemys* are valid genera, and corresponds with Abramson and Lissovsky (2012) in that the subgenus *Alexandromys* should be elevated to genus. However, our study does not support *Phaiomys* as a valid genus, as suggested by Musser and Carleton (2005), but agrees with Liu et al. (2012) in the synonymization of *Phaiomys* into *Neodon*.

In our analyses, *Arvicola* forms a sister-group relationship with monotypic *Ondatra zibethicus* with high support (PP = 1.0) in both data sets. This result is consistent with many other studies based on mitochondrial genes (Conroy and Cook 1999; Bužan et al. 2008; Bannikova et al. 2009), but it differs from results based on nuclear genes (Galewski et al. 2006; Abramson et al. 2009b). Because mitochondrial genes only recover the matrilineal genealogy and many studies now report the introgression of mitogenomes due to ancient interspecific hybridization, the status of *Arvicola* and the validity of the Arvicolini require further study. It is possible that the name Microtini will replace the Arvicolini, and that the latter will be a junior synonym of Ondatrini.

Phylogenetic relationships of *Stenocranius*, which consists of *M. gregalis* only, differ in the analyses of our 2 data sets.

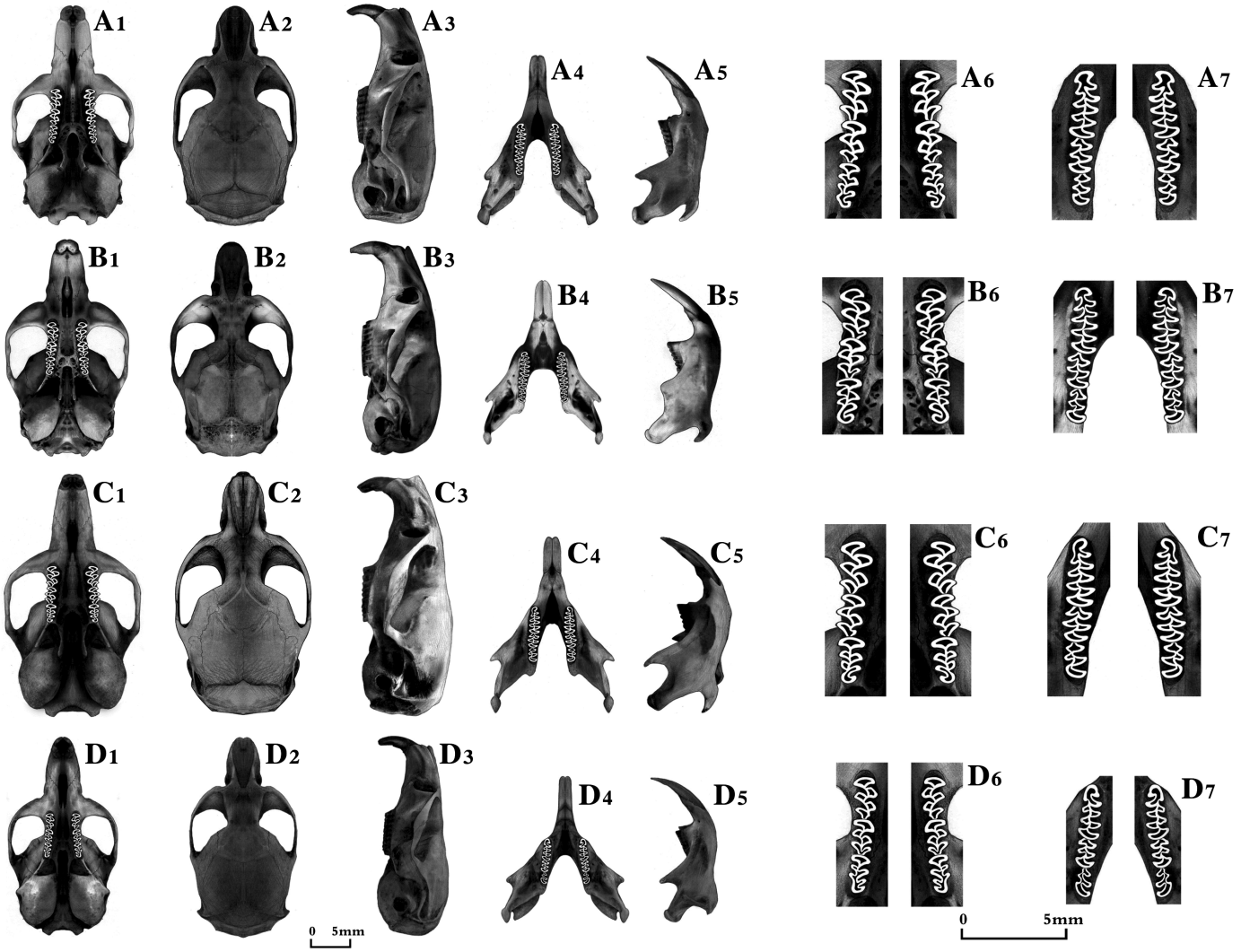

Fig. 6.—Skulls of the new species *Neodon medogensis* and *Neodon nyalamensis* along with those of *Microtus clarkei* and *Volemys millicens*. A) *N. medogensis*; B) *N. nyalamensis*; C) *M. clarkei*; and D) *V. millicens*. 1: ventral view; 2: dorsal view; 3: lateral view; 4: lower jaw (ventral); 5: lower jaws (lateral); 6: upper toothrow; and 7 lower toothrow.

Thus, one of our results conflicts with the opinion that *Lasiopodomys* includes *Stenocranius* (Abramson and Lissovsky 2012; Petrova et al. 2015). Further work is necessary.

Hinton (1923) described *M. clarkei*. Zagorodnyuk (1990) assigned it to *Volemys* but Musser and Carleton (2005) returned it to *Microtus*. Our specimen is from the divide between the Kiukiang (Dulong in Chinese) and Salween (Nujiang in Chinese) rivers in Gongshan county at the junction of Xizang (Tibet) and Yunnan, which is near the type locality. Phylogenetically, the species falls in the middle of *Neodon*. Skulls, teeth, and penises of the species align with members of *Neodon*, although distinct differences exist, as expected in the diagnoses of species (Figs. 6C and 7C; Tables 2–4). The canonical discriminant analysis also places all specimens of *M. clarkei* into *Neodon*. Accordingly, we assign *M. clarkei* to *Neodon*, as *N. clarkei*. Luo et al. (2004) used *M. clarkei* (AY641526) as an outgroup member in their phylogeny of *Eothenomys*. However, our analysis places the animal within *N. irene*, as did the study of Liu et al. (2012). Bannikova et al. (2010) used the same sequence when investigating Asian Arvicolinae and also obtained these results. Our recent communication with Dr. Luo has confirmed that their specimen of *M. clarkei* was, in fact, a misidentified *N. irene*.

Feng et al. (1986) recorded *V. millicens* from southeastern Xizang. Some of our specimens from Mêdog look identical to *V. millicens* (Liu et al. 2010). Compared to 20 topotypes collected in 2013, Tibetan specimens have a much larger HBL, much lower TL/HBL, and different M^1, M^3, and M_3 patterns. For example, the 1st lower molar of *V. millicens* has 5 inner and 4 outer angles, but the Tibetan specimens have 6 inner and 5 outer angles; the 3rd upper molar of *V. millicens* has 3 outer angles, but the Tibetan specimens have 4 angles; and the 3rd lower molar of *V. millicens* has 2 outer angles, but the Tibetan specimens have 3 outer angles (Figs. 6D and 7D; Tables 2–4). Genealogically, our Tibetan specimens fall in *Neodon* with high support and not with topotypes of *V. musseri* and *V. millicens*. Thus, below we describe the Tibetan *millicens* as a new species. It consists of the unidentified taxon from Mêdog.

Table 4.—Comparison of glans penes between 2 new species, *Microtus clarkei*, and *Volemys millicens*. Mean values (in mm) are followed by ranges (in mm) in parenthesis.

	Volemys millicenus	*Microtus clarkei*	Unidentified taxon from Mêdog	Unidentified taxon from Nyalam
n	5	1	4	6
LG	3.48 (3.30–3.60)	4.30	3.90 (3.80–4.00)	4.42 (4.20–4.50)
DG	1.80 (1.70–2.00)	2.40	2.30 (2.20–2.40)	2.58 (2.40–2.80)
Proximal baculum	Bone, sturdy, anterior part pole-like and the top even, proximal part rhombic.	Bone, anterior part trumpet-like. The top circinal.	Bone, very sturdy, anterior part pole-like and the top even, proximal part trapezium-like.	Bone, very sturdy, anterior part pole-like and the top even, proximal part shovel-like.
Distal baculum	Bone, sturdy, proximal part bulging and dentate in 2 sides.	Cartilaginous, and sturdy.	Bone, stick-like, proximal bulging.	Bone, stick-like, proximal bulging.
Lateral baculum	Bone, stick-like.	Cartilaginous, stick-like.	Bone, stick-like, short.	Bone, stick-like, only slightly ossified.
Dorsal papilla	Three forks, 2 of them located the back.	Coniform, single.	Coniform, single.	50% coniform, and 50% two forks.
Outer crater papilla	4–6 every side, obvious.	2 on every sides, unobvious.	No obvious outer crater papilla.	2–3 on every sides, unobvious.
Urethral lappet	Three forks; the middle fork very short.	Two forks.	Two forks.	Three forks and the same height. Very deeply divided and finger-like.
TLBB	3.58 (3.50–3.70)	4.00	3.56 (3.50–3.60)	4.68 (4.60–4.80)
PBL	2.36 (2.30–2.50)	2.80	2.48 (2.45–2.50)	3.02 (2.90–3.15)
WPBB	1.34 (1.20–1.60)	1.50	1.50 (1.40–1.60)	1.58 (1.40–1.80)
WPBM	0.33 (0.30–0.50)	0.45	0.63 (0.60–0.65)	0.47 (0.40–0.60)
HPBB	0.66 (0.50–0.80)	0.50	0.54 (0.45–0.60)	0.58 (0.45–0.70)
DBL	1.16 (1.00–1.20)	1.00	1.00 (0.90–1.10)	1.63 (1.60–1.70)
WDB	0.52 (0.48–0.60)	0.50	0.41 (0.30–0.50)	0.57 (0.40–0.70)
LBL	0.79 (0.70–0.90)	0.70	0.68 (0.42–1.10)	0.51 (0.35–0.75)

Zagorodnyuk (1990) erected *Volemys* for *V. clarkei*, *V. kikuchii*, *V. millicens*, and *V. musseri*. Musser and Carleton (1993) recognized the genus, but subsequently moved *V. clarkei* and *V. kikuchii* to *Microtus* and retained only *V. millicens* and *V. musseri* in the genus (Musser and Carleton 2005). Chen et al. (2012) resolved *V. musseri* and *P. bedfordi* as sister-taxa with high support based on analyses of nuclear *GHR* and *IRBP* and suggested the taxonomic status of *Volemys* needed further study. Our analyses resolve *Volemys* as containing *V. musseri* and *V. millicens.* However, *V. clarkei* assigns to *Neodon* and *V. kikuchii* assigns to *Microtus*. This result supports, in part, the opinions of Musser and Carleton (2005).

Musser and Carleton (2005) only recognized 4 species in *Neodon*: *N. sikimensis*, *N. forresti*, *N. irene*, and *N. juldaschi*. *Neodon juldaschi* has since been removed from *Neodon* (Bannikova et al. 2010; Liu et al. 2012) and assigned to *Blanfordimys*. Our analyses resolve a monophyletic *Blanfordimys*, but this renders *Microtus* a paraphyletic genus. Thus, the status of *Blanfordimys* requires further study.

Our matrilineal genealogy supports recognition of *Proedromys* as a valid genus that includes *P. bedfordi* and *P. liangshanensis*. Our result disagrees with that of Chen et al. (2012), who questioned the status of *P. liangshanensis* due to low nodal support from *cytb*. Further, *P. liangshanensis* and *P. bedfordi* did not cluster together on their *GHR* and *IRBP* tree. In contrast, our analyses of *cytb* and the concatenated data assign them with high support to the same lineage. The 2 species consistently differ from one another morphologically (Liu et al. 2007), and there is no evidence of hybridization from the mtDNA data. They appear to constitute 2 species.

The species delimitation analysis shows that *N. sikimensis*, *N. irene*, *N. leucurus*, unidentified taxon from Nyalam, and *V. musseri* constitute a monophyletic taxon. However, this result contradicts the well-studied morphology. Some previous studies have reported that GMYC tends to deliver an unrealistically high out-count (Kekkonen and Hebert 2014; Michonneau 2015). Therefore, we continue to recognize *N. sikimensis*, *N. irene*, and *N. leucurus*, the unidentified taxon from Nyalam, and *V. musseri*. *Microtus arvalis*, *M. gregalis*, *M. kikuchii*, and *M. oeconomus* may comprise multiple species because of deep mtDNA divergences. They need further study especially because all sequences of *M. kikuchii* and the deeply divergent sequences of *M. gregalis*, *M. arvalis*, and *M. oeconomus* are from GenBank.

Previously, Liu et al. (2007) described *P. liangshanensis* from the Liangshan Mountains, Sichuan, China and stated that the front of the incisors did not have grooves. However, most individuals in a subsequent collection possess them. Thus, *P. bedfordi* and *P. liangshanensis* differ from one another as follows: HBL and TL/HBL of *P. liangshanensis* are much larger than in *P. bedfordi*; 3rd upper molar of *P. bedfordi* with a bean-like tooth-loop, which in *P. liangshanensis* is composed of several triangles; the distal baculum of *P. bedfordi* is stick-like, but the distal baculum of *P. liangshanensis* is cap-like.

Our analyses of K2P genetic distances obtain divergences that typically separate species of mammals, which have a mean interspecific gap of 7.8% (± 4.5—Meier et al. 2008). Our gaps fall within this variation. Although this measure may misidentify species, our divergences exceed the upper limit of intraspecific variation, the overlap between inter- and intraspecific mean variation, and the overlap between inter- and lowest intraspecific divergence. Thus, K2P distances also suggest that the newly sampled populations are undescribed species.

The 2 new species are described as follows:

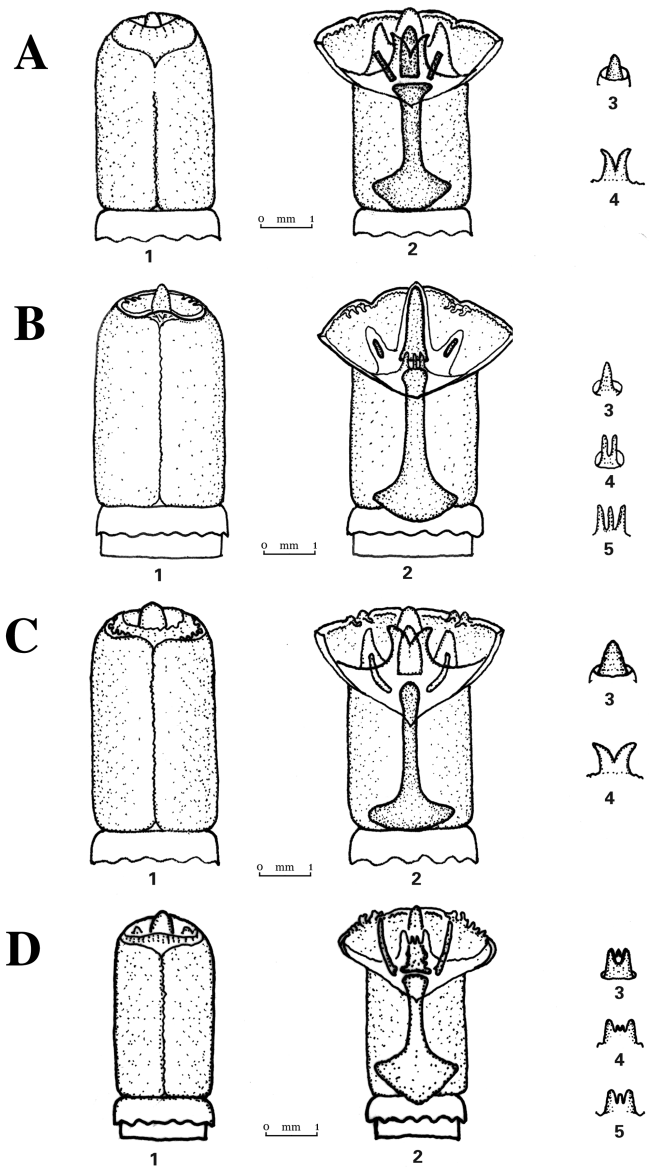

Fig. 7.—Comparison of the glans penis of the new species *Neodon medogensis* and *Neodon nyalamensis* with *Microtus clarkei* and *Volemys millicens*. A) *N. medogensis*; B) *N. nyalamensis*; C) *N. clarkei*; and D) *V. millicens*. 1: glans; 2: mid-ventral cut view; 3: dorsal papilla (A, C); 4: urethral lappet (A, C); 3, 4: dorsal papilla (B, D); 5: urethral lappet (B, D).

Neodon medogensis, new species

Holotype.—Adult male, Field number MT009 (Museum number SAF08810), collected from Mêdog (Motuo) county, Xizang (Tibet), by Yang Liu on 14 May 2008. The specimen was prepared as a skin with cleaned skull, translucent baculum, and deposited in Sichuan Academy of Forestry.

Type locality.—Mêdog (Motuo) county; 29.73815°N, 95.67496°E; 3,410 m a.s.l.

Measurements of holotype.—Weight, 39.2 g; HBL, 110 mm; TL, 44 mm; HFL, 20 mm; EL, 14 mm; SGL, 26.81 mm; SBL, 245.39 mm; CBL, 26.79 mm; ZB, 15.87 mm; IOW, 3.75 mm; MB, 12.47 mm; SH, 10.46 mm; ABL, 6.80 mm; LMxT, 6.41 mm; LMbT, 6.24 mm; M-M, 5.58 mm; ML, 19.88 mm; LEPILM, 9.19 mm.

Paratypes.—Six specimens (2 ♂♂, 4 ♀♀), skins with skulls, and male specimens with glans penis. Four specimens (MT001 [SAF08802], ♂; MT003 [SAF08804], ♀; MT006 [SAF08807], ♀; MT007 [SAF08808], ♀) from type locality, collected by Yang Liu. Two specimens (XZ11288 [SAF11473], ♀; XZ11289 [SAF11474], ♀) from 6 km southwest of type locality, Mêdog (Motuo) county, Xizang, 29.70939°N, 95.58225°E, 2,770 m, collected by Rui Liao.

Additional specimens.—Seven specimens (3 ♂♂, 4 ♀♀), 4 of which (MT002 [SAF08803], ♂; MT004 [SAF08805], ♀; MT005 [SAF08806], ♀; MT008 [SAF08809], ♂, with skulls broken) are from the type locality, collected by Yang Liu, and 3 of which (XZ11287 [SAF11472], ♂; XZ11290 [SAF11475], ♀; XZ11291 [SAF11476], ♀, with skulls broken) are from 6 km southwest of type locality, 29.70939°N, 95.58225°E, 2,770 m, Mêdog (Motuo) county, collected by Rui Liao.

Geographic distribution.—Known only from 2 sites in Mêdog (Motuo) county (Fig. 1).

Etymology.—The name is derived from the Mêdog (Motuo) county.

Diagnosis.—An arvicoline rodent with typical palate of *Microtus*. First lower molar usually with 4 closed triangles and 6 inner and 5 outer angles. Second upper molar with a posterior inner angle, and 3 inner and 3 outer angles. Third upper molar usually with 4 inner and 4 outer angles, while 30% of specimens with 4 inner and 3 outer angles. First transversal loop of the 3rd lower molar with an outer angle, 3rd lower molar with only 3 outer and inner angles. Tail comparatively long, nearly 48% of HBL (average 47.6%). Baculum unique with the proximal part flask-shaped, and distal part bulging and even; distal baculum tongue-shaped and sturdy; lateral bacula short stick-shaped. This species is much like *V. millicens* in tooth pattern in that the 1st lower molar of both species have 4 closed triangles, the 1st upper molar has 3 inner and 3 outer angles, and the 2nd upper molar has 3 inner and 3 outer angles. However, the 1st lower molar of *N. medogensis* has 6 inner and 5 outer angles versus *V. millicens* possessing 5 inner and 4 outer angles. The 3rd upper molar of the new species has 4 inner and 4 outer angles versus *V. millicens* having 4 inner and 3 outer angles. *Neodon medogensis* and *N. fuscus* have 4 closed triangles in the 1st lower molar but they differ from each other in tooth pattern and measurements. All other species of *Neodon* have 3 or 5 closed triangles in their lower molars.

Description.—General pelage color of holotype black-brown. Fur fine and about 10 mm long; proximal part of fur black-gray, distal part brown. Very few guard hairs. Venter and dorsum with obvious boundary. Ventral gray-white with black base; color from throat to belly and anus uniform. Margin of lip gray-white. Pelages of paratypes same as holotype.

Mystacial vibrissae mostly white, but some black, 20–25 each side. Shortest vibrissa about 5 mm and longest ones about 20 mm.

Ears project slightly above pelage; rim on front of ears covered with dense gray fur; back of ears with dense gray fur. Top of tail black-gray and underside light gray-white. Hairs on

tip of tail slightly longer. Forelimb hairs gray. Hindlimb pelage gray and lustrous. Claws yellow-white. Five palmar and 6 plantar pads. Females with 8 mammae consisting of 2 pectoral pairs and 2 inguinal pairs.

Skull sturdy (Fig. 6, A1–3), straight in dorsal profile; nasal slightly arced, brain case orbicular. Nasal bones broad anteriorly and narrow posteriorly and even on end. Posterior and anterior frontal bones broad but narrow in middle. Interparietal broad and rhombus-shaped, the mid-anterior part protruding to frontals. Distinct ridges in interorbital space; old specimen with 2 ridges forming a crest. Two ridges behind temporal and above auditory bulla. Zygomatic arches sturdy. Auditory bullae medium-sized. Middle of incisive foramen slightly broad anteriorly and posteriorly narrow. Posterior palate typical of *Microtus*, continuing as a narrow bridge, and separating 2 lateral pits. Many small holes in palatine and pterygoid; mandibles sturdy (Fig. 6, A4–5).

Upper incisors are orange in color. First upper molar (Fig. 6, A6) with 5 closed triangles (2 inner and 3 outer), forming 3 outer and 3 inner angles. Second upper molar with 4 closed triangles (2 inner and 2 outer), last triangle with an inner angle forming 3 inner and 3 outer angles (Fig. 6, A6), but in some specimens, the inner angles vestigial. Third upper molar of 80% of the specimens transverse prism-like followed by 2 small outer, and a larger inner closed triangles, and a C-shaped loop forming an outer angle; this configuration forms 4 inner and 4 outer angles. The remaining specimens same as former but C-shaped loop without outer angles, forming 4 inner and 3 outer angles.

Lower incisors relatively long, extruding out the mandible 8.95 ± 0.766 mm on average (LEPILM). First lower molar (Fig. 6, A7) with 4 closed triangles in front of posterior transverse space; anterior space large and anomalistic forming 3 inner angles and 2 outer angles; this molar with 6 inner and 5 outer angles. Second lower molar (Fig. 6, A7) with 3 outer and inner angles. Third lower molar (Fig. 6, A7) with 3 transverse lobes, the anterior-most of which with an external projection; 3 inner and 3 outer angles.

Glans penis (Fig. 7, A1–4) slender (Table 4). Exterior of glans pole-shaped with a ventral groove. Outer crater papillae absent. Urethral lappet forks into 2 branches. Dorsal papilla cone-shaped, as high as outer dorsal crater. Proximal baculum bony, broad and flask-shaped, distal part even. Distal baculum also bony and tongue-shaped. Lateral bacular processes bone, short stick-shaped (Table 4).

Habitat.—Type locality has bamboo about 3 m in height; coverage about 75%. The other location has original coniferous forest with spruce. Height of the trees is about 20 m; coverage about 30%. Two localities have thick humus and very loose soil. The holes of this species are about 20 mm in diameter and usually dug under rotten wood.

Neodon nyalamensis, new species

Holotype.—Adult female, Field number XZ13055 (Museum number SAF13517) collected from Nyalam (Nielamu) county, Xizang, by Rui Liao and Wang Tie on 7 November 2013. The specimen was prepared as a skin with cleaned skull and deposited in Sichuan Academy of Forestry.

Type locality.—Nyalam (Nielamu) county; 28.08152°N, 85.99854°E; 3,200 m a.s.l.

Measurements of holotype.—Weight, 36 g; HBL, 106 mm; TL, 46 mm; HFL, 19 mm; EL, 13 mm; SGL, 27.08 mm; SBL, 25.32 mm; CBL, 26.62 mm; ZB, 15.72 mm; IOW, 3.42 mm; MB, 11.90 mm; SH, 10.07 mm; ABL, 6.75 mm; LMxT, 6.42 mm; LMbT, 6.51 mm; M-M, 5.58 mm; ML, 19.48 mm; LEPILM, 8.89 mm.

Paratypes.—Thirteen specimens (9 ♂♂, 4 ♀♀), skins with skulls, and male specimens with glans penises. Six specimens (XZ13046 [SAF13508], ♂; XZ13047 [SAF13509], ♂; XZ13048 [SAF13510], ♂; XZ13049 [SAF13511], ♂; XZ13050 [SAF13512], ♂; XZ13051 [SAF13513], ♂) from the type locality. Seven specimens (XZ13066 [SAF13528], ♂; XZ13067 [SAF13529], ♂; XZ13077 [SAF13539], ♂; XZ13071 [SAF13533], ♀; XZ13072 [SAF13534], ♀; XZ13073 [SAF13535], ♀; XZ13076 [SAF13538], ♀) collected from 2 km north of the type locality, Nyalam (Nielamu) county, 28.12550°N, 85.98440°E, 3,630 m a.s.l.

Additional specimens.—Eight specimens (2 ♂♂, 6 ♀♀) with skull broken, including 4 specimens (XZ13052 [SAF13514], ♀; XZ13053 [SAF13515], ♀; XZ13054 [SAF13516], ♀; XZ13056 [SAF13518], ♀) from type locality and 4 specimens (XZ13068 [SAF13530], ♂; XZ13069 [SAF13531], ♂; XZ13074 [SAF13536], ♀; XZ13075 [SAF13537], ♀) from 2 km north of the type locality, Nyalam county.

Geographic distribution.—Presently known from Nyalam (Nielamu) county (Fig. 1).

Etymology.—The name is derived from Nyalam (Nielamu) county.

Diagnosis.—An arvicoline rodent with palate typical of *Microtus*. First lower molar usually with 3 closed triangles and 6 inner and 5 outer angles. First upper molar with very obvious posterior inner angle, forming a minor tooth rim in some specimens. First upper molar with 4 inner and 3 outer angles. Second upper molar with very obvious posterior inner angle, forming 3 inner and outer angles. Third upper molar usually with 4 inner and outer angles. First transverse loop of 3rd lower molar with an obvious outer angle, resulting in 3 inner and outer angles. Tail about 40% of HBL. Glans penis with 3 outer crater papillae on all sides. Proximal baculum trumpet-shaped and distal end circinal; distal baculum sword-shaped and sturdy; lateral bacula slightly ossified only. Regarding both new species, 1st lower molar of *N. medogensis* has 4 closed triangles versus 3 in *N. nyalamensis*; anterior-most teeth-loop of *N. medogensis* trefoil, but semicircular in *N. nyalamensis*; 1st upper molar of *N. medogensis* with 3 inner and outer angles and no posterior inner angle, but *N. nyalamensis* has 4 inner and 3 outer angles and a very distinct posterior inner angle; *N. medogensis* has TL/HBL of nearly 48%, but that of *N. nyalamensis* about 40%; glans penis of the new species exhibits many differences. Compared with the remaining 5 species of *Neodon*, the 1st lower molar of *N. nyalamensis* has 3 closed triangles, but *N. clarkei*, *N. fuscus*, and *N. linzhiensis* have 4 or 5 closed

triangles; the 1st upper molar of *N. nyalamensis* has a very distinct posterior inner angle, forming 4 inner angles but those of *N. irene*, *N. leucurus*, and *N. sikimensis* do not have posterior inner angles and only 3 inner angles.

Description.—General pelage of holotype gray brushing with pale yellow. Fur hairs fine and long, about 8–10 mm. Guard hairs absent. Venter more lightly colored than dorsum. Ventral fur black-gray; color from throat to belly and anus uniform. Transition between darker dorsal and lighter ventral pelage not abrupt. Pelages of paratypes same as holotype.

Mystacial vibrissae mostly white, but some black, 18–20 on each side. Shortest vibrissa about 6 mm, and longest about 25 mm.

Almost bare ears project slightly above the pelage. Top of tail black-gray and underside light gray-white. Hairs on tip of tail slightly longer. Forelimb and hindlimb hairs black-gray. Claws yellow-white and translucent. Five palmar and 6 plantar pads. Females with 8 mammae consisting of 2 pectoral pairs and 2 inguinal pairs.

Skull sturdy (Fig. 6, B1–3), dorsal profile forming an arc, brain case circular. Nasals broad anteriorly and narrow posteriorly. Posterior margin of nasals pointed, protruding in front of the maxilla. Posterior and anterior of frontal bone broad, while narrow in middle. Interparietal bone rectangular, with mid-anterior part protruding to frontals. Weak interorbital ridges form a faint crest in older specimens. Zygomatic arches sturdy. Bulla behind temporal and above auditory. Auditory bullae medium-sized. Incisive foramen long and narrow, 1.4 mm wide and 5.4 mm long. Posterior palate typical of *Microtus*, with 2 obvious lateral pits. Many small foramen in palatine and pterygoid; mandibles sturdy (Fig. 6, B4–5).

Upper incisors orange. First upper molar (Fig. 6, B6) with 5 closed triangles (2 inner and 3 outer), and last triangle with obvious inner angle, some triangles forming a lobe resulting in 4 outer and 3 inner angles. Second upper molar with very obvious poster-inner angle, forming 3 inner and 3 outer triangles (Fig. 6, B6). Third upper molar in 80% of specimens with 4 inner and 4 outer angles, and 20% with 4 inner and 3 outer angles (Fig. 6, B6).

Lower incisors medium-sized, extruding out the mandible 8.19 ± 0.490 mm in average. First lower molar (Fig. 6, B7) with 3 closed triangles in front of the posterior transverse space, which has 6 inner and 5 outer angles. Second lower molar (Fig. 6, B7) with 3 outer and 3 inner angles has 4 closed triangles in front of the posterior transverse space. Third lower molar with 3 inner and 2 outer angles has 3 transverse lobes; anterior-most lobe obviously projects externally.

Glans penis (Fig. 7, B1–5) broad relative to length (Table 4). Exterior of glans pole-shaped and with a ventral groove. Three outer crater papillae on all sides. Urethral lappet forks into 3 branches. Dorsal papilla cone-shaped. Proximal baculum bony, broad, trumpet-shaped with circinal distal tip. Distinct distal baculum bony and sword-shaped; lateral bacular processes only slightly ossified (Table 4).

Habitat.—At the type locality, the vegetation consists of secondary coniferous forest with spruces, about 6 m high and with 40% coverage. Below the canopy, bamboo shrub about 1.5 m high covers 70%. Vegetation of the other site is bamboo shrub with 1.5 m height and 50% coverage. Two sites are valley shrubs with huge stones and the soil is arenarious. Holes usually dug under rocks and among bamboos.

Nomenclatural statement.—A life number was obtained for the new species *Neodon medogensis* and *Neodon nyalamensis*: urn:lsid:zoobank.org:pub:08801F67-911B-447C-91FA-4BFA171FA149.

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Supplementary Data

Supplementary Data SD1.—Samples used in this study and accession numbers.

Supplementary Data SD2.—Specimens examined morphologically in this study.

Supplementary Data SD3.—Character loading and percentage of variance explained on the 1st 3 components of the principal component analysis; morphological measurements from adult specimens of 7 clades.

Supplementary Data SD4.—Student's *t*-test for equality of means for 17 measurements between 2 unidentified taxa.

Literature Cited

Abramson, N. I., V. S. Lebedev, A. A. Bannikova, and A. S. Tesakov. 2009b. Radiation events in the subfamily Arvicolinae (Rodentia): evidence from nuclear genes. Doklady Biological Science 428:458–461.

Abramson, N. I., V. S. Lebedev, A. S. Tesakov, and A. A. Bannikova. 2009a. Supraspecies relationships in the subfamily Arvicolinae (Rodentia, Cricetidae): an unexpected result of nuclear gene analysis. Molekulyarnaya Biologiya 43:834–846 (in Russian).

Abramson, N. I., and A. A. Lissovsky. 2012. Subfamily Arvicolinae. Pp. 220–275 in The mammals of Russia: a taxonomic and geographic references (I. Y. Pavlinov and A. A. Lissovsky, eds.). KMK Scientific Press Ltd., Moscow, Russia.

Altschul, S. F., et al. 1997. Gapped BLAST and PSIBLAST: a new generation of protein database search programs. Nucleic Acids Research 17:3389–3402.

Bannikova, A. A., et al. 2010. Molecular phylogeny and evolution of the Asian lineage of vole genus *Microtus* (Rodentia: Arvicolinae) inferred from mitochondrial cytochrome b sequence. Biological Journal of the Linnaean Society 99:595–613.

Bannikova, A. A., V. S. Lebedev, and F. N. Golenishchev. 2009. Taxonomic position of Afghan vole (subgenus *Blanfordimys*) by the sequence of the mitochondrial *cytb* gene. Russian Journal of Genetics 45:91–97.

Brandley, M. C., A. Schmitz, and T. W. Reeder. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Systematic Biology 54:373–390.

Bužan E. V., B. Krystufek, B. Hänfling, and W. F. Hutchinson. 2008. Mitochondrial phylogeny of Arvicolinae using comprehensive

taxonomic sampling yields new insights. Biological Journal of the Linnean Society 94:825–835.

Chen, W. C., H. B. Hao, Z. Y. Sun, Y. Liu, S. Y. Liu, and B. S. Yue. 2012. Phylogenetic position of the genus *Proedromys* (Arvicolinae, Rodentia): evidence from nuclear and mitochondrial DNA. Biochemical Systematics and Ecology 42:59–68.

Conroy, C. J., and J. A. Cook. 1999. MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents. Journal of Mammalian Evolution 6:221–245.

Conroy, C. J., and J. A. Cook. 2000. Molecular systematic of a Holarctic rodent (*Microtus*: Muridae). Journal of Mammalogy 81:344–359.

Cummings, M. P., M. C. Neel, and K. L. Shaw. 2008. A genealogical approach to quantifying lineage divergence. Evolution 62:2411–2422.

Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics 10:315–319.

Feng, Z. J., G. Q. Cai, and C. L. Zheng. 1986. The mammals of Xizang. Science Press, Beijing (in Chinese).

Fujisawa, T., and T. G. Barraclough. 2013. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: a revised method and evaluation on simulated data sets. Systematic Biology 62:707–724.

Galewski, T., M. Tilak, S. Sanchez, P. Chevret, E. Paradis, and E. J. P. Douzery. 2006. The evolutionary radiation of Arvicolinae rodents (voles and lemmings): relative contribution of nuclear and mitochondrial DNA phylogenies. BMC Evolutionary Biology 6:80.

Hillis, D. M., D. D. Pollock, J. A. McGuire, and D. J. Zwickl. 2003. Is sparse taxon sampling a problem for phylogenetic inference? Systematic Biology 52:124–126.

Hinton, M. A. C. 1923. On the voles collected by Mr. G. Forrest in Yunnan, with remarks upon the genera *Eothenomys* and *Neodon* and upon their allies. Annals and Magazine of Natural History Series 9 6:146–163.

Hinton, M. A. C. 1926. Monograph of the voles and lemmings (Microtinae) living and extinct. Printed by Order of the Trustees of the British Museum, London, United Kingdom.

Hooper, E. T. 1958. The male phallus in mice of the genus Peromyscus. Miscellaneous Publications of the Museum of Zoology, University of Michigan 623:1–18

Irwin, D. M., T. D. Kocher, and A. C. Wilson. 1991. Evolution of the cytochrome-b gene of mammals. Journal of Molecular Evolution 32:128–144.

Jaarola, M., et al. 2004. Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 33:647–663.

Jaarola, M., and J. B. Searle. 2002. Phylogeography of field voles (*Microtus agrestis*) in Eurasia inferred from mitochondrial DNA sequences. Molecular Ecology 12:2613–2621.

Kekkonen, M., and P. D. Hebert. 2014. DNA barcode-based delineation of putative species: efficient start for taxonomic workflows. Molecular Ecology Resources 14:706–715.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. Journal of Molecular Evolution 16:111–120.

Kocher, T. D., et al. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of National Academy of Sciences of the United States of America 86:6196–6200.

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

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

Lidicker, W. Z. 1968. A. phylogeny of New Guinea rodent genera based on phallic morphology. Journal of Mammalogy 49:609–643.

Liu, S. Y., Z. Y. Sun, Y. Liu, Z. X. Fan, P. Guo, and R. W. Murphy. 2012. A new vole from Xizang, China and the molecular phylogeny of the genus *Neodon* (Cricetidae: Arvicolinae). Zootaxa 3235:1–22.

Liu, S. Y., Z. Y. Sun, Z. Y. Zeng, and E. M. Zhao. 2007. A new species (*Proedomys*: Aricolinae: Murida) from the Liangshan Mountains of Sichuan Province, China. Journal of Mammalogy 5:1170–1178

Liu, S. Y., M. Zhang, and Z. Y. Sun. 2010. Biodiversity of Gongbu Natural Reserve of Xizang. Southwest Normal University Press, Chongqing (in Chinese).

Luo, A. R., et al. 2010. Performance of criteria for selecting evolutionary models in phylogenetics: a comprehensive study based on simulated datasets. BMC Evolutionary Biology 10:242.

Luo, J., et al. 2004. Molecular phylogeny and biogeography of Oriental voles: genus *Eothenomys* (Muridae, Mammalia). Molecular Phylogenetics and Evolution 33:349–362.

Martine, Y., G. Gerlach, C. Schlotterer, and A. Meyer. 2000. Molecular phylogeny of European muroid rodents based on complete cytochrome b sequences. Molecular Phylogenetics and Evolution 1:37–47.

Martínková, N., and J. Moravec. 2012. Multilocus phylogeny of arvicoline voles (Arvicolini, Rodentia) shows small tree terrace size. Folia Zoologica 61:254–267.

Meier, R., G. Zhang, and F. Ali. 2008. The use of a mean instead of smallest interspecific distances exaggerates the size of the 'barcoding gap' and leads to misidentification. Systematic Biology 57:809–813.

Michonneau, F. 2015. Cryptic and not-so-cryptic species in the complex "*Holothuria* (*Thymiosycia*) *impatiens*" (Forsskål, 1775) (Echinodermata: Holothuroidea). bioRxiv beta the preprint server for biology. doi:http://dx.doi.org/10.1101/014225.

Miller, G. S. 1896. The genera and subgenera of voles and lemmings. North American Fauna 12:1–84.

Monaghan, M. T., et al. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. Systematic Biology 58:298–311.

Musser, G. G., and M. D. Carleton. 1993. Subfamily Arvicolinae. Pp. 501–535 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). 2nd ed. Smithsonian Institution Press, Washington, D.C.

Musser, G. G., and M. D. Carleton. 2005. Family Cricetidae. Pp. 995–1039 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). 3rd ed. The Johns Hopkins Press, Baltimore, Maryland.

Pääbo, S., and A. C. Wilson. 1988 Polymerase chain reaction reveals cloning artefacts. Nature 334:387–388.

Petrova, T. V., E. S, Zakharov, R. Samiya, and N. I. Abramson. 2015. Phylogeography of the narrow-headed vole *Lasiopodomys* (*Stenocranius*) *gregalis* (Cricetidae, Rodentia) inferred from mitochondrial cytochrome b sequences: an echo of Pleistocene prosperity. Journal of Zoological Systematics and Evolutionary Research 53:97–108.

Pons, J., et al. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology 4:595–609.

Robovsky, J., V. Ricankova, and J. Zrzavy. 2008. Phylogeny of Arvicolinae (Mammalia, Cricetidae): utility of morphological and molecular data sets in a recently radiating clade. Zoologica Scripta 37:571–590.

Ronquist, F. R., and J. P. Huelsenbeck. 2003. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 19:1572–1574.

Sikes, R. S., W. L. Gannon, and The Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.

Smith, A. T., and Y. Xie. 2009. A guide to the mammals of China. Hunan Education Publishing House, Changsha (in Chinese).

State Council Decree. 1992. Wildlife protective enforcement regulation of The People's Republic of China. The Ministry of Forestry of PR China (in Chinese).

Swofford, D. L. 2001. PAUP*: phylogenetic analysis using parsimony (* and other methods). Version 4.0b8. Sinauer Associates, Sunderland, Massachusetts.

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

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876–4882.

Weisrock, D. W., et al. 2010. Delimiting species without nuclear monophyly in Madagascar's mouse lemurs. PLoS ONE 3:e9883.

Yang, A. F., and L. X. Fan. 1988. Phallic morphology of 13 species of the family Muridae from China with comments on its taxonomic significance. Acta Theriologica Sinica 4:275–285 (in Chinese).

Yang, A. F., S. Y. Liu, and L. X. Fan. 1992. Eight species of rodents glans penis morphological study of *Meriones* and *Microtus*. Acta Theriologica Sinica 1:31–42 (in Chinese).

Zagorodnyuk, I. V. 1990. Karyotypic variability and systematics of the gray voles (Rodentia, Arvicolini). Communication 1. Species composition and chromosomal numbers. Vestnik Zoologii 2:26–37 (in Russian).

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