

NEW INFORMATION FOR SYSTEMATICS, TAXONOMY, AND PHYLOGEOGRAPHY OF THE RODENT GENUS *APODEMUS (SYLVAEMUS)* IN UKRAINE

STEVEN R. HOOFER,* SERGEY GASCHAK, YELENA DUNINA-BARKOVSKAYA, JULIA MAKLUK, HEATHER N. MEEKS,
JEFFREY K. WICKLIFFE, AND ROBERT J. BAKER

Department of Biological Sciences and the Museum of Texas Tech University, Texas Tech University,
Lubbock, TX 79409, USA (SRH, YD-B, HNM, JKW, RJB)

International Radioecology Laboratory, Slavutych, 07100 Ukraine (SG, JM)

Present address of JKW: Department of Preventive Medicine and Community Health,
University of Texas Medical Branch, Galveston, TX 77555-1110, USA

In 2004, we collected 211 specimens of European field mice (genus *Apodemus*; subgenus *Sylvaemus*) from 16 localities in northern, western, central, and southern Ukraine, including the Crimean Peninsula. We used cytochrome-*b* mitochondrial DNA sequence data to investigate species diversity, distributional patterns, and taxonomy of *Apodemus* in Ukraine. Sequence data proved useful for species-level discrimination, because the 11 species examined were defined by low levels of intraspecific variation (mean range, 0.00–3.35%) and by high levels of interspecific variation (mean range, 5.37–18.9%). We identified the 211 specimens to 1 of 4 species: *A. flavicollis* ($n = 121$), *A. sylvaticus* ($n = 34$), *A. uralensis* ($n = 16$), and *A. witherbyi* ($n = 40$). Although all 4 species are known from Ukraine, this study provides new information about the ranges and population identity for these species in southeastern Europe. For example, our analyses substantiate the marked genetic structure reported for *A. sylvaticus* and document the presence of northern and southern continental lineages of this species in Ukraine that are hypothesized to have been isolated from each other in glacial refugia approximately 1.5 million years ago (>5% genetic divergence). Banks of the Dneiper River in northern Ukraine represent an area of secondary contact for the 2 lineages. Additionally, although application of *A. witherbyi* as a species-level name is debatable, examination of our genetic and morphological data supports its validity and priority over junior synonyms *A. hermonensis* and *A. iconicus*.

Key words: *Apodemus*, cytochrome *b*, distribution, Rodentia, *Sylvaemus*, systematics, taxonomy, Ukraine

Systematics and phylogeography of Palearctic field mice (genus *Apodemus*) have received much attention during the last 3 decades, yet fundamental questions about the morphological, genetic, and distributional boundaries of species in the group remain unanswered. This is particularly true in the western Palearctic, where different authors at different times have recognized from 2 to 15 *Apodemus* species in the subgenus *Sylvaemus* (Corbet 1978; Filippucci et al. 1989, 1996; Mezhzherin 1997; Michaux et al. 2002; Musser and Carleton 1993, 2005; Musser et al. 1996; Orlov et al. 1996; Vorontsov et al. 1989, 1992; Zagorodnyuk et al. 1997).

Taxonomic problems in the subgenus *Sylvaemus* are rooted in morphological crypsis and misidentification of specimens,

resulting in confusion regarding species identifications and debates about actual species diversity and phylogenetic relationships in the taxonomic literature. For example, different populations or individual specimens of the long-tailed field mouse (*A. sylvaticus*) have been described or recognized as >50 different species by various authorities (reviewed in Musser and Carleton 2005). Although comparative genetic techniques have provided a new and sharper view into population identity and geographic structuring, they have yet to resolve debates about *Apodemus* taxonomy and systematics. New examinations of *Apodemus (Sylvaemus)* diversity and systematics also should include adequate nomenclatural solutions with careful consideration of existing type specimens (Krystufek 2002).

Of the new genetic characters and techniques used for taxon identification, mitochondrial cytochrome-*b* sequences represent the most important single source of comparative material for *Apodemus* in terms of phyletic utility, cost, and availability.

* Correspondent: srhooper@hotmail.com

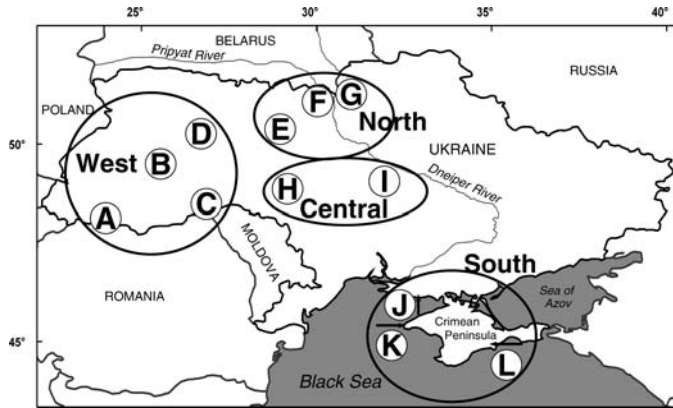


FIG. 1.—Trapping localities (A–L) in northern, western, central, and southern Ukraine, including the Crimean Peninsula. A = Rakhiv; B = Terebovlya; C = Klementy; D = Ezyaslav; E = Korostychev; F = Chornobyl; G = Nedanchichy; H = Pechera; I = Smela; J = Hladkovka, Krasnoye, and Ozernoe; K = Olenevka; L = Kluchevoe, Nasypnoe, Stary Krym, and Solnechnaya Dolina. Localities correspond with Table 2 and are further detailed in Appendix I.

More than 700 partial or complete cytochrome-*b* sequences representing 18 *Apodemus* species are available in GenBank. Cytochrome-*b* sequences can be extremely useful in species-level discrimination, providing that inter- and intraspecific variability are nonoverlapping and analyses include a phylogenetic context (Baker and Bradley 2006; Bradley and Baker 2001). For *Apodemus*, studies so far indicate that differences between species are 3–4 times greater than differences among individuals within species (e.g., Michaux et al. 2002).

Distributional boundaries of *Apodemus* species remain unresolved in many regions of Europe. Among these areas are Ukraine (including the Crimean Peninsula), other parts of Mediterranean Europe, and the Caucasus, all historically identified as regions of high diversity and endemism (Cagnin et al. 1998; Cheylan 1990; Krystufek and Griffiths 2002; Mayr 1963; Vereshchagin 1959), and where some widespread species are characterized by overlapping convergence of body sizes and pectoral spot expressions (e.g., *A. flavicollis* and *A. sylvaticus*—Filippucci et al. 1989). In the past, as many as 8 species of the subgenus *Sylvaemus* were thought to occur in Ukraine: *A. arianus*, *A. flavicollis*, *A. fulvipectus*, *A. mosquensis*, *A. ponticus*, *A. sylvaticus*, *A. uralensis*, and *A. vohlynensis* (Michaux et al. 2002; Musser and Carleton 1993; Musser et al. 1996; Orlov et al. 1996; Vorontsov et al. 1992). Furthermore, additional species (e.g., *A. ciscaucasicus*, *A. epimelas*, *A. hermonensis*, *A. hyrcanicus*, *A. iconicus*, *A. mystacinus*, and *A. wardi*), thought to occur in the Balkans, Caucasus, and mountainous regions of Asia Minor (Krystufek and Mozetic Francky 2005; Macholán et al. 2001; Michaux et al. 2005a), might reach their distributional limits in the Carpathian Mountains of western Ukraine or the mountains of Crimean Peninsula. In contrast, the most recent synthesis of morphological and genetic information for *Apodemus* (Musser and Carleton 2005) recognizes 4 *Apodemus* (*Sylvaemus*) species in Ukraine: *A. flavicollis* (including *arianus*), *A. sylvaticus* (including *vohlynensis*), *A. uralensis* (including *ciscaucasicus*

and *mosquensis*), and *A. witherbyi* (including *fulvipectus*, *hermonensis*, and *iconicus*).

We present genetic data for 211 new specimens of *Apodemus* (*Sylvaemus*) from Ukraine. Our goals are to identify the species of *Apodemus* under study through comparative analysis of published cytochrome-*b* sequence data, to test previous hypotheses about *Apodemus* diversity in Ukraine, to examine distributional patterns of *Apodemus* in Ukraine, and to discuss our results in the context of past and current *Apodemus* systematics and taxonomy.

MATERIALS AND METHODS

Specimens examined.—From June through August 2004, we collected 280 specimens of *Apodemus* from 16 localities in northern (2), western (4), central (2), and southern (8) Ukraine (Fig. 1; Appendix I). Of these, 69 were *A. agrarius*, a member of the subgenus *Agrarius* that is easily distinguished from members of *Sylvaemus* by its conspicuous black dorsal stripe. We also collected specimens representing *Cricetus*, *Crocidura*, *Microtus*, *Mustela*, and *Mus* at these same localities. We collected liver samples from all specimens and stabilized the DNA in 5 ml of lysis buffer (Longmire et al. 1997). Voucher specimens are deposited in the Museum of Texas Tech University (TTU), Lubbock, Texas, and the International Radioecology Laboratory, Slavutysh, Ukraine. Tissue samples from all specimens are deposited at TTU. All procedures were approved by the animal care and use committee of Texas Tech University and follow the guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998).

We retrieved 224 cytochrome-*b* sequences from GenBank (Appendix I). Of these, 171 represent partial sequences for specimens of *A. flavicollis* (98) and *A. sylvaticus* (73) collected in the region surrounding Chornobyl, Ukraine, as part of longitudinal studies examining exposure to radioactivity (Dunina-Barkovskaya 2004; Makova et al. 2000). We included these data when examining frequencies and distributions of haplotypes in Ukraine. Of the remaining 53 sequences from GenBank, 20 represent specimens of *A. flavicollis* (10) and *A. sylvaticus* (10) from other European countries, 25 represent other members of the subgenus *Sylvaemus* available in GenBank (3 *alpicola*, 4 *epimelas*, 2 *hermonensis*, 3 *iconicus*, 6 *mystacinus*, 2 *pallipes*, and 5 *uralensis*), and 8 represent specimens of the subgenera *Gurkha* (3 *gurkha*) and *Apodemus* (3 *agrarius* and 2 *speciosus*). We used the latter 8 sequences as outgroups in phylogenetic analyses (Chelomina 1998; Filippucci et al. 2002; Liu et al. 2004; Michaux et al. 2002; Serizawa et al. 2000). Sequences from GenBank originally were generated by Jansa et al. (1999), Krystufek and Mozetic Francky (2005), Makova et al. (2000), Martin et al. (2000), Michaux et al. (2002, 2003, 2004, 2005a), Serizawa et al. (2000), and Suzuki et al. (2003, 2004), and lists of specimens examined including voucher information are accessible in each of those publications.

We did not include the 8 GenBank sequences available for *A. ponticus* and *A. fulvipectus* from Georgia (AF249761–AF249768—Hille et al. 2002). Although these 8 sequences are the only published cytochrome-*b* data available for either

species, they have been shown to be unreliable (Hille et al. 2002; Reutter et al. 2003), perhaps representing data from nuclear copies (pseudogenes) of cytochrome-*b*, as postulated for some sequences of *A. sylvaticus* in Martin et al. (2000; see Liu et al. 2004 and Michaux et al. 2002). Furthermore, they lack the 1st 127 characters (of 392), corresponding to more than one-third of the parsimony-informative characters in this study.

Molecular methods.—We extracted genomic DNA from liver tissue with standard phenol methods (Longmire et al. 1997). We used standard polymerase chain reaction methods (e.g., Liu et al. 2004) and primers L14724 and H15915R (Irwin et al. 1991) to amplify the complete cytochrome-*b* gene. We purified double-stranded polymerase chain reaction amplicons by using the QIAquick PCR Purification Kit (Qiagen, Inc., Chatsworth, California) and sequenced both strands with Big-Dye version 3.1 chain terminators, followed by electrophoresis on a 3100-Avant Genetic Analyzer (Applied Biosystems, Inc., Foster City, California). We sequenced only the 1st 392 base pairs, with the appropriate external primer and primer MVZ 04 (Smith and Patton 1993), because the majority of comparative data available from GenBank is the 1st 392–800 base pairs. We assembled resulting, overlapping fragments in AssemblyLIGN 1.0.9 software (Oxford Molecular Group PLC 1998).

Phylogenetic analysis.—We performed multiple sequence alignment in Clustal X software (Thompson et al. 1997) and viewed alignments in MacClade software (version 4.0—Maddison and Maddison 2002) to ensure insertions–deletions or stop codons were absent in both our new sequences and those from GenBank. We coded nucleotides as unordered, discrete characters, and multiple states as polymorphisms. In PAUP* software (test version 4.0b10—Swofford 2002), we examined level of phylogenetic signal via the g_1 -statistic (Hillis and Huelsenbeck 1992) for 100,000 randomly drawn trees. In Collapse 1.2 software (Posada 2004), we calculated numbers and frequencies of cytochrome-*b* haplotypes among all Ukraine samples (new and from GenBank) thereby removing unnecessary redundancy in further analyses.

To identify the 211 unknown *Apodemus*, we 1st constructed a neighbor-joining phenogram in PAUP* software (test version 4.0b10—Swofford 2002) using substitution models (e.g., Kimura 2-parameter and Tamura–Nei) typical of other studies of *Apodemus* that examined cytochrome-*b* data (e.g., Michaux et al. 2003). We also computed Kimura 2-parameter distances for all pairwise comparisons among haplotypes to facilitate comparisons with previous studies. We then performed a series of more detailed phylogenetic analyses to examine node support in the neighbor-joining tree and the correspondence between phenetic (neighbor-joining) and phylogenetic methods. For this, we used Bayesian analysis implemented in

MrBayes 2.01 software (Huelsenbeck and Ronquist 2001) and maximum-likelihood and parsimony analyses implemented in PAUP* software (test version 4.0b10—Swofford 2002). The general time reversible (GTR) model with allowance for gamma distribution of rate variation (Γ) and for proportion of invariant sites (I) best fit the cytochrome-*b* data based on the Akaike information criterion test implemented in Modeltest 3.06 software (Posada and Crandall 1998).

Because of computational time constraints, we were able to analyze the entire data set only with Bayesian methods. We ran 2×10^6 generations with 1 cold and 3 incrementally heated Markov chains, random starting trees for each chain, and trees sampled (saved) every 100 generations. We treated model parameters as unknown variables (with uniform priors) to be estimated in each Bayesian analysis (Leaché and Reeder 2002). We ran sets of 2 independent analyses for each specified outgroup (*A. agrarius*, *A. gurkha*, and *A. speciosus*) with burn-in values (initial set of unstable generations to be ignored) based on empirical evaluation of likelihoods converging on stable values. We calculated a 50% majority-rule consensus tree from the sample of stabilized trees in PAUP* software (test version 4.0b10—Swofford 2002) and obtained branch lengths via the “sumt” option in MrBayes software.

Based on initial analyses, we truncated the data set to include just 2–5 sequences per putative species, selecting haplotypes to best represent the range of diversity within species (i.e., most divergent haplotypes). Our purpose was to reduce the number of taxa and the amount of homoplasy so as to reduce computation time and permit bootstrap analysis (250 iterations) under both parsimony and maximum-likelihood optimality criteria (Felsenstein 1985). For maximum-likelihood analysis, we used the GTR + Γ + I model and parameters, neighbor-joining starting trees, and tree-bisection-reconnection branch swapping. For parsimony analysis, we treated all characters and substitution types with equal probability, and used starting trees by simple addition and tree-bisection-reconnection branch swapping. We regarded supported nodes as those with bootstrap values $\geq 70\%$ and posterior probabilities ≥ 0.95 .

RESULTS

Sequence alignment of the 1st 392 base pairs of the cytochrome-*b* gene for 211 specimens generated in this study plus the 224 retrieved from GenBank was unequivocal and without stop codons. Some character states were missing in 1 or more taxa, corresponding to 37 partial GenBank sequences representing 1 specimen of *A. alpicola*, 2 *A. epimelas*, 6 *A. flavicollis*, 2 *A. hermonensis*, 3 *A. iconicus*, 6 *A. mystacinus*, 1 *A. pallipes*, 1 *A. uralensis*, and 15 *A. sylvaticus*. The majority of these partial sequences lacked 5–10 characters at the 5' end.

FIG. 2.—Neighbor-joining tree based on 1st 392 base pairs of the cytochrome-*b* gene and Kimura 2-parameter distances, identifying all 211 specimens of *Apodemus* (subgenus *Sylvaemus*) collected across Ukraine in 2004 to 1 of 4 species: *A. flavicollis* (haplotypes *Afl* 1–30); *A. sylvaticus* (*As* 1–10); *A. uralensis* (*Au* 1–4); and *A. witherbyi* (*Aw* 1–4). Haplotypes for *A. flavicollis* and *A. sylvaticus* include the additional specimens from previous studies near Chornobyl. Frequencies and distributions of haplotypes are detailed in Table 2. Asterisks (*) indicate Bayesian posterior probabilities ≥ 0.95 based on analysis of entire data set. 1a, 1b, 2a, and 2b correspond to *A. sylvaticus* lineages identified in Michaux et al. (2003, 2005b); both northern (2b) and southern (1a) continental lineages of *A. sylvaticus* are present in Ukraine.

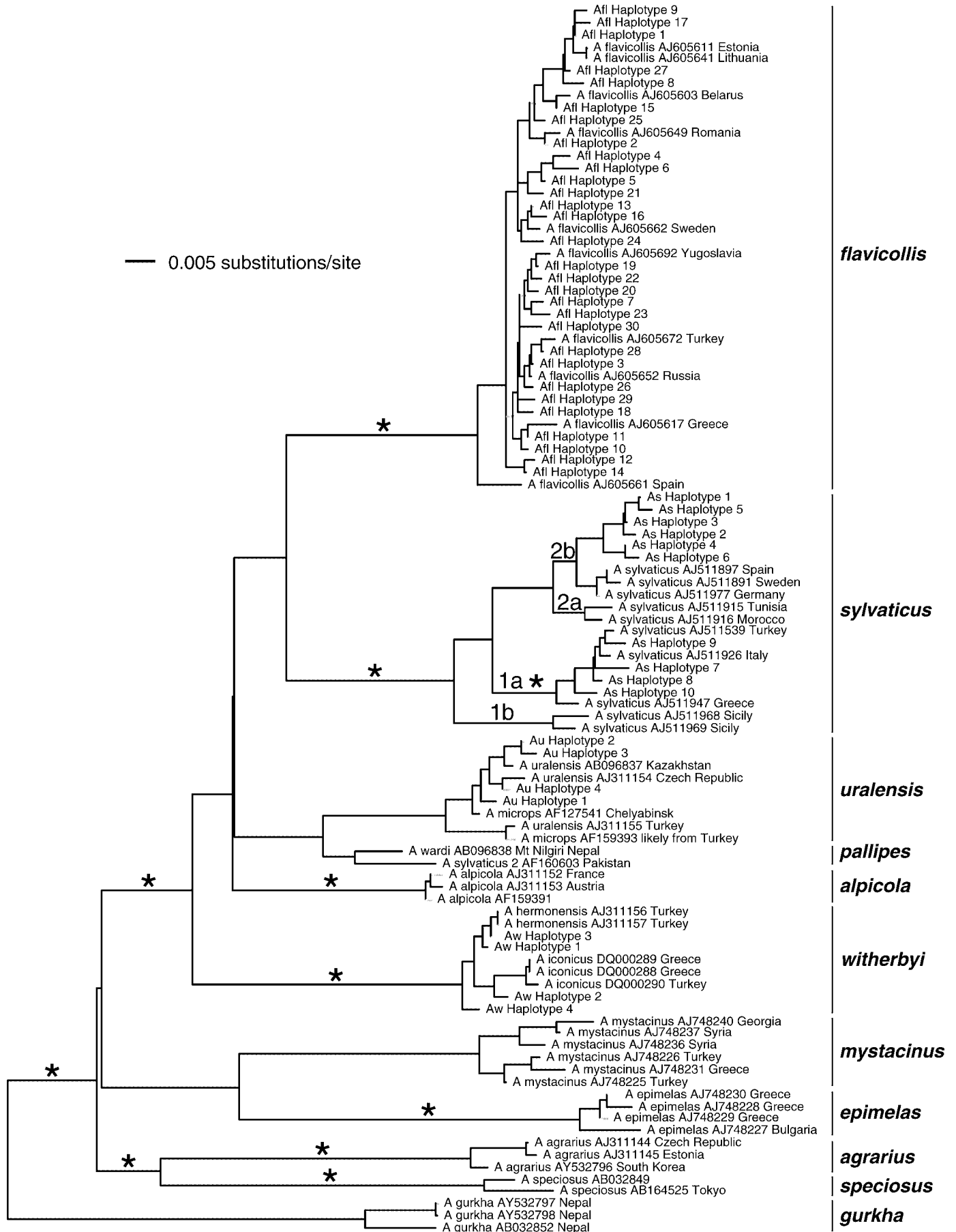


TABLE 1.—Mean percent cytochrome-*b* distance (Kimura 2-parameter) within (diagonal; in bold type) and among (below diagonal) *Apodemus* species.

Taxon	1	2	3	4	5	6	7	8	9	10
<i>agrarius</i>	0.59									
<i>speciosus</i>	13.47	1.81								
<i>epimelas</i>	17.45	18.79	0.89							
<i>mystacinus</i>	17.88	17.28	12.88	2.04						
<i>alpicola</i>	13.43	16.09	16.40	14.49	0.00					
<i>pallipes</i>	13.15	12.67	18.07	15.31	8.14	2.00				
<i>uralensis</i>	14.69	15.17	16.95	15.10	9.12	5.43	1.48			
<i>flavicollis</i>	16.68	16.28	18.06	16.61	8.08	7.88	10.01	1.01		
<i>sylvaticus</i>	17.01	17.12	18.67	17.39	12.94	11.27	14.12	10.91	3.35	
<i>witherbyi</i>	15.29	16.55	16.08	17.02	9.83	11.76	11.36	12.75	12.66	1.00

Of the 392 characters, 218 were constant and 124 were parsimony informative, with nucleotide variation distributed across codon positions as expected for protein-coding genes (22 substitutions at 1st positions, 3 at 2nd positions, and 99 at 3rd positions). Level of phylogenetic signal, based on the g_1 statistic (-0.3055), was significant ($P < 0.01$).

Neighbor-joining analysis revealed 11 clusters that we interpret as species-level groups (Fig. 2): *A. agrarius*, *A. alpicola*, *A. epimelas*, *A. flavicollis*, *A. gurkha*, *A. mystacinus*, *A. pallipes*, *A. speciosus*, *A. sylvaticus*, *A. uralensis*, and *A. witherbyi*. Kimura 2-parameter distances within each of the 11 clusters averaged 1.64%, ranging from 0.00% (within *alpicola*) to 3.35% (within *sylvaticus*). Distances among the 11 clusters averaged 13.93%, ranging from 5.43% (*pallipes* versus *uralensis*) to 18.79% (*epimelas* versus *speciosus*; Table 1). On average, distances between closely related *Sylvaemus* species (*flavicollis*, *pallipes*, *sylvaticus*, *uralensis*, and *witherbyi*) were 5–6 times greater than distances within species. However, based on pairwise comparisons of all haplotypes, the lower limit was 1.3 times greater distance between versus within species (6.35% maximum distance within *A. sylvaticus* versus 8.37% minimum distance between *A. sylvaticus* and *A. flavicollis*).

Bayesian analysis of the entire data set yielded similar topology as neighbor-joining, although there were differences involving weakly supported, alternative branching orders of mostly terminal taxa within species. Bayesian likelihoods reached stationarity before 100,000 generations (i.e., burn-in = 1,000), thinning the data to 19,000 sample points. Topology and posterior probabilities for nodes and model parameters agreed for all sets of runs regardless of choice of outgroup (2 runs each).

Eight of the 11 species-level clusters were supported by parsimony bootstrapping, maximum-likelihood bootstrapping, and Bayesian posterior probabilities based on analysis of the truncated data set (Fig. 3); all 8 also were supported by Bayesian posterior probabilities based on analysis of the entire data set (Fig. 2). The 3 exceptions were *A. mystacinus*, *A. uralensis*, and *A. pallipes*; the former 2 received support from parsimony bootstrap analysis only, and the latter received no support from all 3 analyses (Fig. 3). Three sister-species relationships were supported in 1 or more analyses: *A. pallipes*–*A. uralensis*, *A. epimelas*–*A. mystacinus*, and *A. agrarius*–*A. speciosus*. Support for higher-level relation-

ships by 1 or more analyses was limited to a clade containing all sampled members of subgenus *Sylvaemus*, and a sister relationship between the *A. epimelas*–*A. mystacinus* clade and a clade containing the remainder of *Sylvaemus* (Fig. 3). Overall, supported topologies (i.e., $\geq 70\%$ bootstrap value, ≥ 0.95 Bayesian posterior probability) obtained from all optimality criteria agreed, and there were no supported conflicts.

Based on cytochrome-*b* analyses, we identify all 211 specimens of *Apodemus* (subgenus *Sylvaemus*) collected across Ukraine in 2004 to 1 of 4 species (Table 2; Figs. 2 and 3): *A. flavicollis* ($n = 121$) from all regions except southern mainland; *A. sylvaticus* ($n = 34$) from all regions except Crimean Peninsula; *A. uralensis* ($n = 16$) from southern region only, including both mainland and Crimean Peninsula; and *A. witherbyi* ($n = 40$) from southern region only, including both mainland and Crimean Peninsula. Number of haplotypes within species from Ukraine, including the 179 sequences of *A. flavicollis* (99) and *A. sylvaticus* (80) from previous Chernobyl studies, ranged from 4 within *A. uralensis* and *A. witherbyi* to 30 within *A. flavicollis* (Table 2). Frequency and distribution of haplotypes within species varied considerably, with some haplotypes unique to specific regions of Ukraine (e.g., Crimean Peninsula) and others found across all regions in which the species was collected (Table 2).

DISCUSSION

Identification and phylogeographic patterns of Apodemus in Ukraine.—All species examined are defined by low levels of intraspecific variation (mean range, 0.00–3.35%) and high levels of interspecific variation (mean range, 5.37–18.9%), corresponding with DNA sequence results in previous studies of *Apodemus* (Chelomina 1998; Chelomina et al. 1998; Libois et al. 2001; Liu et al. 2004; Michaux et al. 2002, 2003, 2004, 2005a; Serizawa et al. 2000). We recognize 4 *Apodemus* (*Sylvaemus*) species in the material examined from Ukraine, *A. flavicollis*, *A. sylvaticus*, *A. uralensis*, and *A. witherbyi*. Although application of these species epithets is debatable (see “Taxonomy” below), it is consistent with detailed morphological and distributional descriptions in the literature and the most recent synthesis of *Apodemus* taxonomy (Musser and Carleton 2005). All 4 species previously were known from Ukraine, and our data provide new information about the distributional limits of these species in southeastern Europe.

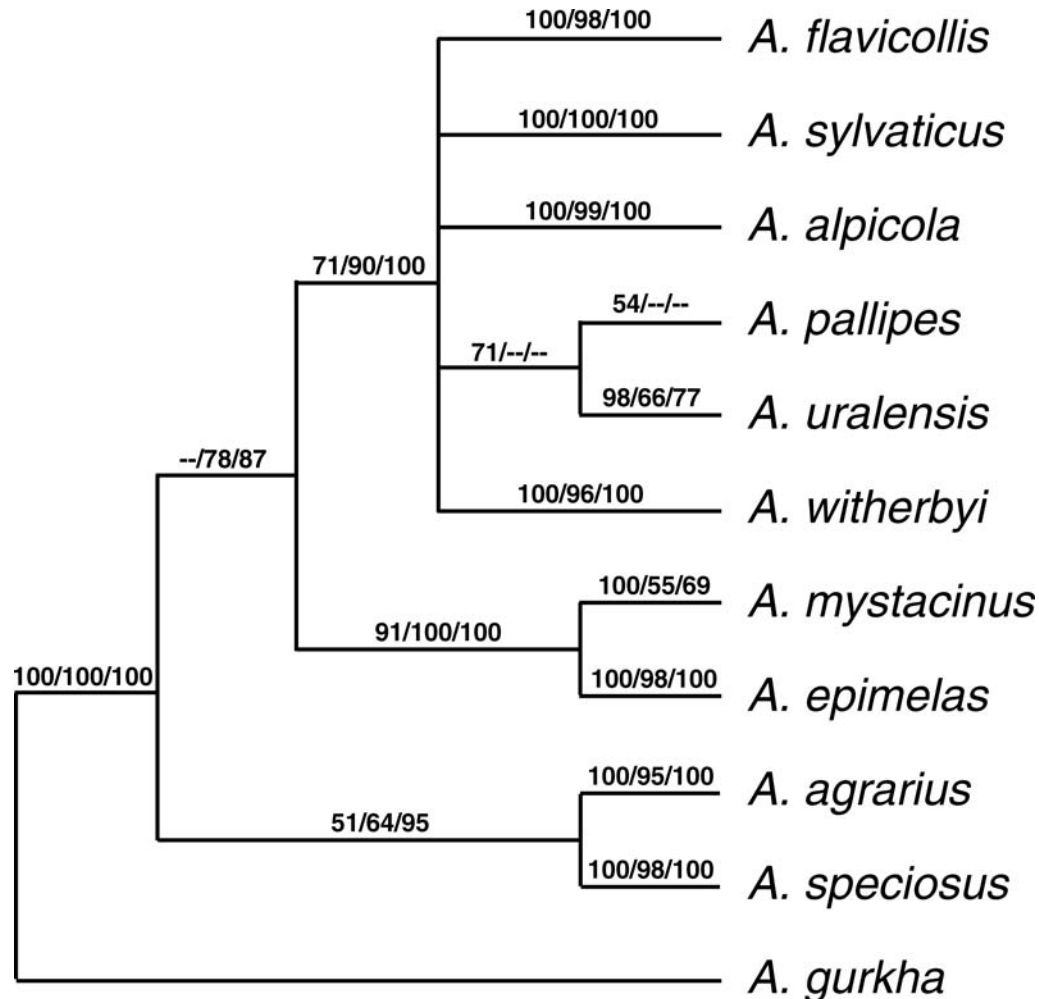


FIG. 3.—Cladogram showing nodal support for each species-level clade based on 3 types of analyses of a truncated taxon set (see text). Parsimony bootstrap values are listed 1st, followed by maximum-likelihood bootstrap values and Bayesian posterior probabilities. Bootstrap values are percentages of 250 iterations. Only nodes supported by 1 or more of the analyses (bootstrap value $\geq 70\%$ or posterior probabilities ≥ 0.95) are shown. Dashes indicate unsupported nodes.

Apodemus flavicollis is by far the most abundant and widespread *Apodemus* species that we collected in Ukraine, as it is perhaps in all of Europe (Mitchell-Jones et al. 1999). We collected specimens of *A. flavicollis* in all habitat types and sampled regions of Ukraine except steppe areas of the southern mainland (Table 2). We detected relatively high levels of haplotype diversity in *A. flavicollis* relative to the other 3 species, which agrees with Michaux et al. (2004, 2005b), who also found high cytochrome-*b* haplotype diversity in *A. flavicollis* from throughout its vast European distribution.

In contrast, the other 3 small-bodied species of *Apodemus* (*sylvaticus*, *uralensis*, and *witherbyi*) have more restricted distributions and habitat preferences. Although all 3 species co-occur in the southern mainland Ukraine, distribution is limited in other areas (Table 2). For example, our samples of *A. witherbyi* are predominately from the Crimean Peninsula, in both arid grasslands (Olenevka) in the west and forested areas of the Crimean Mountains in the southeast. We collected only 5 individuals north of the peninsula, all in dense Russian olive thickets of the southern mainland. Thus, *A. witherbyi*, a species

thought to be widespread in the Middle East, apparently reaches its northwestern limits in the Crimean Peninsula and southern Ukraine.

Our analyses indicate that *A. sylvaticus* is the only small-bodied species occurring in forested areas along sampled riparian floodplains of central, western, and northern regions of Ukraine, and this species is most prevalent in the northern region (Table 2). In the southern mainland, *A. sylvaticus* is also fairly common in dense Russian olive thickets (Krasnoye and Ozernoe). However, we did not collect this species in the Crimean Peninsula. Its absence in Crimea also has been documented in other studies (Mezhzherin and Lashkova 1992; Mezhzherin et al. 2002; Orlov et al. 1996). Furthermore, *A. sylvaticus* is virtually unknown from areas southeast of Ukraine. Recent studies with extensive sampling across Asia Minor and Iran found just 2 specimens of *A. sylvaticus* along the Black Sea coast in western Anatolia (Filippucci et al. 1996; Macholán et al. 2001). Thus, the species appears to be rare in this region, where it likely reaches its southeastern distributional limits (Mezhzherin 1997).

TABLE 2.—Continued.

Taxon and haplotypes	South																
	West			North		Central		Mainland		Crimean Peninsula							
	Rakhiv	Klementy	Terebovlya	Ezyaslav	Korostyshev	Chornobyl	Nedanchichy	Pechera	Smela	Hladkovka	Krasnoye	Ozerno	Olenevka	Kluchevoe	Nasypnoe	Stary Krym	Sol. Dolina
Afl 20	1																
Afl 21	1																
Afl 22		1															
Afl 23		1															
Afl 24							4										
Afl 25							1										
Afl 26									1								
Afl 27																	
Afl 28							5							1			
Afl 29															3		
Afl 30															1		4
																	1

Apodemus uralensis was the least common of the 4 species, with 14 of the 16 specimens collected during 1 night in a mature hardwood forest in the southern mainland (Hladkovka; Table 2). We collected the other 2 specimens in the southeastern part of the Crimean Peninsula; 1 in a mature hardwood forest in the Crimean Mountains (Stary Krym), the other nearby in a dense shelterbelt (Kluchevoe). *A. uralensis* is known from each of these general areas, but it is thought to be more common in eastern Ukraine, east of the Dneiper River (Mezhzherin 1997; Mezhzherin et al. 2002; Naglov 1995). Otherwise, the species is widely distributed in Central Europe, European Russia, northwest China, and in humid, mountainous areas of Asia Minor (reviewed in Musser and Carleton 2005). Although *A. uralensis* has been reported from the Carpathian Biosphere Reserve (Kyselyuk 1993), we did not collect this species in the Carpathian Mountains in western Ukraine.

These observations indicate that Ukraine is an important region for *Apodemus* diversity, which is not surprising considering that Ukraine is characterized as a steppe–deciduous forest ecotone (Walter 2002) and the Crimean Peninsula is thought to be a place of high endemism (Cheylan 1990; Krystufek and Griffiths 2002). Further study, aided by genetic identification methods and including additional sampling from eastern Ukraine, will be vital for understanding the distributional patterns of these species and the biogeographical significance of southern Ukraine.

Matrilineal distribution in A. sylvaticus.—Our collections provide missing data critical for interpreting the historical biogeography of *A. sylvaticus*. As discussed by Michaux et al. (2003, 2005b), continental European populations of *A. sylvaticus* are characterized by the highest level of intraspecific cytochrome-*b* sequence variation of any *Apodemus* species and are divided into 2 main lineages that likely separated 1.5–1.6 million years ago (5.4% maximum Kimura 2-parameter distance): a northern lineage (called subclade 2b) that is “widespread from southern Spain in the south to Sweden in the north and the Ukraine in the east”; and a southern lineage (called subclade 1a) that is “limited to Italy, the Balkans and western part of Turkey” (Fig. 2; Michaux et al. 2003:692). They concluded that these 2 lineages represent populations of a single species and explained the ancient divergence between them by isolation of 2 populations in 2 refugia (Iberian peninsula and Italo-Balkan region) during a Quaternary glacial period. Michaux et al. (2003, 2005b) hypothesize that present distribution of the northern clade is the result of postglacial recolonization and rapid expansion from southwestern Europe throughout most of the western Palearctic, whereas that of the southern clade is the result of a much slower, postglacial recolonization, perhaps hindered by the Alps and Carpathians, from the Italo-Balkan region east to Turkey.

Michaux et al. (2003, 2005b) reportedly examined 4 specimens from northern Ukraine, all collected in or near Chornobyl, approximately 5 km west of the confluence of the Pripyat and Dneiper rivers. Haplotypes for the specimens all had northern clade origins and represented the easternmost extent of that clade in Europe. Michaux et al. (2003, 2005b) concluded that the geographical distributions of the northern and southern lin-

eages are entirely nonoverlapping, at least based on the limited availability of samples for eastern European populations. Our cytochrome-*b* analysis not only substantiates the marked genetic structure within *A. sylvaticus*, but it also reveals presence of both the northern (Table 2; haplotypes *As* 1–6) and southern (haplotypes *As* 7–12) lineages in Ukraine. As in Michaux et al. (2003, 2005b), our results indicate that all 73 individuals (*As* 1–6) collected near Chornobyl are part of the northern lineage. In contrast, individuals from southern, central, and western regions of Ukraine, and 1 locality in the northern region (Korostyshev), have southern clade origins (Table 2; Fig. 2). Furthermore, we collected individuals belonging to both northern and southern lineages from the same locality near Nedanchichy, a small village in the northern region 3 km east of the Dneiper River and 40 km northeast of Chornobyl. Thus, the banks of the Dneiper River in northern Ukraine represent an area of secondary contact for the 2 lineages that were isolated from each other approximately 1.5 million years ago.

Discovery of sympatry between the northern and southern forms of *A. sylvaticus* raises the question of whether these are intraspecific genetic groups or different species. Michaux et al. (2003) concluded the former, apparently based on comparisons of other genetic divergence values reported in the literature (Bradley and Baker 2001; Michaux et al. 2002). However, the southern clade may correspond to *A. vohlynensis* Migulin, 1938, a species recognized decades ago based upon morphological features (type locality: Zhytomir District, Ukraine). Orlov et al. (1996) further distinguished *A. vohlynensis* from *A. sylvaticus* based on karyological features for populations in Central Europe (Balkans), Bulgaria, north-central and western Ukraine, and western Russia (~400 km northeast of Chornobyl). Resolving this issue will require further sampling and study of nuclear DNA variation across multiple loci and should involve careful examination of type specimens.

Taxonomy.—Our analysis involves the clade we recognize as *A. witherbyi* (Thomas, 1902), within which are 4 haplotypes (40 individuals) from southern Ukraine ($n = 5$) and Crimean Peninsula ($n = 35$) and 5 sequences from GenBank (Fig. 2). Two of the specimens from GenBank were identified as *A. hermonensis* by Michaux et al. (2002—AJ311156 and AJ311157 from Turkey) and 3 were identified as *A. iconicus* by Krystufek and Mozetic Francky (2005—DQ000288 and DQ000289 from Greece: Rhodes Island; and DQ000290 from Turkey: Bozcaada Island).

For most of the 20th century, *A. witherbyi* has been placed in synonymy with other *Sylvaemus* species or regarded as a subspecies of *A. sylvaticus* (Ellerman 1941) or *A. arianus* (Musser and Carleton 1993). Musser and Carleton (2005) now regard it as the oldest available name for the small-bodied *Sylvaemus* species of southeastern Europe and the Middle East characterized by the diagnostic morphological features listed in Filippucci et al. (1996—as *A. hermonensis*) and Krystufek (2002—as *A. iconicus*). These characters include a distinctly stephanodont upper 1st molar (and upper 2nd molar, but with less frequency), configuration of the palatopterygoid region, lengths of the auditory bulla and maxillary tooththrow, and pattern of pectoral spot.

Zagorodnyuk (1996), Zagorodnyuk et al. (1997), and Mezhzherin (1997) revised *A. witherbyi* (but using the name *A. arianus*) to include the names and ranges of *fulvipectus*, *falzfeini*, and *hermonensis* (but see Vorontsov et al. 1992). Krystufek (2002) provided additional clarity through comparisons among 365 skulls of 3 species from Turkey (*A. flavicollis*, *A. hermonensis*, and *A. uralensis*) and the type specimens of 4 *Apodemus* species (*arianus*, *iconicus*, *sylvaticus*, and *witherbyi*). He demonstrated that *A. arianus* (Blanford, 1881) is not part of *A. uralensis* or *A. hermonensis*, but might represent a junior synonym of *A. flavicollis*; *A. iconicus* Heptner, 1948, is the valid name for the species *A. hermonensis*; and the holotype of *A. witherbyi* (Thomas, 1902) from Iran is possibly identical with *A. hermonensis* (and thus *A. iconicus*), differing slightly in shorter bullae and shorter maxillary tooththrow; if deemed identical, *A. witherbyi* is the valid name for the species, having priority over *A. hermonensis* and *A. iconicus*. Krystufek and Mozetic Francky (2005) further demonstrated the genetic equivalence of *A. hermonensis* and *A. iconicus* through cytochrome-*b* analysis of 3 specimens identified morphologically as *A. iconicus* from Greek and Turkish islands (all 3 are included in this study) and 5 sequences representing *A. hermonensis* and 4 other *Apodemus* (*Sylvaemus*) species.

Our results affirm the morphological and genetic equivalence *A. hermonensis*, *A. iconicus*, and the 40 specimens of *A. witherbyi* we collected in Ukraine. Kimura 2-parameter distances between them ranged from 0.00% to 1.83% (Table 1; Fig. 3). All skin ($n = 13$) and skull ($n = 27$) preparations available in TTU match the diagnostic morphological characters listed for *A. hermonensis/A. iconicus/A. witherbyi* (Filippucci et al. 1996; Krystufek 2002). As reported by Filippucci et al. (1996) for a different series of specimens, frequency of stephanodont upper 1st molar in our specimens was less than 100% (absent in 2 [7.4%] of 27 of the specimens examined). Furthermore, length measurements of the auditory bulla and maxillary tooththrow in our samples averaged 4.3 mm (3.9–4.6 mm) and 3.6 mm (3.4–3.9 mm), respectively, spanning the range of variation evident in type specimens and topotypes of *hermonensis*, *iconicus*, and *witherbyi* (Filippucci et al. 1996; Krystufek 2002; Krystufek and Mozetic Francky 2005; Musser and Carleton 2005). Based on these morphological comparisons and those highlighted in Musser and Carleton (2005:1280) and Krystufek and Mozetic Francky (2005:71), coupled with results from phylogenetic analysis of cytochrome-*b* sequences (this study), we follow Musser and Carleton's (2005) taxonomic arrangement regarding *A. hermonensis* and *A. iconicus* (including *A. fulvipectus*) as junior synonyms of *A. witherbyi*.

We found 4 haplotypes within the 16 specimens that we recognize as *A. uralensis*. Fourteen of these specimens are from southern Ukraine and 2 are from the Crimean Peninsula. In addition, 5 sequences from GenBank are recognized as *A. uralensis* (Fig. 2); 3 of these were deposited in GenBank as *A. uralensis* (AJ311154 from the Czech Republic and AJ311155 from Turkey deposited by Michaux et al. [2002], and AB096837 from Kazakhstan deposited by Suzuki et al. [2003]), and 2 were deposited as *A. microps* (AF127541 from Chelyabinsk, Russia,

deposited by Makova et al. [2000], and AF159393 likely from Turkey deposited by Martin et al. [2000]).

Apodemus uralensis has been regarded as a subspecies of *A. sylvaticus* (Corbet 1978; Ellerman and Morrison-Scott 1951; Pavlinov and Rossolimo 1987) but now is recognized as the valid name for the small-bodied *Sylvaemus* species formerly called *A. microps* and *A. mosquensis* (Musser and Carleton 2005). Placement of *A. microps* as a junior synonym of *A. uralensis* has been supported by multiple morphological and molecular studies (Bellinvia et al. 1999; Filippucci et al. 1996; Macholán et al. 2001; Mezhzherin 1997; Mezhzherin and Mikhailenko 1991; Mezhzherin and Zykov 1991; Mezhzherin et al. 1992; Reutter et al. 2003). Orlov et al. (1996), in reference to specimens from southern Ukraine and Crimean Peninsula, regarded *A. mosquensis* as distinct from *A. uralensis*, but classified it as a species within the superspecies *A. uralensis*.

Our analyses support the synonymy of *A. microps* within *A. uralensis*. Although we have not examined the type specimens, our cytochrome-*b* analysis includes a specimen from Chelyabinsk, Russia (AF127541, labeled as *A. microps*), which is a topotype of *A. uralensis* from the southern Ural Mountains of Russia, and specimens from Czech Republic (near Slovakia, the type locality of the European *A. microps*), Ukraine (Crimea and mainland east of Dneiper River), Kazakhstan, and Turkey. Collectively, these sequences differ by <1.00% Kimura 2-parameter distance, which represents typical intraspecific variation (Baker and Bradley 2006; Bradley and Baker 2001). All available skin ($n = 5$) and skull ($n = 15$) preparations that we recognize as *A. uralensis* have the diagnostic morphological features of *A. uralensis* (Filippucci et al. 1996). In our collection, we find no evidence that 2 small-bodied *Apodemus* (*Sylvaemus*) species with this combination of morphological characteristics co-occur in southern Ukraine. Furthermore, *A. mosquensis* also shares the diagnostic morphological properties of *A. microps* and *A. uralensis* (Filippucci et al. 1996). Thus, although we have not examined the type specimen of *A. mosquensis*, together the above observations provide circumstantial evidence for regarding *A. mosquensis* from southern Ukraine and Crimean Peninsula (Orlov et al. 1996) as *A. uralensis*.

One of the 2 specimens representing *A. pallipes* in our analysis was identified as *A. wardi* by Suzuki et al. (2003—Mount Nilgiri, Pakistan, AB096838), and the other was identified as *A. sylvaticus* by Jansa et al. (1999—Pakistan, AF160603; called *sylvaticus* 2). Musser and Carleton (2005) singled out both of these specimens and the associated cytochrome-*b* sequences. They used morphological, distributional, and molecular evidence to justify the synonymy of *A. wardi* with *A. pallipes*, and they concluded that the *sylvaticus* specimen examined by Jansa et al. (1999) was probably misidentified. Our analyses show close phylogenetic and phenetic similarity between these 2 specimens, their distinction from other *Apodemus* (*Sylvaemus*) species, and phyletic proximity to *A. uralensis* (Darviche et al. 1979; Mezhzherin 1997). However, the branch linking these 2 specimens as *A. pallipes* (Fig. 3) is not supported, so we provisionally follow

Musser and Carleton's (2005) recognition of *A. pallipes* pending additional study with extensive sampling.

Finally, our results provide new information important to an unresolved nomenclatural issue regarding *A. flavicollis*. As discussed in Corbet (1978:134), Krystufek (2002:48), and Musser and Carleton (2005:1267), some authors have used the earlier name *tauricus* Pallas, 1811 (type locality, mountains of Crimea), instead of *flavicollis* Melchior, 1834, even though Corbet (1978) noted that the identity of *tauricus*, because of its inadequate original description, is indeterminable. One of the strongest points for using the name *tauricus* is Zagorodnyuk's claim (in Krystufek 2002) that just 1 species of *Apodemus* (i.e., *A. flavicollis*) occurs on the Crimean Peninsula. Results from our morphological and genetic analyses document the presence of 3 *Apodemus* species on the peninsula, *A. flavicollis*, *A. uralensis*, and *A. witherbyi*, and Mezhzherin (1997) also lists these 3 species as occurring in Crimean Peninsula, although by using the names *A. tauricus* (= *A. flavicollis*), *A. uralensis*, and *A. arianus* (= *A. witherbyi*). The type description of *tauricus* is too vague to apply to *flavicollis*, *witherbyi*, or *uralensis*, and apparently there is no holotype to which *tauricus* is attached (G. G. Musser, in litt.). Without such a voucher specimen, the identity of *tauricus* cannot be demonstrated. Thus, *tauricus* Pallas, 1811, is nomen dubium (International Commission on Zoological Nomenclature 1999:111) and should not replace *A. flavicollis*.

ACKNOWLEDGMENTS

This study is an outgrowth of the efforts, led by R. Chesser, to understand the biodiversity and biological significance of the fauna living in the environment created by the Chernobyl power plant disaster. B. Rodgers and C. Phillips have contributed leadership to the development of the biological database required to design parts of this work. We thank M. Bondarkov, director of the International Radioecology Laboratory, for facilitating studies in Ukraine. We thank N. Aleksashin, A. Brown, E. Buntova, A. Grabanyuk, K. Holmes, V. Kholosha, E. Krysanov, M. Lomakin, V. Maksimenko, V. Pokinchereda, G. Rudenskaya, G. Shursha, V. Stupina, and R. Van Den Bussche for logistical support, assistance in collecting and preparing specimens, or both. We also thank H. Garner and K. MacDonald of TTU for help with tissue and specimen loans, G. Musser and B. Krystufek for helpful discussions and correspondences about *Apodemus*, J. Prichard for assistance in tabulating data, H. Mantilla-Meluk for assistance in generating maps, and R. K. Baker and 2 anonymous reviewers for comments and suggestions that improved the manuscript. Financial support for fieldwork was provided by Department of Energy (RWO 44) to R. Chesser, C. Phillips, and R. Baker of Texas Tech University. Laboratory work was funded through the Biological Database Initiative at Texas Tech University.

LITERATURE CITED

- ANIMAL CARE AND USE COMMITTEE. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* 79:1416–1431.
- BAKER, R. J., AND R. D. BRADLEY. 2006. Speciation in mammals and the genetic species concept. *Journal of Mammalogy* 87:643–662.
- BELLIN VIA, E., P. MUNCLINGER, AND J. FLEGR. 1999. Application of the RAPD technique for a study of the phylogenetic relationships

- among eight species of the genus *Apodemus*. *Folia Zoologica* 48:241–248.
- BRADLEY, R. D., AND R. J. BAKER. 2001. A test of the genetic species concept: cytochrome-*b* sequences and mammals. *Journal of Mammalogy* 82:960–973.
- CAGNIN, M., ET AL. 1998. Comparative study of Spanish and Italian terrestrial small mammal coenoses from different biotopes in Mediterranean peninsular tip regions. *Journal of Biogeography* 25:1105–1113.
- CHELOMINA, G. N. 1998. Molecular phylogeny of forest and field mice of the genus *Apodemus* (Muridae, Rodentia) based on the data on restriction analysis of total nuclear DNA. *Genetika* 34:1286–1292.
- CHELOMINA, G. N., H. SUZUKI, K. TSUCHIYA, K. MORIWAKI, E. A. LYAPUNOVA, AND N. N. VORONTSOV. 1998. Sequencing of the mtDNA cytochrome *b* gene and reconstruction of the maternal relationships of wood and field mice of the genus *Apodemus* (Muridae, Rodentia). *Genetika* 34:650–661.
- CHEYLAN, G. 1990. Endémisme et spéciation chez les mammifères Méditerranéens. *Vie Milieu* 40:137–143.
- CORBET, G. B. 1978. The mammals of the Palaearctic region: a taxonomic review. British Museum (Natural History), London, United Kingdom.
- DARVICHE, D., F. BENMEHDI, J. BRITTON-DAVIDIAN, AND L. THALER. 1979. Données préliminaires sur la systématique biochimique des genres *Mus* et *Apodemus* en Iran. *Mammalia* 43:427–430.
- DUNINA-BARKOVSKAYA, Y. V. 2004. Population genetics of rodents living in the Chornobyl environment based on mitochondrial and nuclear gene sequences. M.S. thesis, Texas Tech University, Lubbock.
- ELLERMAN, J. R. 1941. The families and genera of living rodents. Family Muridae. British Museum (Natural History), London, United Kingdom. Vol. 2.
- ELLERMAN, J. R., AND T. C. S. MORRISON-SCOTT. 1951. Checklist of Palaearctic and Indian mammals 1758 to 1946. Trustees of the British Museum (Natural History), London, United Kingdom.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- FILIPPUCCI, M. G., M. MACHOLÁN, AND J. R. MICHAUX. 2002. Genetic variation and evolution in the genus *Apodemus* (Muridae: Rodentia). *Biological Journal of the Linnean Society* 75:395–419.
- FILIPPUCCI, M. G., S. SIMSON, AND E. NEVO. 1989. Evolutionary biology of the genus *Apodemus* Kaup, 1829 in Israel. Allozymic and biometric analyses with description of a new species: *Apodemus hermonensis* (Rodentia, Muridae). *Bollettino di Zoologia* 56:361–376.
- FILIPPUCCI, M. G., G. STORCH, AND M. MACHOLÁN. 1996. Taxonomy of the genus *Sylvaemus* in western Anatolia—morphological and electrophoretic evidence. *Senckenbergiana Biologica* 75:1–14.
- HILLE, A., D. TARKHNISHVILI, H. MEINIG, AND R. HUTTERER. 2002. Morphometric, biochemical and molecular traits in Caucasian wood mice (*Apodemus/Sylvaemus*), with remarks on species divergence. *Acta Theriologica* 47:389–416.
- HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analysis. *Journal of Heredity* 83:189–195.
- HUELSENBECK, J. P., AND F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17:754–755.
- INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE. 1999. International code of zoological nomenclature. 4th ed. International Trust for Zoological Nomenclature, London, United Kingdom.
- 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.
- JANSA, S. A., S. M. GOODMAN, AND P. K. TUCKER. 1999. Molecular phylogeny and biogeography of the native rodents of Madagascar (Muridae: Nesomyinae): a test of the single-origin hypothesis. *Cladistics* 15:253–270.
- KRYSTUFEK, B. 2002. Identity of four *Apodemus* (*Sylvaemus*) types from the eastern Mediterranean and the Middle East. *Mammalia* 66:43–51.
- KRYSTUFEK, B., AND H. I. GRIFFITHS. 2002. Species richness and rarity in European rodents. *Ecography* 25:120–128.
- KRYSTUFEK, B., AND B. MOZETIC FRANCKY. 2005. Mt. Hermon field mouse *Apodemus iconicus* is a member of the European mammal fauna. *Folia Zoologica* 54:69–74.
- KYSELYUK, A. I. 1993. *Sylvaemus microps* (Rodentia, Muridae) in the East Carpathians. *Vestnik Zoologii* 1994:41–47.
- LEACHÉ, A. D., AND T. W. REEDER. 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Systematic Biology* 51:44–68.
- LIBOIS, R. M., J. R. MICHAUX, M. B. RAMALHINHO, C. MAUROIS, AND M. SARÀ. 2001. On the origin of the northern African wood mouse (*Apodemus sylvaticus*) populations: a comparative study of mtDNA restriction patterns. *Canadian Journal of Zoology* 79:1503–1511.
- LIU, X., F. WEI, M. LI, X. JIANG, Z. FENG, AND J. HU. 2004. Molecular phylogeny and taxonomy of wood mice (genus *Apodemus* Kaup, 1829) based on complete mtDNA cytochrome *b* sequences, with emphasis on Chinese species. *Molecular Phylogenetics and Evolution* 33:1–15.
- LONGMIRE, J. L., M. MALTBIE, AND R. J. BAKER. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. *Occasional Papers, The Museum, Texas Tech University* 163:1–3.
- MACHOLÁN, M., M. G. FILIPPUCCI, P. BENDA, D. FRYNTA, AND J. SÁDLOVÁ. 2001. Allozyme variation and systematics of the genus *Apodemus* (Rodentia: Muridae) in Asia Minor and Iran. *Journal of Mammalogy* 82:799–813.
- MADDISON, D. R., AND W. P. MADDISON. 2002. MacClade 4 (version 4.05). Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- MAKOVA, K. D., A. NEKRUTENKO, AND R. J. BAKER. 2000. Evolution of microsatellite alleles in four species of mice (genus *Apodemus*). *Molecular Ecology* 16:166–172.
- MARTIN, Y., G. GERLACH, C. SCHLÖTTERER, AND A. MEYER. 2000. Molecular phylogeny of European muroid rodents based on complete cytochrome *b* sequences. *Molecular Phylogenetics and Evolution* 16:37–47.
- MAYR, E. 1963. *Animal species and evolution*. Harvard University Press, Cambridge, Massachusetts.
- MEZH ZHERIN, S. V. 1997. Revision of mouse genus *Apodemus* (Rodentia, Muridae) of northern Eurasia. *Vestnik Zoologii* 31(4):29–41.
- MEZH ZHERIN, S. V., G. G. BOYESKOROV, AND N. N. VORONTSOV. 1992. Genetic relations between European and Transcaucasian mice of the genus *Apodemus* Kaup. *Genetika* 28(11):111–121.
- MEZH ZHERIN, S. V., AND E. I. LASHKOVA. 1992. Two closely related mice species—*Sylvaemus sylvaticus* and *S. flavicollis* (Rodentia, Muridae) in an area of their overlapping occurrence. *Vestnik Zoologii* 26(3):33–41.
- MEZH ZHERIN, S. V., E. I. LASHKOVA, AND N. N. TOVPINETS. 2002. Geographic distribution, population densities and habitat preference of the wood mice genus *Sylvaemus* (Rodentia, Muridae) in the territory of Ukraine. *Vestnik Zoologii* 36(6):39–49.
- MEZH ZHERIN, S. V., AND A. G. MIKHAILENKO. 1991. On the species identity of *Apodemus sylvaticus tscherga* (Rodentia, Muridae) of Altay. *Vestnik Zoologii* 25(3):35–44.
- MEZH ZHERIN, S. V., AND A. E. ZYKOV. 1991. Genetical divergence and allozyme variability of mice of the genus *Apodemus* s. lato (Muridae, Rodentia). *Cytologia i Genetika* 25(4):51–58.

- MICHAUX, J. R., E. BELLINIA, AND P. LYMBERAKIS. 2005a. Taxonomy, evolutionary history and biogeography of the broad-toothed field mouse (*Apodemus mystacinus*) in the eastern Mediterranean area based on mitochondrial and nuclear genes. *Biological Journal of the Linnean Society* 85:53–63.
- MICHAUX, J. R., P. CHEVRET, M.-G. FILIPPUCI, AND M. MACHOLAN. 2002. Phylogeny of the genus *Apodemus* with a special emphasis on the subgenus *Sylvaemus* using the nuclear IRBP gene and two mitochondrial markers: cytochrome *b* and 12S rRNA. *Molecular Phylogenetics and Evolution* 23:123–136.
- MICHAUX, J. R., R. LIBOIS, AND M.-G. FILIPPUCI. 2005b. So close and so different: comparative phylogeography of two small mammal species, the yellow-necked fieldmouse (*Apodemus flavicollis*) and the woodmouse (*Apodemus sylvaticus*) in the western Palearctic region. *Heredity* 94:52–63.
- MICHAUX, J. R., R. LIBOIS, E. PARADIS, AND M.-G. FILIPPUCI. 2004. Phylogeographic history of the yellow-necked fieldmouse (*Apodemus flavicollis*) in Europe and in the Near and Middle East. *Molecular Phylogenetics and Evolution* 32:788–798.
- MICHAUX, J. R., E. MAGNANOU, E. PARADIS, C. NIEBERDING, AND R. LIBOIS. 2003. Mitochondrial phylogeography of the woodmouse (*Apodemus sylvaticus*) in the western Palearctic region. *Molecular Ecology* 12:685–697.
- MITCHELL-JONES, A. J., ET AL. 1999. *Atlas of European mammals*. Academic Press, London, United Kingdom.
- MUSSER, G. G., E. M. BROTHERS, M. D. CARLETON, AND R. HUTTERER. 1996. Taxonomy and distributional records of Oriental and European *Apodemus*, with a review of the *Apodemus*–*Sylvaemus* problem. *Bonner Zoologische Beiträge* 46:143–190.
- MUSSER, G. G., AND M. D. CARLETON. 1993. Family Muridae. Pp. 501–755 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. Superfamily Muroidea. Pp. 894–1531 in *Mammal species of the world: a taxonomic and geographic reference* (D. E. Wilson and D. A. Reeder, eds.). 3rd ed. Johns Hopkins University Press, Baltimore, Maryland, Vol. 2.
- NAGLOV, V. A. 1995. Distribution and population density of *Sylvaemus sylvaticus* (Rodentia, Muridae) in Kharkov Oblast'. *Vestnik Zoologii* 29(5–6):87–89.
- ORLOV, V. N., A. I. KOZLOVSKY, R. S. NADJAFOVA, AND N. SH. BULATOVA. 1996. Karyological diagnoses, distribution and evolutionary classification of wood mice of the subgenus *Sylvaemus* (*Apodemus*, Muridae, Rodentia) in Europe. *Zoologicheskii Zhurnal* 75(1):88–102.
- OXFORD MOLECULAR GROUP PLC. 1998. *AssemblyLIGN 1.0.9*. Oxford Molecular Group PLC, Oxford, United Kingdom.
- PAVLINOV, I. YA., AND O. L. ROSSOLIMO. 1987. *Systematics of the mammals of the USSR*. Moscow University Press, Moscow, Russia.
- POSADA, D. 2004. *Collapse 1.2*. Brigham Young University, Provo, Utah.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- REUTTER, B. A., E. PETIT, H. BRÜNNER, AND P. VOGEL. 2003. Cytochrome *b* haplotype divergences in West European *Apodemus*. *Mammalian Biology* 68:153–164.
- SERIZAWA, K., H. SUZUKI, AND K. TSUCHIYA. 2000. A phylogenetic view on species radiation in *Apodemus* inferred from variation of nuclear and mitochondrial genes. *Biochemical Genetics* 38: 27–40.
- SMITH, M. F., AND J. L. PATTON. 1993. The diversification of South American murid rodents: evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biological Journal of the Linnean Society* 50:149–177.
- SUZUKI, H., ET AL. 2003. Molecular phylogeny of wood mice (*Apodemus*, Muridae) in East Asia. *Biological Journal of the Linnean Society* 80:469–481.
- SUZUKI, H., S. P. YASUDA, M. SAKAIZUMI, S. WAKANA, M. MOTOKAWA, AND K. TSUCHIYA. 2004. Differential geographic patterns of mitochondrial DNA variation in two sympatric species of Japanese wood mice, *Apodemus speciosus* and *A. argenteus*. *Genes & Genetic Systems* 79:165–176.
- SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.
- THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAC, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The Clustal X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24:4876–4882.
- VERESHCHAGIN, N. K. 1959. *The mammals of the Caucasus, a history of the evolution of the fauna*. Smithsonian Institution Press, Washington, D.C.
- VORONTSOV, N. N., G. G. BOYESKOROV, S. V. MEZHHERIN, E. A. LYAPUNOVA, AND A. S. KANDAUROV. 1992. Systematics of the Caucasian wood mice of the subgenus *Sylvaemus* (Mammalia, Rodentia, *Apodemus*). *Zoologicheskii Zhurnal* 71(2):119–131.
- VORONTSOV, N. N., S. V. MEZHHERIN, G. G. BOYESKOROV, AND E. A. LYAPUNOVA. 1989. Genetic differentiation of sibling species of wood mice (*Apodemus*) in the Caucasia and their diagnostics. *Doklady Akademii Nauk SSSR* 309(5):1234–1238.
- WALTER, H. 2002. *Walter's vegetation of the earth: the ecological systems of the geo-biosphere*. 4th ed. Springer, New York.
- ZAGORODNYUK, I. V. 1996. What is *Apodemus sylvaticus arianus* (Blanford, 1881)? *Vestnik Zoologii* 30(3):20.
- ZAGORODNYUK, I. V., G. G. BOYESKOROV, AND A. YE. ZYKOV. 1997. Variation and taxonomic status of the steppe forms of genus *Sylvaemus* “*sylvaticus*” (*falzfeini*–*fulvipectus*–*hermonensis*–*arianus*). *Vestnik Zoologii* 31(5–6):37–56.

Submitted 8 July 2006. Accepted 1 November 2006.

Associate Editor was Mark S. Hafner.

APPENDIX I

Specimens examined.—List of *Apodemus* (*Sylvaemus*) specimens collected in Ukraine. Voucher specimens are housed in the Museum of Texas Tech University (TTU) in Lubbock, Texas, and the International Radioecology Laboratory in Slavutych, Ukraine. Tissue samples from all specimens are housed at TTU. Specimens examined are arranged by their general localities mapped in Fig. 1 (letters A–L), then alphabetically by species and chronologically by cytochrome-*b* haplotype identified in Table 2 and Fig. 2. Eight specimens from locality G (near Nedanchichy) and all but 1 of the specimens from locality F (near Chernobyl) were sequenced and deposited in GenBank before this study as part of an unpublished master's thesis (Dunina-Barkovskaya 2004); Genbank accession numbers are listed (AF or AY numbers). We deposited a single representative sequence in Genbank (EF016757–EF016789) for each new cytochrome-*b* haplotype identified in this study (*A. flavicollis* [Af], 9–30; *A. sylvaticus* [As], 8–10; *A. uralensis* [Au], 1–4; and *A. witherbyi* [Aw], 1–4).

A. Uzhgorod District: near Rhakiv (48°01.519'N, 24°10.63'E), 500–650 m.

A. flavicollis ($n = 17$): Af 2—TK96904. Af 11—TK96893. Af 12—TK96873. Af 13—TK96890, 96891, 96894, 96895. Af 15—TK96871, 96901, 96902. Af 16—TK96886. Af 17—

- TK96887. *Afl* 18—TK96888, 96889. *Afl* 19—TK96903. *Afl* 20—TK96892. *Afl* 21—TK96896.
- B. Ternopol District: near Terebovlya, between villages Slobodka and Budanov (49°11.083'N, 25°41.987'E), 245–335 m.
A. flavicollis ($n = 10$): *Afl* 7—TK96856, 96867. *Afl* 11—TK96850, 96852, 96858, 96862, 96870. *Afl* 12—TK96857. *Afl* 13—TK96864. *Afl* 14—TK96869.
- C. Chernivtsy District: near Klementsyy (48°34.170'N, 26°55.557'E), 200–250 m.
A. flavicollis ($n = 8$): *Afl* 3—TK96917, 96923, 96927, 96928. *Afl* 13—TK96926. *Afl* 19—TK96925. *Afl* 22—TK96924. *Afl* 23—TK96929.
A. sylvaticus ($n = 3$): *As* 7—TK96932, 96933. *As* 9—TK96930.
- D. Khmelnytsky District: near Ezyaslav (50°04.337'N, 26°38.396'E), 240 m.
A. flavicollis ($n = 4$): *Afl* 1—TK96821, 9696822. *Afl* 2—TK96817. *Afl* 10—TK96824.
A. sylvaticus ($n = 1$): *As* 8—TK96830.
- E. Zhytomir District: near Korostyshev, between villages Vysoky Kamen and Rudnya-Gorodetskaya (50°22.512'N, 29°12.104'E), 150 m.
A. flavicollis ($n = 3$): *Afl* 1—TK96786. *Afl* 9—TK96769–770.
A. sylvaticus ($n = 9$): *As* 7—TK96768, 96771–96773, 96775, 96779, 96785, 96788, 96789.
- F. Chornobyl District: near Chornobyl (51°44.491'N, 30°06.376'E; 51°38.400'N, 30°06.300'E; and 51°04.469'N, 30°16.117'E).
A. flavicollis ($n = 98$): *Afl* 1—AY158345, AY158348, AY158349, AY158352–158355, AY158357–158366, AY158368, AY158370, AY158375, AY158376, AY158378, AY158380, AY158383–158385, AY158387, AY158388, AY158390, AY158394, AY158395, AY158397, AY158399–158402, AY158404, AY158406–158409, AY158413, AY158416, AY158418–158420, AY158422, AY158425, AY158427, AY158429–158432, AY158435, AY158436, AY158439. *Afl* 2—AY158344, AY158346, AY158347, AY158350, AY158351, AY158356, AY158367, AY158372–158374, AY158377, AY158379, AY158382, AY158386, AY158389, AY158391, AY158396, AY158398, AY158403, AY158405, AY158410, AY158414, AY158415, AY158417, AY158421, AY158423, AY158424, AY158426, AY158440. *Afl* 3—AY158369, AY158371, AY158381, AY158411, AY158412. *Afl* 4—TK67783. *Afl* 5—AY158450. *Afl* 6—AY158428, AY158434, AY158437, AY158438, AY158441. *Afl* 7—AY158433.
- A. sylvaticus* ($n = 73$): *As* 1—AF548662, AF548663, AF548666–548669, AF548671, AF548673–548678, AF548680, AF548682, AF548683, AF548685–548689, AF548698, AF548700–548703, AF548706–548709, AF548711–548715, AF548720–548724, AF548726–548731, AF548735. *As* 2—AF548664, AF548665, AF548670, AF548672, AF548691, AF548692, AF548695, AF548696, AF548699. *As* 3—AF548681, AF548690, AF548697, AF548710, AF548716–548718, AF548736. *As* 4—AF548679. *As* 5—AF548684, AF548693, AF548694, AF548704, AF548705, AF548719, AF548725. *As* 6—AY158462.
- G. Chernigov District: near Nedanchichy (51°58.891'N, 30°85.231'E and 51°50.003'N, 30°85.986'E).
A. flavicollis ($n = 6$): *Afl* 8—AY158442. *Afl* 27—TK96988, 96992, 96996–96998.
- A. sylvaticus* ($n = 10$): *As* 1—AF445747, AF548737. *As* 7—TK81577, AF548732–548734, AF445799, AY158460. *As* 9—TK96984. *As* 10—TK96985.
- H. Vinnitsa District: near Nemirov, between villages Pechera and Sokolec (48°51.901'N, 28°44.657'E), 200–240 m.
A. flavicollis ($n = 18$): *Afl* 3—TK96954, 96956. *Afl* 13—TK96942, 96947. *Afl* 19—TK96940, 96941, 96948, 96955, 96958, 96959, 96961–96963. *Afl* 24—TK96952, 96953, 96960, 96964. *Afl* 25—TK96957.
A. sylvaticus ($n = 1$): *As* 10—TK96951.
- I. Cherkassy District: near Smela (32°02.610'N, 49°13.966'E and 32°06.959'N, 49°12.731'E), 140–200 m.
A. flavicollis ($n = 2$): *Afl* 3—TK96973. *Afl* 26—TK96980.
A. sylvaticus ($n = 1$): *As* 10—TK96972.
- J. Kherson District: near Hladkovka (46°26.220'N, 32°32.495'E and 46°25.084'N, 32°34.143'E), 20–26 m.
A. uralensis ($n = 14$): *Au* 1—TK133759, 133760, 133783, 133792, 133797. *Au* 2—TK133762, 133773, 133784, 133808. *Au* 3—TK133764, 133766. *Au* 4—TK133778–133780.
- Kherson District: near Krasnoye (46°06.775'N, 32°43.533'E), 3 m.
A. sylvaticus ($n = 15$): *As* 7—TK133755, 133761, 133763, 133765, 133767, 133774, 133781, 133782, 133791, 133795, 133796, 133799. *As* 9—TK133768–133770.
A. witherbyi ($n = 4$): *Aw* 1—TK133771, 133785. *Aw* 2—TK133793, 133794.
- Kherson District: near Ozernoe (46°08.434'N, 32°50.490'E and 46°07.485'N, 32°50.436'E), 2–5 m.
A. sylvaticus ($n = 2$): *As* 9—TK133819, 133820.
A. witherbyi ($n = 1$): *Aw* 1—TK133818.
- K. Crimea District: near Olenevka (45°24.363'N, 32°38.348'E and 45°23.537'N, 32°29.753'E), 20–60 m.
A. witherbyi ($n = 12$): *Aw* 1—TK133833–133842. *Aw* 2—TK133845. *Aw* 3—TK133843.
- L. Crimea District: near Kluchevoe (45°03.563'N, 35°11.633'E), 150 m.
A. flavicollis ($n = 9$): *Afl* 3—TK133874, 133893, 133917, 133919, 133920, 133925, 133934, 133935. *Afl* 28—TK133918.
A. uralensis ($n = 1$): *Au* 4—TK133924.
A. witherbyi ($n = 6$): *Aw* 1—TK133852, 133905, 133921–133923. *Aw* 2—TK133904.
- Crimea District: near Nasypnoe (45°03.150'N, 35°16.797'E), 80 m.
A. flavicollis ($n = 3$): *Afl* 3—TK133876, 133896, 133914.
A. witherbyi ($n = 13$): *Aw* 1—TK133853, 133854, 133889, 133895, 133909–133913, 133930–133932. *Aw* 4—TK133926.
- Crimea District: near Sary Krym (45°00.610'N, 35°02.580'E), 440 m.
A. flavicollis ($n = 24$): *Afl* 3—TK133847–133850, 133858–133863, 133867–133870, 133875, 133877, 133879, 133880, 133882, 133888. *Afl* 28—TK133851, 133881, 133916. *Afl* 29—TK133887.
A. uralensis ($n = 1$): *Au* 4—TK133894.
- Crimea District: near Solnechnaya Dolina (44°53.051'N, 35°05.957'E), 100–150 m.
A. flavicollis ($n = 18$): *Afl* 3—TK133941, 133945, 133948, 133949, 133951, 133952, 133955–133957, 133960–133963. *Afl* 28—TK133942, 133946, 133958, 133959. *Afl* 30—TK133954.
A. witherbyi ($n = 4$): *Aw* 1—TK133943, 133944, 133947, 133950.