

## Mt. Hermon field mouse *Apodemus iconicus* is a member of the European mammal fauna

Boris KRYŠTUFEK and Bojana MOZETIČ FRANCKY

Science and Research Centre Koper, University of Primorska, Garibaldijeva 18, SI-6000 Koper, Slovenia;  
e-mails: boris.krystufek@zrs-kp.si, bojana.mozetic@zrs-kp.si

Received 3 January 2005; Accepted 11 April 2005

**Abstract.** The cranial and dental morphology of field mouse from the island of Rhodes suggests that they belong to *Apodemus iconicus* (= *A. hermonensis*) rather than to *A. sylvaticus* or any other *Sylvaemus* species. Morphological identification was supported by partial mitochondrial cytochrome *b* nucleotide sequences (370 bp long DNA fragment). Samples from the islands of Rhodes and Bozcaada shared 98% identity in nucleotide and 100% identity in amino acid sequences with *Apodemus iconicus* from Anatolia.

**Key words:** *Apodemus iconicus*, mt cytochrome *b*, Island of Rhodes, island biogeography

### Introduction

The taxonomy of East European field mice of the genus *Apodemus* started to change considerably in the late 1980s following the application of electrophoretic analyses of allozymes (Filippucci et al. 1989, Mezhzhherin & Zagorodnyuk 1989). It appeared that, contrary to previous belief (e.g. Corbet 1978), *A. sylvaticus* (Linnaeus, 1758) is mainly absent from the Asiatic coasts of the eastern Mediterranean, being represented instead by a newly described *A. hermonensis* Filippucci, Simson & Nevo, 1989. It was shown subsequently (Kryštufek 2002) that *A. hermonensis* is predated by several names, of which *A. iconicus* Heptner, 1948 (*nomen novum* to replace *tauricus* Barrett-Hamilton, 1900, not Pallas, 1811) poses no doubt about its taxonomic identity. The oldest name for *A. hermonensis* is possibly *Mus sylvaticus witherbyi* Thomas, 1902, from Iran, which, however, differs from the Anatolian *A. iconicus* in smaller bullae (Kryštufek 2002). The above nomenclatorial solution has been mainly ignored (cf. Filippucci et al. 2002; for the opposing view see Čolak 2003).

What is common to all genetically based studies of eastern Mediterranean field mice is their ignorance of island populations (Filippucci et al. 1996, 2002, Michaux et al. 2002). Özkán & Kryštufek (1999) showed that wood mice from the island of Bozcaada, offshore north-western Asia Minor, match morphologically *A. hermonensis* (= *iconicus*) and also questioned the correctness of other reports of *A. sylvaticus* for the eastern Aegean island archipelago. The Atlas of European Mammals (Mitchell-Jones et al. 1999) excluded Bozcaada from the geographic scope of Europe. In the evident lack of taxonomic revision of field mice from the eastern Aegean islands of Greece, *A. sylvaticus* is thus mapped for the archipelago (Montgomerly 1999). *Apodemus sylvaticus* was reported for Rhodes by Pieper (1966).

In this communication, we provide first evidence on the occurrence of *A. iconicus* from the Island of Rhodes, which is an addition to the faunas of Greece, as well as of Europe (sensu Mitchell-Jones et al. 1999).

## Material and Methods

Specimens were collected between April 26 and May 2, 2004, using small kill traps baited with peanut butter. They were obtained on two localities on the western coast of the Island of Rhodes: Kamiros (36°20'N 27°55'E) and Kattavia (35°58'N 27°45'E). The habitat was Mediterranean shrubland between sea level and c. 50 m a.s.l. Associated species were *Crociodura suaveolens*, *Rattus rattus* and *Mus macedonicus* (first record for the Island). Rocky habitats and forests on rocky ground more inland were inhabited by *Apodemus mystacinus*.

Material was processed in a standard mammalogical way (carded skins, skulls) and tissue samples (muscle, liver) were preserved in ethanol. Skulls were preserved in ethanol and cleaned subsequently by *Dermestes* beetles. Cranial measurements were taken by a vernier calliper (to the nearest 0.1 mm) and drawings of dentition were done under a dissecting microscope using a drawing device. Morphological identification was based on cranial and dental characters given by Filippucci et al. (1996), also using comparative material from Anatolia (in the Slovenian Museum of Natural History) whose identity was assessed by electrophoretic analyses.

Total DNA from the ethanol preserved tissue samples (in the case of the Bozcaada specimen from the standard museum skin sample) was extracted on an ABI PRISM 6100 apparatus for nucleic acid isolation following the manufacturer's instructions. A cytochrome *b* fragment was amplified using primers L 14771 (5'–CAACATTCGTAAAACCCACC–3') and H 15149 (5'–AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA–) (Irwin et al. 1991). Amplification by polymerase chain reaction (PCR) was performed on a Perkin Elmer thermo cycler using AmpliTaq Gold polymerase (Applied Biosystems). The initial denaturation step at 95°C for 15 minutes was followed by 40 cycles of 1 min. at 94°C, 1 min. annealing at 55°C, and 1 min. elongation at 72°C. PCR products and negative controls were checked on a 1.5% agarose gel. Double stranded products were purified with an ULTRA PCR Clean up purification kit following the protocol of the manufacturer (Abgene). Sequencing was performed on an ABI PRISM 310 automated DNA sequencer (Applied Biosystems). Each DNA fragment was sequenced in both directions, using the same primers as for PCR amplifications.

**Table 1.** References of *Apodemus* tissues. <sup>1)</sup> reported as *A. hermonensis*; <sup>2)</sup> as *A. mystacinus*.

Species	Geographic origin	Sample symbols	GenBank accession numbers
<i>A. iconicus</i>	Greece, Is. of Rhodes	AI-1	DQ000288
<i>A. iconicus</i>	Greece, Is. of Rhodes	AI-2	DQ000289
<i>A. iconicus</i>	Turkey, Is. Bozcaada	AI-3	DQ000290
<i>A. iconicus</i> <sup>1)</sup>	Turkey, Doğubayazıt	AI-4	AJ311157
<i>A. uralensis</i>		AU	AB096837
<i>A. sylvaticus</i>		AS	AF159395
<i>A. flavicollis</i>	Greece, Mt. Olympus	AF	AJ631968
<i>A. epimelas</i> <sup>2)</sup>	Greece, Peloponnisos	AE	AJ311132

Computer based nucleotide and amino acid search of the GenBank databases were performed with the BLAST search program (Altschul et al. 1990). Previously published cytochrome *b* sequences for other *Apodemus* species (Table 1) were downloaded from the gene bank and aligned with the new sequences using Clustal W (Thompson et al. 1994). Using the Translate expasy program (Gasteiger et al. 2003) for the vertebrate mitochondrial gene code, nucleotide sequences were translated into amino acid ones. The latter were subsequently compared with cytochrome *b* protein sequences selected from the EMBL data gene bank.

Phylogenetic trees were inferred using the neighbour-joining (NJ) method (Saitou & Nei 1987) as implemented in TREECON (Van de Peer & De Wachter 1994). The significance of the various phylogenetic lineages was assessed by bootstrap analyses (1000 replications).

## Results

### Morphology

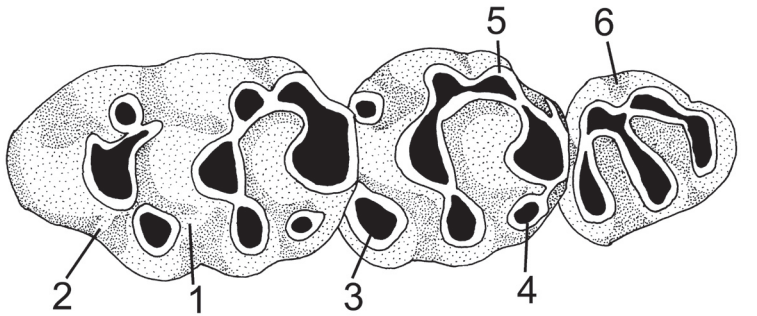
Field mice from the Island of Rhodes were small (Table 2), thus matching *A. sylvaticus* in this respect, their tail, however, exceeded head and body length (111–120%). The bivariate plot of maxillary tooth-row length against the length of bullae (not shown) placed our specimens within the *A. iconicus* polygon (cf. Filippucci et al. 1996, Kryštufek 2002). The upper molars show all the diagnostic properties of *A. iconicus* (cf. Filippucci et al. 1996). On the 1<sup>st</sup> upper molar these involved a stephanodont pattern (pt. 1 on Fig. 1) and presence of a t1bis between cusps t1 and t2 (pt. 2). Characteristic features on the 2<sup>nd</sup> upper molar were a large cusp t1 (pt. 3), and well-developed cusps t7 (pt. 4) and t9 (pt. 5). The 3<sup>rd</sup> upper molar was relatively large and with a deep labial fold (pt. 6).

**Table 2.** External and cranial measurements (in mm; body mass in grams) of *Apodemus iconicus* from the Island of Rhodes. \* teeth moderately worn; \*\* teeth much worn; M – male, F – female.

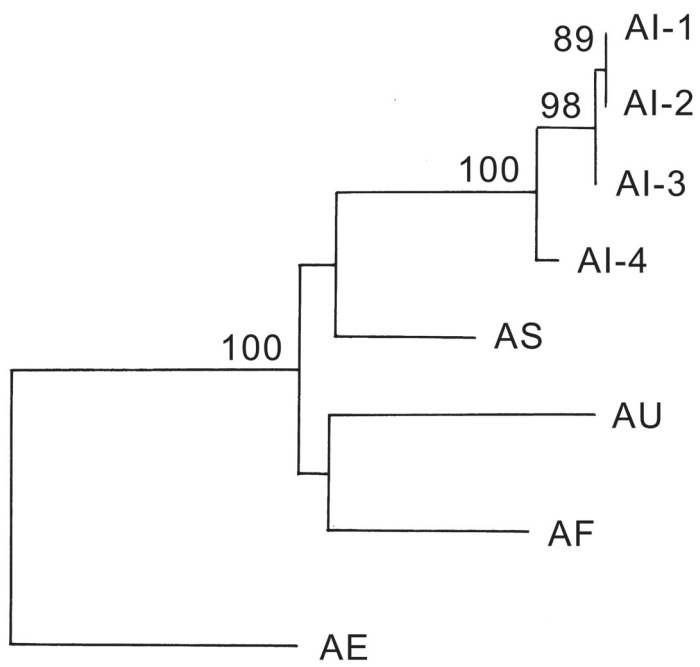
Sex	F**	M**	M*	M*
Body mass	26	>27	22.5	19.5
Head & body	103	102	91	92
Tail	118	113	101	110
Hind foot	22.6	22.7	22.8	23.4
Ear	15.3		14.6	16.0
Condylobasal length	23.4	24.0	21.6	22.3
Maxillary tooth-row	3.7	3.8	3.6	3.9
Length of bullae	4.6	4.6	4.4	4.6

### Cytochrome *b* sequences

The comparison of partial mitochondrial cytochrome *b* nucleotide sequences revealed that both samples of field mice collected on the Island of Rhodes were the same haplotype (identical along a 370 bp long DNA fragment encoding cytochrome *b*). Both sequences differed from the wood mouse from the Island of Bozcaada in one nucleotide only. The island



**Fig. 1.** Left upper molar row in *Apodemus iconicus* from the Island of Rhodes. See text for further explanation. Scale bar = 1 mm.



**Fig. 2.** Neighbour-joining tree derived from a 370 bp long DNA fragment encoding mt cytochrome *b* in four *Apodemus* (*Sylvaemus*) species and rooted on *A. epimelas*. It represents the bootstrap consensus following 1000 replicates; nodes with confidence values greater than 70% are indicated. For sample identities see Table 1.

samples shared 98% identity in nucleotide and 100% identity in amino acid sequences with *Apodemus iconicus* from Doğubayazıt (reported as *A. hermonensis*), thus entirely supporting the above morphological identification (Fig. 2).

**Discussion**

From the zoogeographical point, the presence of *A. iconicus* on the Island of Rhodes does not pose much surprise. The entire eastern Aegean island chain, from Lesbos in the north to Rhodes in the south, became separated from the western Anatolian mainland only quite

recently (Storch 2004). Thus, Anatolia, rather than southeast Europe was the most likely faunal source for the colonisation of the entire island archipelago. As a matter of fact, these islands show clear zoogeographical affinities to Asia Minor. Among mammals, these involve *Nannospalax nehringi* on Bozcaada and Gökçeada (Kryštufek & Vohralík 2001), *Sciurus anomalus* on Lesbos (Ondrias 1966) and on Gökçeada (Özkan 1999), *Meriones tristrami* on Kos (Pieper 1966), and *Erinaceus concolor rhodius* (not *E. roumanicus*; own results) and *Eptesicus bottae* (Helversen 1999) on Rhodes. In addition, *Apodemus mystacinus* (and not *A. epimelas* of the Balkan peninsula) is widespread on the islands offshore Asia Minor (Storch 2004). We predict that phylogeographic studies will reveal further examples in support of the colonisation of these islands from Anatolia also among species, which are common to southeast Europe and to Anatolia. In any case, the idea that the islands of the western Anatolian coast are biogeographically part of Turkey is not a new one (Laar & Daan 1967).

#### Acknowledgements

We thank Dr. Dušan Kordiš for help in reconstructing phylogenetic trees and to Mr. Mojmir Štangelj for designing the figures.

#### LITERATURE

- ALTSCHUL S. F., GISH W., MILLER W., MYERS E. W. & LIPMAN D. J. 1990: Basic local alignment search tool. *J. Mol. Biol.* 215: 403–410.
- ÇOLAK E. 2003: A study on *Apodemus iconicus* Heptner, 1948 (Mammalia: Rodentia) in Turkey. *Tr. J. Zool.* 27: 61–63.
- CORBET G. B. 1978: The mammals of the Palaearctic region: a taxonomic review. *British Museum (Natural History), London.*
- FILIPPUCCI M. G., SIMSON S. & NEVO E. 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). *Boll. Zool.* 56: 361–367.
- FILIPPUCCI M. G., STORCH G. & MACHOLÁN M. 1996: Taxonomy of the genus *Sylvaemus* in western Anatolia – morphological and electrophoretic evidence. *Senckenberg. biol.* 75: 1–14.
- FILIPPUCCI M. G., MACHOLÁN M. & MICHAUX J. R. 2002: Genetic variation and evolution in the genus *Apodemus* (Muridae: Rodentia). *Biol. J. Linn. Soc.* 75: 395–419.
- GASTEIGER E., GATTIKER A., HOOGLAND C., IVANYI I., APPEL R. D. & BAIROCH A. 2003: ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* 31: 3784–3788.
- HELVERSEN von O. 1999: *Eptesicus bottae* (Mammalia, Chiroptera) auf der Insel Rhodos. *Bonn. zool. Beitr.* 48: 113–121.
- IRWIN D. M., KOCHER T. D. & WILSON A. C. 1991: Evolution of cytochrome b gene of mammals. *J. Mol. Evol.* 32: 128–144.
- KRYŠTUFEK B. 2002: Identity of four *Apodemus* (*Sylvaemus*) types from the eastern Mediterranean and the Middle East. *Mammalia* 66: 43–51.
- KRYŠTUFEK B. & VOHRALÍK V. 2001: Mammals of Turkey and Cyprus. Introduction, checklist, Insectivora. *Znanstveno-raziskovalno središče Republike Slovenije Koper, Koper.*
- LAAR van V. & DAAN S. 1967: The etruscan shrew, *Suncus etruscus* (Savi, 1822), found on Samos, Greece. *Z. Säugetierkd.* 32: 174–175.
- MEZHHERIN S. V. & ZAGORODNYUK I. V. 1989: A new species of mice of the genus *Apodemus* (Rodentia, Muridae). *Vestnik Zool.* 1989(4): 55–59 (in Russian with English summary).

- MICHAUX J. R., CHEVRET P., FILIPPUCCI M.-G. & MACHOLÁN M. 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. *Mol. Phylo. Evol.* 23: 123–136.
- MITCHELL-JONES A. J., AMORI G., BOGDANOWICZ W., KRYŠTUFEK B., REIJNDERS P. J. H., SPITZENBERGER F., STUBBE M., THISSEN J. B. M., VOHRALÍK V. & ZIMA J. 1999: The Atlas of European Mammals. *Poyser Ltd, London*.
- MONTGOMERY W. I. 1999: *Apodemus sylvaticus* (Linnaeus, 1758). In: Mitchell-Jones A. J., Amori G., Bogdanowicz W., Kryštufek B., Reijnders P.J.H., Spitzenberger F., Stubbe M., Thissen J.B.M., Vohralík V. & Zima J. (eds), The Atlas of European Mammals. *Poyser Ltd, London*: 274–275.
- ÖZKAN B., 1999. Rodent fauna of Imbros and Tenedos (Mammalia: Rodentia). *Tr. J. Zool.* 23: 133–147.
- ÖZKAN B. & KRYŠTUFEK B. 1999: Wood mice, *Apodemus* of two Turkish islands: Gökçeada and Bozcaada. *Folia Zool.* 48: 17–24.
- ONDRIAS J. 1966: The taxonomy and geographical distribution of the rodents of Greece. *Säugetierkd. Mitt.* 14: 1–136.
- PIEPER H. 1966: Über einige bemerkenswerte Kleinsäuger-Funde auf der Inseln Rhodos und Kos. *Acta Biol. Hellenica* 1: 21–28.
- SAITOU N. & NEI M. 1987: The Neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.
- STORCH G. 2004: Late Pleistocene rodent dispersal in the Balkans. In: Griffiths H.I., Kryštufek B. & Reed J.M. (eds), Balkan biodiversity. Pattern and Process in the European hotspot. *Kluwer Academic Publishers, Dordrecht*: 135–145.
- THOMPSON J. D., HIGGINS D. G. & GIBSON T. J. 1994: Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Res.* 22: 4673–4680.
- VAN DE PEER Y. & DE WACHTER R. 1994: TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Applic. Biosci.* 10: 569–570.