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False phylogenies on wood mice due to cryptic cytochrome-*b* pseudogene

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ABSTRACT

The phylogeny and phylogeography of the Old World wood mice (subgenus *Sylvaemus*, genus *Apodemus*, Muridae) are well-documented. Nevertheless, the distributions of species, such as *A. fulvipectus* and *A. ponticus* remain dubious, as well as their phylogenetic relationships with *A. sylvaticus*. We analysed samples of *Apodemus* spp. across Europe using the mitochondrial cytochrome-*b* gene (*cyt-b*) and compared the DNA and amino-acid compositions of previously published sequences. The main result stemming from this study is the presence of a well-differentiated lineage of *Sylvaemus* including samples of various species (*A. sylvaticus*, *A. fulvipectus*, *A. ponticus*) from distant locations, which were revealed to be nuclear copies of the mitochondrial *cyt-b*. The presence of this cryptic pseudogene in published sequences is supported by different pathways. This has led to important errors in previous molecular trees and hence to partial misinterpretations in the phylogeny of *Apodemus*.

1. Introduction

The phylogeny and phylogeography of the Old World wood mice (subgenus *Sylvaemus*, genus *Apodemus*, Muridae) are well-documented in numerous publications involving the mitochondrial cytochrome-*b* gene (*cyt-b*; e.g. Martin et al., 2000; Hille et al., 2002; Michaux et al., 2002, 2003, 2004, 2005; Reutter et al., 2003; Balakirev et al., 2007; Hoofer et al., 2007; Suzuki et al., 2008) or both mitochondrial and nuclear genes (e.g. Michaux et al., 2002, 2005; Suzuki et al., 2008). The western Eurasian species of the subgenus *Sylvaemus*, i.e. the alpine mouse *A. alpicola*, the wood mouse *A. sylvaticus*, the yellow-necked mouse *A. flavicollis*, the pygmy woodmouse (*A. uralensis*), the yellow-breasted mouse *A. witherbyi*, the Mt Hermon mouse *A. hermonensis* (a synonym of *A. witherbyi;* see Musser and Carleton, 2005) and the Caucasus mouse *A. ponticus* have been revealed to be genetically closely related (Michaux et al., 2002; Suzuki et al., 2008).

Within the species, the phylogeography of *A. flavicollis*, based on the *cyt-b* (Michaux et al., 2004) showed two well differentiated clades, a first in Turkey, Syria, Israel, and Iran (Southern of the Caucasus), and a second including western, central, and eastern Europe, and Russia (Fig. 1). For *A. alpicola*, a species restricted to the alpine region, Michaux et al. (2002) and Reutter et al. (2003) documented its monophyly. A thorough phylogeography of *A. sylvaticus*, based on the *cyt-b* (Michaux et al., 2003), highlighted a clear pattern with slightly divergent sub-clades involving different geographic areas such as (i) western, northern and central Europe, (ii) Italy and Balkans, or (iii) Sicily. Interestingly, Reutter et al. (2003), based on restricted sampling, found two additional well differentiated sequences of *A. sylvaticus* (G3.4 and G3.1), both from Karlsruhe in the southern part of Germany, from animals clearly identified as *A. sylvaticus* in the field. The first one (G3.4) was considered to belong to a lineage that separated very early, before the split separating the Italian animals (Michaux et al., 2003) and animals from the Pyreneans to the Ukraine. The authors were not able to explain the syntropic occurrence of these two lineages. The second one (G3.1) revealed to be closely related to some sequences ascribed to *A. fulvipectus* (but the identity is still uncertain) and *A. ponticus* from Georgia (Hille et al., 2002) and to a unique sequence of *A. sylvaticus* from Konstanz, Germany (Martin et al., 2000), a locality 150 km away from Karlsruhe. Both tissue samples were from biopsies of released animals without preserved voucher specimens. Reutter et al. (2003) suggested that an unrecognized population of *A. fulvipectus* might occur in southern Germany. As these sequences were fully coding the authors excluded the presence of a nuclear pseudogene and refuted the possibility of a DNA contamination in their laboratory, as they had never handled samples of *A. fulvipectus* from the Caucasus before. Moreover, the situation in Georgia, as studied by Hille et al. (2002), was all but clear. The assignment of some samples by morphology was not concordant

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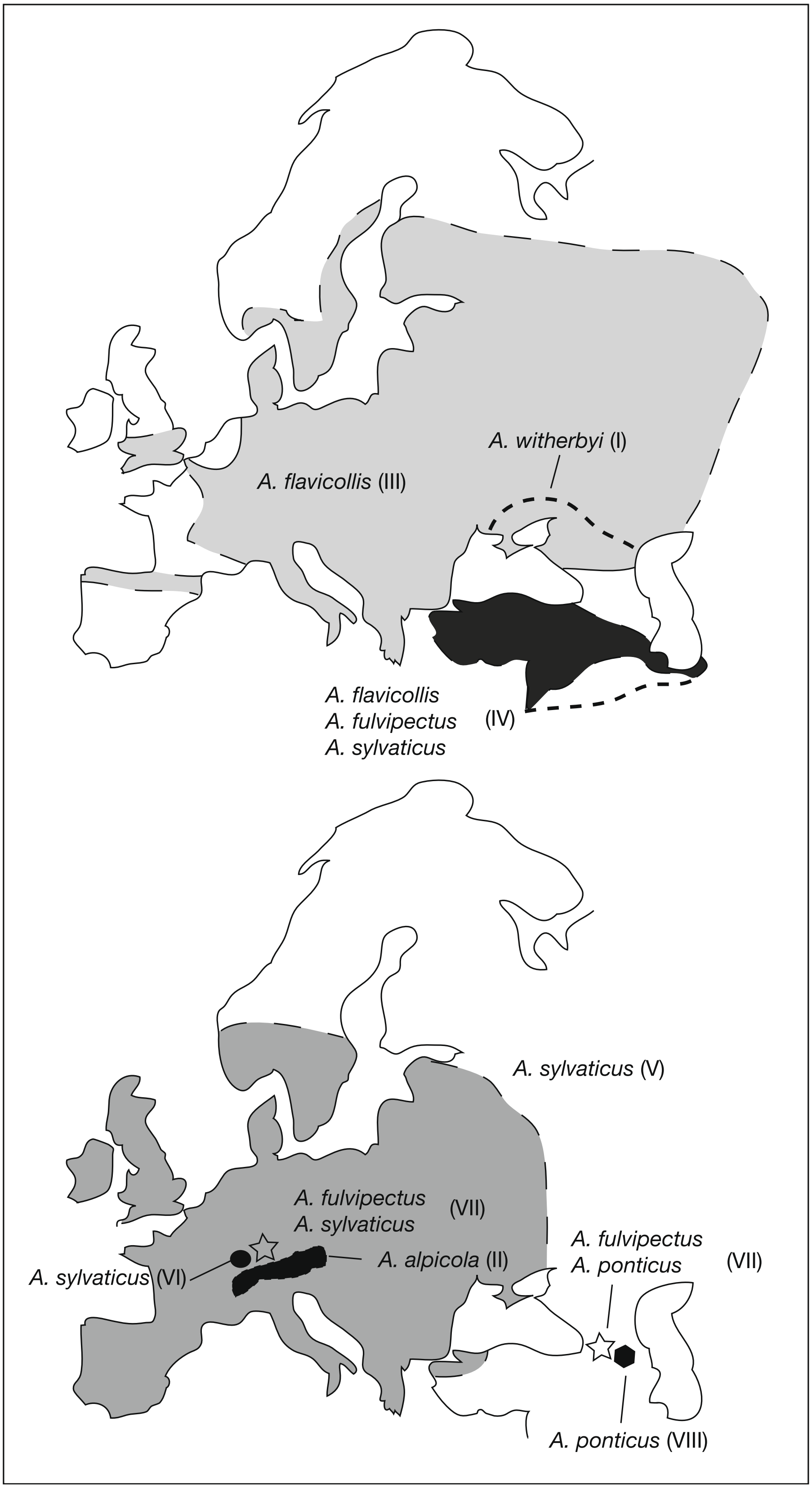

Fig. 1. Distribution of *Apodemus* spp. in Europe and the Near East, according to previous publications based on molecular phylogenetic studies and corresponding lineage (I–VIII) of the present study. Areas of lineages I–V are shaded. The locations of the samples of lineage VI and VII shown by dot, stars or polygon.

with the genetic assignment (*cyt-b*), especially the assignment of *A. ponticus* and *A. flavicollis* from the same locality appeared often problematic. Finally, a recent phylogenetic study (Suzuki et al., 2008) including some of these sequences yielded similar results, which led the authors to the conclusion that the systematics of these species needs to be revised.