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## Importance of Superoxide Dismutase in Distinguishing of *Apodemus flavicollis* and *Apodemus sylvaticus* (Mammalia: Rodentia) in Thrace

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**Abstract:** Specimens of *Apodemus flavicollis*, *Apodemus sylvaticus* and *Apodemus agrarius* in Thrace were analyzed based on superoxide dismutase (SOD). Sod fixed for A alleles in *A. flavicollis*, B alleles in *A. sylvaticus* and C alleles in *A. agrarius* in Turkish Thrace and very clearly separated two sibling species *A. flavicollis* and *A. sylvaticus* along with *A. agrarius*.

**Key words:** *Apodemus*, taxonomy, allele, Thrace

### INTRODUCTION

Of the genus *Apodemus*, *Apodemus flavicollis*, *Apodemus sylvaticus*, *Apodemus iconicus*, *Apodemus uralensis*, *Apodemus mystacinus* and *Apodemus agrarius* live in Turkey. *A. mystacinus* and *A. agrarius* are morphologically the most distinguishable species of the genus *Apodemus* in Turkey. Contrary to this, distribution and taxonomic identification of *A. sylvaticus* and *A. flavicollis* are problematic (Filippucci *et al.*, 1996; Macholán *et al.*, 2001). Özkan and Krystufek (1999) recorded *A. sylvaticus* from Thrace along with *A. flavicollis*, based on morphological aspects. According to Filippucci (1992), morphological characters do not allow identification of *A. sylvaticus* and *A. flavicollis*. A single enzyme locus, superoxide dismutase, may be a distinguishing character for sibling species, especially for *A. flavicollis* and *A. sylvaticus*.

The aim of this study is to contribute to taxonomy, population genetics of the genus *Apodemus* species in Thrace.

### MATERIALS AND METHODS

Superoxide dismutase (SOD) of eighteen specimens of the genus *Apodemus* from Thrace was analysed, with three of *A. sylvaticus* from Edirne (n = 1) and Büyükkarıştıran (Tekirdağ) (n = 2), ten of *A. flavicollis* from Velikaköprüsü (Kırklareli) (n = 10) and five of *A. agrarius* from İğneada (Kırklareli) (n = 5) (Fig. 1).

The muscles were used for this enzyme system. Tissues were kept at -70°C until use. The muscle extracts were mixed with 10% sucrose+stain (Brom phenol blue/BPB) at 1:1 ratio (Prabhakaran and Kamble, 1993).

Consort E 863 model vertical slab gel electrophoresis device was used for electrophoresis. Polyacrilamide gels were prepared in a concentration of 7.5% resolving gel and 4% stacking gel as suggested by Sambrook *et al.* (1989). Electrode buffer solution was prepared with 0.025 M Tris, 0.192 M Glycine pH: 8.3 (Laemmli, 1970). Twenty microliter from each sample was loaded to the gels. 8 V cm<sup>-1</sup> constant voltage was applied to stacking gel and voltage was adjusted to 15 V cm<sup>-1</sup> when the tracking dye reached to the resolving gel. After electrophoresis, gel was stained for SOD with the method suggested by Harris and Hopkinson (1976). Gel was then exposed to light. After the darkening of the gel background, SOD bands appeared as achromatic zones.

### RESULTS

*A. flavicollis* and *A. agrarius* were collected from deciduous forests and *A. sylvaticus* from the destroyed areas in the edge of forests and cultivated areas.

Single SOD locus was determined in the genus *Apodemus*. Three alleles (A, B, C) were observed and three electrophoretic bands were scored. *A. flavicollis* is homozygous for the slowest of the three alleles (A) while *A. agrarius* is homozygous for the fastest of the three alleles (C). *A. sylvaticus* has intermediate moved alleles (B). It is impossible to deduce anything about the subunit structure as there are no heterozygotes present. The three species are genetically different at this locus. *A. flavicollis*, *A. sylvaticus* and *A. agrarius* formed the separated banding region on the gel. *A. flavicollis* fixed for A alleles, *A. sylvaticus* for B alleles and *A. agrarius* for C alleles (Fig. 2).

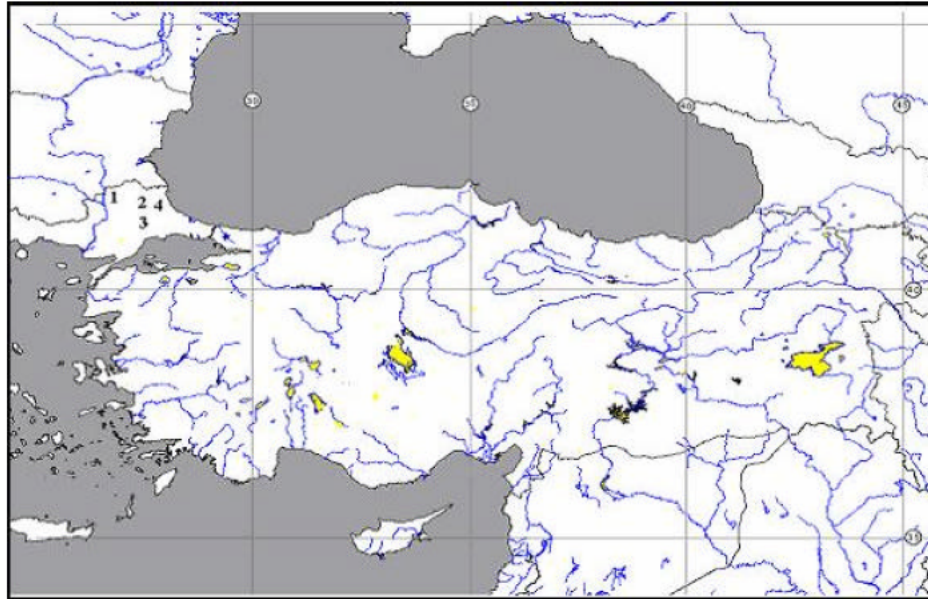


Fig. 1: The map showing the localities of *Apodemus* specimens examined in Thrace. 1. Edirne, 2. Pınarhisar, 3. Büyükkarıştıran, 4. Velikaköprüsü and İğneada

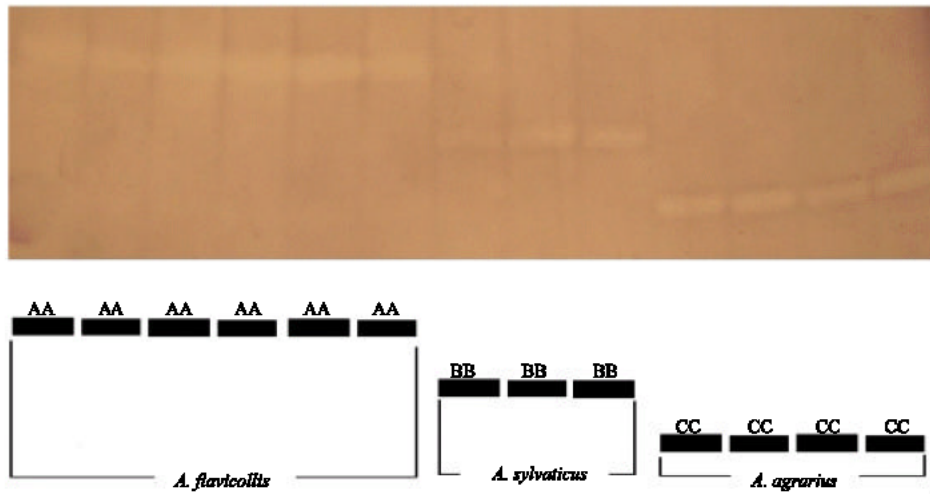


Fig. 2: Zymogram (above) and its diagrammatic representation (bottom) of the gel for muscle superoxide dismutase. AF: *A. flavicollis*, AS: *A. sylvaticus*, AA: *A. agrarius*

### DISCUSSION

Özkan and Krystufek (1999) recorded *A. flavicollis* from Istranca Mountains and *A. sylvaticus* from Edirne. Results of present study are consistent with that of Özkan and Krystufek (1999).

Britton-Davidian *et al.* (1991) analyzed *A. sylvaticus* from France, Greece, Italy and Spain; *A. flavicollis* from France and *A. agrarius* from Bulgaria and Greece based

on SOD aspects and found slow allele for *A. flavicollis*, fast allele for *A. agrarius* and an allele between slow and fast alleles for *A. sylvaticus* as in present study. Britton-Davidian *et al.* (1991) revealed allele fixation for SOD in 67 specimens of *A. sylvaticus*, 19 specimens of *A. flavicollis* and 18 specimens of *A. agrarius*. In present study as in work of Britton-Davidian *et al.* (1991), SOD was fixed for A allele for *A. flavicollis*, B allele for *A. sylvaticus* and C allele for *A. agrarius*. In Turkish Thrace, *A. flavicollis* and

*A. agrarius* live in same habitat and need same ecological requirements, whereas *A. sylvaticus* lives in different habitat; the destroyed forests and cultivated areas. This shows that SOD does not change based on habitat differentiation in Turkish Thrace. Therefore SOD seems to be an important character in separation of two sibling species; *A. sylvaticus* and *A. flavicollis* in Turkish Thrace. Çolak *et al.* (2005) showed that morphometrics did not distinguish *A. flavicollis* from *A. sylvaticus*, whereas patterns of esterase (EST) clearly separated the sibling species *A. flavicollis* from *A. sylvaticus* as well as *A. agrarius*. They also stated that SOD seems to be a second taxonomic character for *A. flavicollis* and *A. sylvaticus*, after EST.

In conclusion, SOD locus very clearly separated sibling species *A. flavicollis* and *A. sylvaticus* as well as *A. agrarius*.

#### ACKNOWLEDGMENTS

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#### REFERENCES

- Britton-Davidian, J., M. Vahdati, F. Benmehdi, P. Gros, V. Nance, H. Croset, S. Guerassimov and C. Triantaphyllidis, 1991. Genetic differentiation in four species of *Apodemus* from Southern Europe: *A. sylvaticus*, *A. flavicollis*, *A. agrarius* and *A. mystacinus* (Muridae, Rodentia). *Zeitschrift für Säugetierkunde*, 56: 25-33.
- Çolak, R., E. Çolak and N. Yiğit, 2005. Morphometric, karyotypic and electrophoretic analysis of the Genus *Apodemus* Kaup, 1826 (Mammalia: Rodentia) in Thrace. *Turk. J. Zool.*, 29: 147-153.
- Filippucci, M.G., 1992. Allozyme variation and divergence among European, Middle Eastern and North African species of the genus *Apodemus* (Rodentia, Muridae). *Isr. J. Zool.*, 38: 193-218.
- Filippucci, M.G., G. Storch and M. Macholán, 1996. Taxonomy of genus *Sylvaemus* in western Anatolia morphological and electrophoretic evidence (Mammalia: Rodentia: Muridae). *Senckenbergiana Biol.*, 75: 1-14.
- Harris, H. and D.A. Hopkinson, 1976. *Handbook of Enzyme Electrophoresis in Human Genetics*. North-Holland Publishing Company, Amsterdam.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, 227: 680-685.
- Macholán, M., M.G. Filippucci, P. Benda, D. Frynta and J. Sadlova, 2001. Allozyme variation and systematics of the genus *Apodemus* (Rodentia: Muridae) in Asia Minor and Iran. *J. Mammal.*, 82: 799-813.
- Özkan, B. and B.B. Kryštufek, 1999. Wood mice, *Apodemus* of two Turkish islands: Gökçeada and Bozcaada. *Folia Zool.*, 48: 17-24.
- Prabhakaran, S.K. and S.T. Kamble, 1993. Activity and electrophoretic characterization of esterase in insecticide-resistant and susceptible strains of German cockroach (Dictyoptera: Blattellidae). *J. Econ. Entomol.*, 86: 1009-1013.
- Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. *Molecular Cloning, a Laboratory Manual*. 2nd Edn., Cold Spring Harbor Laboratory Press, New York.