Acta Zoologica Academiae Scientiarum Hungaricae 55 (1), pp. 31–38, 2009

MORPHOMETRIC KEY FOR THE DISCRIMINATION OF TWO WOOD MICE SPECIES, *APODEMUS SYLVATICUS* AND *A. FLAVICOLLIS*

BARČIOVÁ, L.[1,2] and MACHOLÁN, M.[3,4]

[1]*Department of Zoology, Faculty of Science, University of South Bohemia*
Branišovská 31, 370 05 České Budějovice, Czech Republic
E-mail: lenka_barciova@yahoo.com
[2]*Department of Biological Disciplines, Faculty of Agriculture, University of South Bohemia*
Studentská 13, 370 05 České Budějovice, Czech Republic
[3]*Institute of Animal Physiology and Genetics ASCR*
Veveří 97, 602 00 Brno. E-mail: macholan@iach.cz
[4]*Institute of Botany and Zoology, Masaryk University, Brno, Czech Republic*

Sixteen cranial measurements were examined in 311 genetically identified specimens of *Apodemus sylvaticus* and *A. flavicollis* in order to establish a simple identification key diagnosing these two morphologically similar species using the classification tree technique. The resulting identification key includes three cranial measurements only (length of the lower tooth row, condylobasal length, length of bulla tympanica) and enables separation of the species under investigation with 98.3% accuracy. Moreover, some previously proposed identification criteria for *A. sylvaticus* and *A. flavicollis* are reviewed and their applicability is discussed.

Key words: *Apodemus*, discrimination, classification trees

INTRODUCTION

The wood mouse, *Apodemus sylvaticus* (LINNAEUS, 1758), and the yellow-necked mouse, *A. flavicollis* (MELCHIOR, 1834), are two common rodent species inhabiting a major part of Europe. Although various aspects of their biology have been intensively studied for several decades, the task of their morphological discrimination is not satisfactorily resolved to date. The two members of the subgenus *Sylvaemus* can be easily distinguished in northern areas (e.g. URSIN 1956, REINWALDT 1957), but their morphological identification is impeded by the reverse north-south clinal variation in body size (NIETHAMMER 1969, ALCÁNTARA 1991) and the occurrence of other *Sylvaemus* species in central, southern, and south-eastern Europe. Both species also have similar ecology and karyotypes (ZIMA & KRÁL 1984), and therefore the reliable discrimination of *A. flavicollis* and *A. sylvaticus* individuals is generally possible only by means of biochemical or molecular analysis. However, analyses of cranial measurements have been only rarely carried out on genetically identified specimens (GEMMEKE & NIETHAMMER

1981, FERNANDES *et al.* 1991, MEZHZHERIN & LASHKOVA 1992, BARČIOVÁ & MACHOLÁN 2006).

Furthermore, most papers focused on morphometric discrimination between *A. sylvaticus* and *A. flavicollis* have been restricted to bivariate comparisons of linear measurements and various indices (e.g. HAITLINGER & RUPRECHT 1967, STEINER 1968, GEMMEKE & NIETHAMMER 1981, STORCH & LÜTT 1989). The main difficulty of this approach is an unbiased assessment of boundary values between the species and therefore, multivariate analyses such as linear discriminant function analysis (DFA) or classification trees seem to be more suitable for assigning individual mice to species. In a previous paper (BARČIOVÁ & MACHOLÁN 2006), we applied the former technique to a set of cranial and dental measurements as well as to a set of dorsal and ventral landmarks and reviewed results of some older studies dealing with multivariate morphometric identification of Central European *Sylvaemus* species (*A. sylvaticus*, *A. flavicollis*, *A. uralensis*, and *A. alpicola*). The latter method, classification tree analysis, has been rather neglected in studies of interspecific variation of mammal species, even though it has several advantages over DFA, e.g. the consideration of the non-additive interactions among explanatory variables or no assumption of any specific distribution of the data.

The classification tree technique was applied to a set of morphometric characters measured on the skulls of genetically identified specimens of *A. sylvaticus* and *A. flavicollis* in order to establish a simple and optimal key for their morphological identification.

MATERIAL AND METHODS

The studied specimens came from 14 sites scattered across the Czech Republic (Table 1) and are deposited at the Department of Zoology, Charles University in Prague (14 specimens from the site Ruda) and in the private collection of L. B.

A total of 311 specimens (229 *A. flavicollis* and 82 *A. sylvaticus*) belonging to age classes II–IV according to ADAMCZEWSKA-ANDRZEJEWSKA (1967) were examined. The specific status of all individuals investigated was identified with standard horizontal starch gel electrophoresis and scoring four diagnostic allozyme markers: lactate dehydrogenase 1 (*Ldh1*, E.C. 1.1.1.27), isocitrate dehydrogenase 1 (*Idh1*, E.C. 1.1.1.42), superoxide dismutase 1 (*Sod1*, E.C. 1.15.1.1), and nucleoside phosphorylase (*Np*, E.C. 2.4.2.1).

Sixteen cranial and dental characters were examined (Fig. 1). Values of FI, L5, L10 and L11 were obtained from images of the ventral side of the skull using the TpsDig program (ROHLF 2001); all others were measured with a slide caliper with precision of 0.1 mm. The paired characters were always taken on the right side of the skull in order to avoid variation due to potential asymmetry.

The significance of sexual dimorphism in interspecific analyses was estimated with univariate two-way ANOVAs (factors species, sex and their interaction) using the Statistica program package (StatSoft 2001). An identification key based on the cranial measurements was produced according to

the result of the classification tree analysis, which was performed in the Classification trees module in Statistica (C&RT-style exhaustive search for univariate splits). In general, classification and regression trees are non-parametric types of regression models in which the tree represents a sequence of binary partitions of the dataset into subsets of increasing homogeneity (LEPŠ & ŠMILAUER 2003). Each splitting follows a simple rule based on a single quantitative or qualitative explanatory variable. In the case of classification trees, the dependent variable is qualitative and identifies the observed class of the specimens. The optimal length of the classification tree was determined with a minimal cost-complexity cross-validation pruning (for details, see LEPŠ & ŠMILAUER 2003).

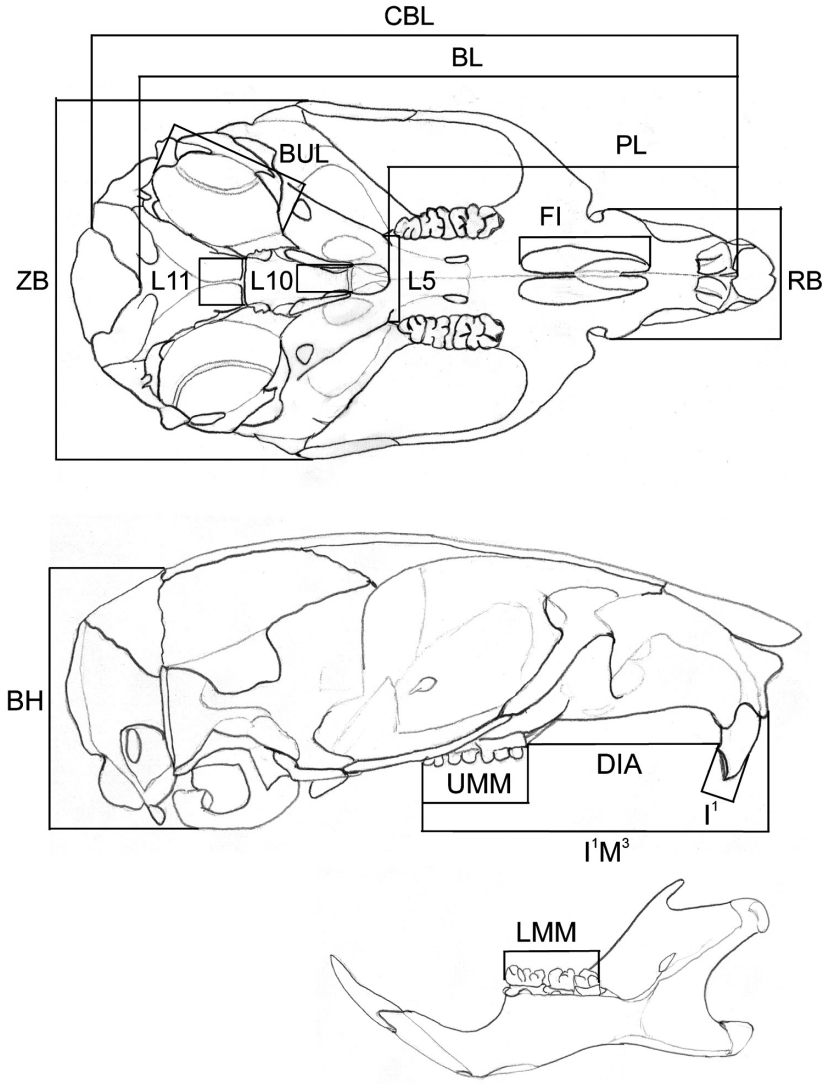

Fig. 1. Cranial measurements used in this study

Table 1. List of sites with their geographic coordinates and corresponding number of investigated specimens.

Site	Latitude	Longitude	No. of specimens	
			A. flavicollis	*A. sylvaticus*
Nové Hrady	48°48' N	14°48' E	24	–
Havraníky	48°49' N	16°00' E	28	–
Havranické vřesoviště	48°49' N	16°01' E	8	–
Stromovka	48°58' N	14°28' E	–	28
Rudolfov	48°59' N	14°32' E	15	6
Vyšatov	49°00' N	14°23' E	5	1
Vomáčka	49°04' N	14°21' E	19	11
Lužnice	49°05' N	14°45' E	11	–
Ruda	49°09' N	14°42' E	20	11
Malý Jordán	49°26' N	14°41' E	4	–
Běstvina	49°50' N	15°35' E	65	–
Litvínov	50°35' N	13°36' E	–	25
Javořina	50°38' N	13°33' E	26	–
Malá Skála	50°39' N	15°12' E	4	–

RESULTS

The results of univariate two-way ANOVAs (not shown) demonstrated significant morphometric differences between the species for most of the measured characters (except L5, L11, FI) and no effect of intraspecific sexual dimorphism (interaction species*sex) except LTP and UMM. Nevertheless, the amount of variability of LTP and UMM explained by the sexual dimorphism is relatively low in comparison with that explained by specific status (cf. the *F* values) and therefore the sexes were pooled for the classification tree analysis.

The individuals of *A. flavicollis* are on average larger than those of *A. sylvaticus*, but the specific ranges of the particular measurements show greater or smaller overlap and no one character can *per se* serve as a diagnostic criterion between the studied species.

The classification tree analysis of all measurements resulted in a simple tree with four terminal nodes. The overall misclassification rate for this tree is 1.7%, i.e. only five of 301 individuals (three *A. sylvaticus* and two *A. flavicollis*) were assigned to incorrect species. According to the splitting rules, the following identification key between *A. sylvaticus* and *A. flavicollis* was created (the number of *A.*

flavicollis (F) and *A. sylvaticus* (S) classified in each terminal node are given in brackets):

1	LMM is less than or equal to 3.8 mm		2
–	LMM is greater than 3.8 mm		3
2	CBL is less than or equal to 23.3 mm	*A. sylvaticus*	[F: 2; S: 71]
–	CBL is greater than 23.3 mm	*A. flavicollis*	[F: 11; S: 2]
3	BUL is less than or equal to 4.4 mm	*A. sylvaticus*	[F: 0; S: 4]
–	BUL is greater than 4.4 mm	*A. flavicollis*	[F: 210; S: 1]

DISCUSSION

The postnatal growth in *Apodemus* species continues throughout the whole life span, hence the correction for the size- and/or age-related variation is necessary. Since the most intensive growth takes place during the first four weeks after birth and then gradually slows down (NIETHAMMER 1969), exclusion of specimens without well-developed third upper molars (age class I) from morphometric analyses seems to be sufficient for minimization of this source of variation. This approach was successfully used, for example, by STORCH and LÜTT (1989) and REUTTER *et al.* (1999) in three *Sylvaemus* species, *A. sylvaticus, A. flavicollis* and *A. alpicola*. Sexual dimorphism represents another unwanted source of morphometric variation in interspecific comparisons. Nevertheless, the effect of sex was found to be negligible in our sample of *A. sylvaticus* and *A. flavicollis*, both for univariate (this study) and multivariate data (BARČIOVÁ & MACHOLÁN 2006).

All authors studying the interspecific morphometric variation in *Sylvaemus* species came to the same conclusion that it was impossible to separate *A. flavicollis* from *A. sylvaticus* using single external or cranial characters, though some tooth measurements were found to be very suitable for discrimination, in particular I^1 (HAITLINGER & RUPRECHT 1967), I^1M^3 (DEMETER & LÁZÁR 1984) or LMM (present study).

Several bivariate scatterplots and/or morphometric indices based on two or more measurements were suggested as useful identification tools for the determination of *A. flavicollis* and *A. sylvaticus* individuals. The most frequently used criterion is the length of foramen incisivum (FI), relative to a measurement representing general size such as condylobasal length (CBL: NIETHAMMER 1969) or the distance between I^1 and M^3 (I^1M^3: TVRTKOVIĆ 1976), the latter being more suitable

for the identification of wood mice remains from owl pellets. KRYŠTUFEK and STOJANOVSKI (1996) successfully applied this method to the material from the Balkans, satisfying results were also achieved by DEMETER and LÁZÁR (1984) in Hungary. NOVÁ (unpubl. MSc. Thesis) was able to discriminate only adults from the Czech Republic (Votice district), whereas GEMMEKE and NIETHAMMER (1981) were unable to separate biochemical diagnosed individuals originating from southern Italy.

Other potentially useful scatterplots have been suggested such as the plot relating UMM and LTP (AMTMANN 1965), UMM and BUL (FILIPPUCCI *et al.* 1996), UMM and LMM (PANZIRONI *et al.* 1994), BUL and CBL (MEZHZHERIN & LASHKOVA 1992), and those using indices such as [I^1 + UMM] plotted against DIA (STORCH & LÜTT 1989) or [UMM × breadth of M^3] plotted against [CBL × ZB] (STEINER 1968). Except for the last one, the proposed discriminant criteria can be applied to the data analyzed in this paper. However, all the scatterplots show overlap between the two species including the plot relating UMM and BUL that appeared to be the most successful one in the discrimination of investigated specimens. Finally, the index *MI* = [(UMM + length of palatal bridge + interorbital breadth) – FI] proposed by FILIPPUCCI *et al.* (1984) has also been found to be of little use (POPOV 1993, NOVÁ, unpubl. MSc. Thesis, MACHOLÁN, M. unpubl. data).

Equivocal results of proposed diagnostic criteria may be caused by geographic variation and/or methodological deficiencies such as inadequate sample sizes, violation of the sample randomness due to the frequently practised elimination of "atypical" specimens or the imprecise description of measured sections hampering repeatability of measurements. The identification key suggested in this study is based on a sample of 301 unambiguously identified individuals and involves three cranial measurements only: LMM, CBL and BUL. STEINER and RACZYŃSKI (1976) tested repeatability of several skull measurements in *Apodemus* and concluded that the condylobasal length (CBL) was the most accurately measured cranial character and the length of the tooth row (LMM) was also characterized by a high level of accuracy, whereas the length of bulla tympanica was not included in their analysis. So it can be concluded that in spite of the relative simplicity of the proposed key, the misclassification rate is surprisingly low (1.7%). Admittedly, it should be noted that both the identification key and the discrimination power can be guaranteed only for the populations under study, so one should be careful in extrapolating the results to other parts of the *A. sylvaticus* and *A. flavicollis* ranges. Obviously, populations from other areas should also be analyzed, yet we hope the criteria presented in this paper can be useful for the discrimination of the two species, at least in central Europe.

*

Acknowledgements – We thank P. NOVÁ for providing us with supplemental material from the site Ruda and JULIA WARDROPPER for English correction. This research was partially supported by the Mattoni Award for Biodiversity and Conservation (L. B.).

REFERENCES

ADAMCZEWSKA-ANDRZEJEWSKA, K. A. (1967) Age reference model for Apodemus flavicollis (Melchior, 1834). *Ekologia Polska – seria A* **15**: 787–790.

ALCÁNTARA, M. (1991) Geographical variation in body size of the wood mouse Apodemus sylvaticus L. *Mammal Review* **21**: 143–150.

AMTMANN, E. (1965) Biometrische Untersuchungen zur introgressiven Hybridisation der Waldmaus (Apodemus sylvaticus Linné, 1758) und der Gelbhalsmaus (Apodemus tauricus Pallas, 1811). *Zeitschrift für zoologische Systematik und Evolutionsforschung* **3**: 103–156.

BARČIOVÁ, L. & MACHOLÁN, M. (2006) Morphometric study of two species of wood mice Apodemus sylvaticus and A. flavicollis (Rodentia: Muridae): traditional and geometric morphometric approach. *Acta Theriologica* **51**: 15–27.

DEMETER, A. & LÁZÁR, P. (1984) Morphometric analysis of field mice Apodemus: character selection for routine identification (Mammalia). *Annales Historico-naturales Musei Nationalis Hungarici* **76**: 297–322.

FERNANDES, C., ENGELS, H., ABADE, A. & COUTINHO, M. (1991) Zur genetischen und morphologischen Variabilität der Gattung Apodemus (Muridae) im Westen der Iberischen Halbinsel. *Bonner zoologische Beiträge* **42**: 261–269.

FILIPPUCCI, M. G., CRISTALDI, M., TIZI, L. & CONTOLI, L. (1984) Morphological and morphometric data, about Apodemus (Sylvaemus) population of central and southern Italy tested by electrophoresis. *Supplemento alle Ricerche di Biologia della Selvaggina* **9**: 85–126 [in Italian with English abstract]

FILIPPUCCI, M. G., STORCH, G. & MACHOLÁN, M. (1996) Taxonomy of the genus Sylvaemus in western Anatolia – morphological and electrophoretic evidence (Mammalia: Rodentia: Muridae). *Senckenbergiana biologica* **75**: 1–14.

GEMMEKE, H. & NIETHAMMER, J. (1981) Die Waldmäuse Apodemus sylvaticus und A. flavicollis vom Monte Gargano (Süditalien). *Zeitschrift für Säugetierkunde* **46**: 162–168.

HAITLINGER, R. & RUPRECHT, A. L. (1967) The taxonomic value of teeth measurements in the subgenus Sylvaemus Ognev & Vorobiev, 1923. *Acta Theriologica* **12**: 180–187.

KRYŠTUFEK, B. & STOJANOVSKI, L. (1996) Apodemus sylvaticus stankovici is a synonym of Apodemus flavicollis. *Folia Zoologica* **45**: 1–7.

LEPŠ, J. & ŠMILAUER, P. (2003) *Multivariate analysis of ecological data using CANOCO.* Cambridge University Press, Cambridge, 282 pp.

MEZHZHERIN, S. V. & LASHKOVA, E. I. (1992) Diagnostics, geographic variation and distribution of two closely related mice species – Sylvaemus sylvaticus and S. flavicollis (Rodentia, Muridae) in an area of their overlapping occurrence. *Vestnik Zoologii* **3**: 33–41 [in Russian with English abstract]

NIETHAMMER, J. (1969) Zur Frage der Introgression bei den Waldmäusen Apodemus sylvaticus und A. flavicollis (Mammalia, Rodentia). *Zeitschrift für zoologische Systematik und Evolutionsforschung* **7**: 77–127.

PANZIRONI, C., CERONE, G., CRISTALDI, M. & AMORI, G. (1994) A method for the morphometric identification of southern Italian populations of Apodemus (Sylvaemus). *Hystrix (n.s.)* **5**: 1–16.

POPOV, V. V. (1993) Discriminant criteria and comparative study on morphology and habitat selection of Apodemus sylvaticus (Linnaeus, 1758) and Apodemus flavicollis (Melchior, 1834) (Mammalia, Rodentia, Muridae) in Bulgaria. *Acta Zoologica Bulgarica* **46**: 100–111.

REINWALDT, E. (1957) Studien am Schädel der schwedischen Waldmäuse, Apodemus sylvaticus (Linné, 1758) und Ap. flavicollis (Melchior, 1834). *Säugetierkundliche Mitteilungen* **5**: 100–103.

REUTTER, B. A., HAUSSER, J. & VOGEL, P. (1999) Discriminant analysis of skull morphometric characters in Apodemus sylvaticus, A. flavicollis, and A. alpicola (Mammalia; Rodentia) from the Alps. *Acta Theriologica* **44**: 299–308.

ROHLF, F. J. (2001) *TpsDig*, version 1.30. Dept. Ecology and Evolution, State University of New York at Stony Brook.

StatSoft Inc. (2001) *Statistica (data analysis software system)*, version 6. www.statsoft.com.

STEINER, H. M. (1968) Untersuchungen über die Variabilität und Bionomie der Gattung Apodemus (Muridae, Mammalia) der Donau-Auen von Stockerau (Niederösterreich). *Zeitschrift für wissenschaftliche Zoologie* **177**: 1–96.

STEINER, H. M. & RACZYŃSKI, J. (1976) Wiederholbarkeit von Messungen und individueller Messfehler bei craniometrischen Untersuchungen an Apodemus. *Acta Theriologica* **21**: 535–541.

STORCH, G. & LÜTT, O. (1989) Artstatus der Alpenwaldmaus, Apodemus alpicola Heinrich, 1952. *Zeitschrift für Säugetierkunde* **54**: 337–346.

TVRTKOVIĆ, N. (1976) The variability of the postero-external supplemental tubercle (T_{12}) on the first and second upper molars in the species Apodemus sylvaticus (Linné, 1758) and Apodemus flavicollis (Melchior, 1834) from western Yugoslavia. *Periodicum biologorum* **78**: 91–100.

URSIN, E. (1956) Geographical variation in Apodemus sylvaticus and A. flavicollis (Rodentia, Muridae) in Europe, with special reference to Danish and Latvian populations. *Biologiske Skrifter Det Kongelige Danske Videnskabernes Selskab* **8**(4): 1–46.

ZIMA, J. & KRÁL, B. (1984) Karyotypes of European mammals II. *Acta scientiarum naturalium Academiae scientiarium Bohemicae – Brno* **18**: 1–62.

Received December 3, 2007, accepted June 16, 2008, published March 31, 2009