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Article

In vivo classification of two closely related species of mice, mound-building mouse (*Mus spicilegus*) and house mouse (*Mus musculus*)

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ABSTRACT – Correct identification of similar, closely related species with overlapping distribution is a crucial point in field biology. In small mammal studies, species identification is particularly problematic in population studies using trapping where live animals need to be identified. The aim of our research was to develop a method making the classification of the two Hungarian mouse species, mound-building mouse (*Mus spicilegus*) and house mouse (*Mus musculus*) possible based on morphometric characters. The basis to obtain reference data was the captive populations of caged animals housed in our laboratory where the true species classification was known for every animal. Body weight, body length, tail length, and tail diameter were measured for 56-56 individuals from both species. From these measurements the ratio of the body length/tail length was also calculated. Besides, the sex and age of these animals were also recorded. Data analysis consisted of stepwise discriminant procedure and discriminant analysis, respectively. The stepwise discriminant procedure restricted the morphometric characters to the ratio of the body length/tail length and tail diameter. Performing the discriminant analysis to these body measures a perfect classification was obtained even using cross-validation. Thus, applying the obtained discriminant function to the classification of any live trapped mice is feasible.

Keywords: small mammal studies, live-trapping, classification

INTRODUCTION

Rodents (*Rodentia*) is one of the most populous order in the Mammalian class, and due to their excellent adaptability, its representatives can be found on all continents (*Bihari*, 2007). They play an essential role in the ecosystem as the primary food for many of our protected and highly protected species of carnivorous birds (*Bihari,* 2007).

One of the most common methods of surveying live rodents is by trapping them with various devices. Trapping small mammals is a direct method that besides faunistic and point mapping tasks allows us to estimate total population size as well.

The genus *Mus* belongs to the family of Mice (*Muridae*) and consists mainly of nocturnal terrestrial species (*Musser and Carleton*, 1993). In Hungary, the

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Mus genus is represented by two species: the eastern subspecies of the house mouse (*Mus musculus musculus* Linneaus 1758) and the mound-building mouse (*Mus spicilegus spicilegus* Petényi 1882). Based on external morphological features the two mice species are difficult to distinguish (*Demeter et al.,* 1996). In Hungary, both the mound mouse and the house mouse are 5-8 cm long, the color of the back coat is gray or greyish brown. Characters such as coat color, body, tail, or leg length are poorly distinguished from relatives of the genus Mus (*Auffray and Britton-Davidian,* 2012). The first scientific description of the mound-building mouse was published by a paleontologist-zoologist János Petényi Salamon (*Petényi,* 1882). The species can play an essential role in nature conservation as an indicator of traditional farming, as in Hungary the individuals of this species can typically be encountered in abandoned agricultural areas (*Bihari,* 2004; *Sokolov et al.,* 1998), where they build their mount during the winter (*Simenovska-Nikolova and Gerasimov,* 2000). On the contrary, the house mouse invades human settlements as the temperature drops. However, both species of mice spend the growing season on the agricultural landscape. Identifying the two species in non-laboratory conditions (with genetic testing) has been limited so far to owl sputum analysis, where the remnant of the animal (e.g. skull bones) is examined in its original habitat. In addition to owl sputum analysis, it would be important to find morphometric differences in live-trapped animals to help species' classification. Hence, an accurate picture of the distribution of mound-building mice and house mice in Hungary could be obtained. The aim of our research was to develop a method making the classification of the two Hungarian mouse species, mound-building mouse (*Mus spicilegus*) and house mouse (*Mus musculus*) possible based on morphometric characters.

MATERIAL AND METHODS

The present study was carried out at the rodent laboratory of the Kaposvár Campus (of the Hungarian University of Agriculture and Life Sciences). The animals were kept in accordance with current legislation, the study is not an animal experiment. The number of animals used in the study was reduced as little as possible (1998. XXVIII. Hungarian law on the protection and welfare of animals).

The founders of our captive mouse populations were wild-caught mound-building mice and house mice from several populations throughout Hungary. The descendants of the founder animals were bred for several generations forming our two captive laboratory populations where the age and species of all animals were known.

Animals were housed in standard T4 laboratory polycarbonate rodent boxes on a 12-12 hour reverse day-night cycle. Red neon fluorescent lamps provided the night illumination. The holding temperature is standard 20-22 ° C and humidity is 30-60%. For bedding, wood shavings (Lignocell J. Rettenmaier and Söhne GmbH, Rosenberg, Germany) and hay were used. Complete rodent feed (Ssniff S8106-SO11 Spezialdiäten GmbH, Soest, Germany) and water *ad libitum* were available.

From the two available captive mouse populations, 56 adult mound-building mice and 56 adult house mice were randomly selected within the species with a sex ratio of 1:1. As no sexual dimorphism was found in a preliminary analysis the sexes were not used in the later statistical analysis.

During the data collection, the species, sex, and age of the individuals were recorded, and their body weights were measured on a lab analytical digital balance scale. The body length of the individuals was measured in millimeters from the tip of the nose to the anus. This measurement was performed with a 3 cm diameter wide and 10 cm long transparent cylinder open at each end and scaled at every 1 mm. The tail length of the animals was measured with a digital caliper from the anus to the tip of the tail to the nearest 0,01 mm (*Figure* 1).

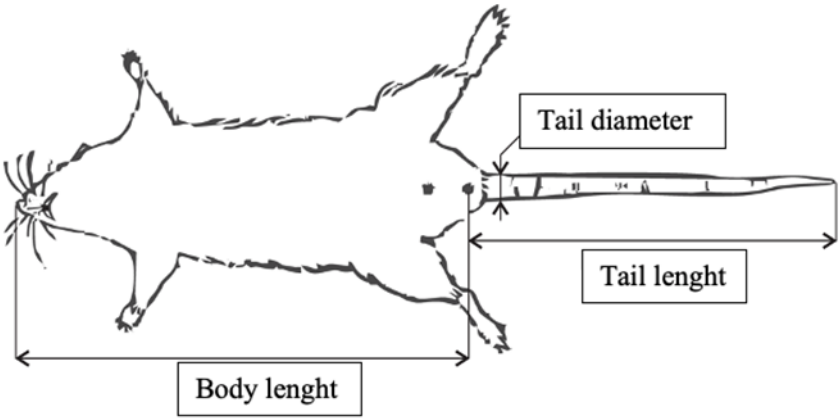

Figure 1. Body variables measured on a live animal. Body length: from nose to anus. Tail length: from the anus to the end of the tail. Tail diameter: the diameter measured at the base of the tail.

Using a digital caliper measuring with two decimal places, we measured the tail diameter to place in the tail base. The body length/tail length ratio and the body length-tail length difference were derived from the measured recordings. The group means of all measured traits were depicted using Excel figures.

Analysis of the above mentioned morphometric characters consisted of stepwise discriminant procedure and discriminant analysis, respectively applying the STEPDISC (*SAS Institute Inc.,* 2013) and DISCRIM (*SAS Institute Inc.,* 2013) procedures of the SAS 9.4 statistical software where the known species of the animals served as the class variable. The equality of variance-covariance matrices of the different species and the equality of their vector means were tested with the POOL and MANOVA options of the DISCRIM procedure. To ensure the reliability of the calculated discrimination function classification of the analyzed animals were tested using cross-validation.

RESULTS AND DISCUSSION

The means of the measured morphometric characters were depicted by the two examined species (*Figures 2-5*). It can be seen that several group means were similar and there was an obvious overlapping between the species for all traits.

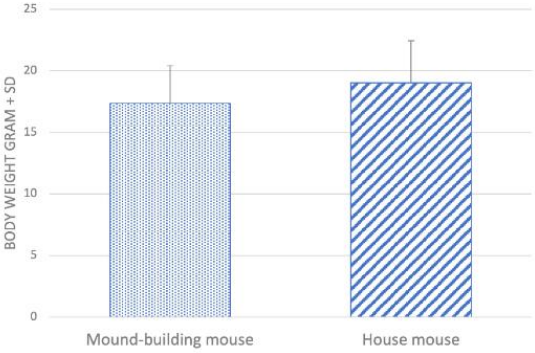

Figure 2. Bodyweight of the two species

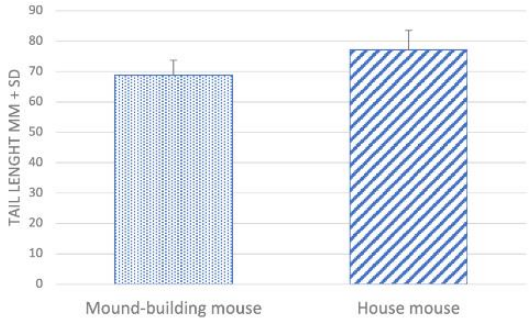

Figure 3. Tail length of the two species

Using the STEPDISC procedure a stepwise discriminant analysis was performed to select a subset of the measured morphometric characters for use in discriminating between the species. In this study, the FORWARD option was used and at each step, PROC STEPDISC entered the variable that contributed most to the discriminatory power of the model. The results of the procedure being provided in *Table 1.* Based on these results the DISCRIM procedure was performed using only the tail diameter and the body length/tail length ratio, respectively.

Table 1
Stepwise selection summary

Step	Morphometric Character	Partial R square	F value	Pr > F
1	Tail diameter	0.84	587	0.0001
2	Body length/tail length ratio	0.12	16	0.0001

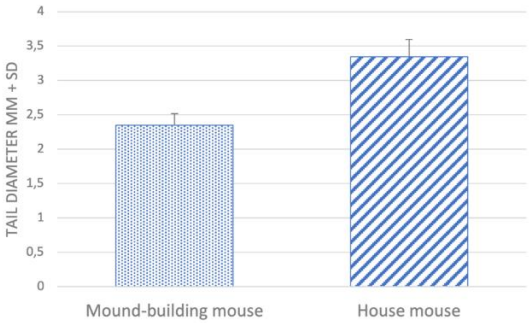

Figure 4. Tail diameter of the two species

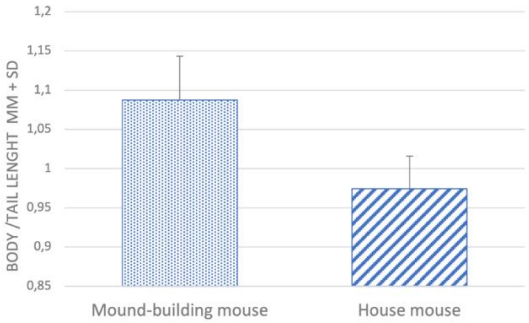

Figure 5. Body length/tail length ratio of the two species

Based on the discriminant analyis both the variance-covariance matrices (Chi^2 = 0.0019) and the vector of means (Wilks lambda = 0.0001) were different thus quadratic discriminant analysis was used. Based on the calculated discriminant function classification of the reference data without and with cross-validation is presented in *Tables 1-2*, respectively. It can be seen that the applied quadratic discriminant function resulted that 100% of the measured individuals could be properly assigned to their species even with cross-validation.

Table 2.
Classification summary for calibration data

Number of observations classified into species			
From: Species	1	2	Total
1	56	0	56
2	0	56	56
Total	56	56	112

Table 3.
Cross-validation summary

Number of observations classified into species			
From: Species	1	2	Total
1	56	0	56
2	0	56	56
Total	56	56	112

Regarding the body sizes of living individuals of the two socially different mouse species, we tested several variables which could help the differentiation of the two species. The first trait was body weight, but we do not consider this a good determinant, as body weight varies with age, sex, and even seasonality. Based on preliminary analysis body length did not differ between the two species, but the tail length did, although the difference in tail length alone does not allow the identification of the two species on the field. If we examine the length of the tail in relation to the length of the body, we see a difference between the two species. The tail of the mound-building mouse is significantly shorter than that of the house mouse, where the body length and tail length are nearly the same or slightly longer than the body. *Lindquist* (2003) found a similar difference in relative tail length in the two American mouse species he studied, the deer mouse (*Peromyscus maniculatus*) and the very similar white-footed mouse (*Peromyscus leucopus*). The morphology of the deer mouse and the white-footed mouse is very similar, challenging to distinguish from each other, just as in the case of the mound-building and the house mouse. In field studies, the two American species can be distinguished from each other based on relative tail length, the tail of the deer mouse is longer than that of the white-footed

mouse (*Horner,* 1954). Our other derived variable i.e., the difference between body length and tail length, is also a well-measurable marker on the field; the tail of a mound-building mouse is shorter than its body, and the tail of a house mouse is longer than the body.

A significant difference was obtained for the tail diameter. The tails of house mice are much thicker than those of mound-building mice. *Sokolov* (1998) also mentions that the tail of the mound-building mouse is the thinnest within the genus Mus, but we have not had quantified data on it so far. The tail diameter of the house mice is over 3 mm at the base of the tail, while that of the mound-building mice is around 2 mm. The question arises whether different tail lengths and thicknesses may be related to the different habitat use of the two species. The tail may be an important factor in overcoming certain field obstacles. Thus, we would like to continue investigating this question, as we know that house mice move to buildings for the winter (*Carlsen*, 1993), where they need the ability to climb, while mound-building mice spend the whole year on abandoned fields (*Bihari,* 2004, *Sokolov et al.,* 1998). In previous open-field tests comparing the two species, *Sokolov et al.* (1990) found that house mice climb on landmarks while exploring the terrain while the mound-building mice remain on the ground.

Concerning species classification based on mouse morphometric analysis, only few studies were found. Based on mouse skulls recovered from owl-pellets *Cserkész et al.* (2008) managed to distinguish *Mus spicilegus* from Mus musculus with almost 100% success based on discriminant function analysis of cranial measurements. Analyzing different body measures (various length, width, and height) of live animals in several *M. m. domesticus* populations *Slabova and Frynta* (2007) also received very high (93-97%) reclassification success for the Czech and Syrian mouse populations applying discriminant function analysis.

It is important to note that the data of our study only characterize the Hungarian population because our data are from this range; there are local differences within the species in the geographic range of the species complex, so our findings can only be applied to the Hungarian population. Based on this knowledge, we have the opportunity to examine the mound-building mice during the field trapping of small mammals; thus, we could get an overall picture of the condition of the Hungarian populations. With the growth of intensive agriculture, the habitat of mound-building mice decreases, and in the absence of the set-aside of arable land, the success of their overwintering may also decrease significantly.

CONCLUSIONS

It can be concluded that the detected morphological differences between the mound-building and the house mouse, may have great importance in the reliable classification of the two species in field studies. The two species could be distinguished based on the tail diameter and the body length/tail length ratio. These variables are well-measurable in the field and from preliminary studies, it is known that these traits are not sex-dependent. Further studies are needed on the possible age effect on these variables because, in field studies, adult and juvenile individuals can also be encountered, where body proportions may be different.

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