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(Short communication)

RETRIEVING *PEROMYSCUS BULLATUS* FROM *P. DIFFICILIS* (RODENTIA: CRICETIDAE) THROUGH CRANIAL MORPHOMETRY IN A MAMMAL COLLECTION

RECUPERANDO ESPECÍMENES DE *PEROMYSCUS BULLATUS* DE *P. DIFFICILIS* (RODENTIA: CRICETIDAE) MEDIANTE MORFOMETRÍA CRANEAL EN UNA COLECCIÓN MASTOZOOLÓGICA

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ABSTRACT. *Peromyscus bullatus* and *Peromyscus difficilis* are sympatric and phenotypically similar rodents. Because of this, misidentification of both species is frequent in the field and in mammal collections. In this study, *P. bullatus* specimens were retrieved from the *P. difficilis* specimens, in the mammal collection of the Instituto de Investigaciones Biológicas of the Universidad Veracruzana, through the analysis of morphometric measurements of the tympanic bulla, proposed by González-Ruíz *et al.* (2005) as discrimination key variables between both species.

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RESUMEN. La identificación errónea de ejemplares de *Peromyscus bullatus* como *Peromyscus difficilis* es frecuente, tanto en el campo como en las colecciones mastozoológicas, debido a que ambas especies son simpátricas y similares fenotípicamente. En este estudio se recuperaron ejemplares de *P. bullatus* incorrectamente catalogados como *P. difficilis* en la colección mastozoológica del Instituto de Investigaciones Biológicas de la Universidad Veracruzana, mediante el análisis de medidas morfométricas de la bula timpánica, propuestas por González-Ruíz *et al.* (2005) como claves para la discriminación entre ambas especies.

Peromyscus is one of the most representative genera of rodents in North America (Bedford & Hoekstra, 2015) displaying a wide range of morphological, physiological, and behavioral variation (Kirkland & Layne, 1989). Nevertheless, for some species, overlapping of morphological characters, color pattern, and shared distribution areas may make it difficult to accurately identify them in the field and scientific collections (Ramírez-Pulido *et al.*, 2001; González-Ruíz *et al.*, 2005). This is the case of the Southern rock deer mouse *Peromyscus difficilis* (J. A. Allen, 1891), widely distributed from Chihuahua to Oaxaca (Fernández *et al.*, 2010), and the Perote mouse *Peromyscus bullatus* (Osgood, 1904), endemic to the Oriental basin in Veracruz and Puebla (González-Ruíz & Álvarez-Castañeda, 2005). *Peromyscus difficilis* is an abundant species found mainly in rocky volcanic areas with xerophytes and in forests with pinyon pines, junipers, and oaks. On the other hand, populations of *P. bullatus* are small, isolated, and restricted to flat sandy arid areas with grasses and scattered trees (Galindo-Leal & Krebs, 2004; González-Ruíz *et al.*, 2005). According to Mexican legislation, the Perote mouse is classified under special protection and is considered critically endangered by the IUCN (Álvarez-Castañeda, 2018; SEMARNAT, 2018). To contribute to the correct discrimination of *P. bullatus* and *P. difficilis*, our objective was to evaluate the usefulness of using tympanic bulla morphometric measures, recommended by Osgood (1904) and Hoffmeister (1951), and corroborated by González-Ruíz *et al.* (2005) as key characters for the accurate discrimination between these species. For this purpose, we search for *P. bullatus* specimens erroneously identified as *P. difficilis* in the mammal collection of the Instituto de Investigaciones Biológicas of the Universidad Veracruzana (IIB – UV). Since misassignment of *P. bullatus* specimens as *P. difficilis* is frequent (González-Ruíz *et al.*, 2005), we expected to find at least one miscataloged *P. bullatus* specimen, achieving hence an accurate discernment between studied species with the proposed tympanic bulla measures.

We obtained morphometric data from 109 adult specimens (according to Hoffmeister, 1951) cataloged as *P. difficilis* in the IIB-UV mammal collection (SEMARNAT VER-MAM-191-10-06); 52 females and 57 males. All specimens were from the region of San Antonio Limón (Totalco), currently a Protected Natural Area (Gobierno del Estado de Veracruz, 2016), in central Veracruz, Mexico. From each specimen's tag, we obtained sex and body measurements: total length – TL, tail length – CL, hindfoot length – HL, ear length – EL. We measured seven dimensions from the right side of craniums: occipito-nasal length (OL), braincase breadth (BC), postorbital constriction (PC), nasal length (NL), maxillary tooth row length (LX), bulla length (BL), and bulla breadth (BW) after Santos-Moreno *et al.* (1998) and González-Ruíz *et al.* (2005). All measurements were taken using an electronic caliper (Mitutoyo series 500; 0.01 mm accuracy) by the same person (IF). We calculated the hindfoot to ear length ratio (HL-EL) and the occipito-nasal length to bulla length ratio (OL-BL; González-Ruíz *et al.*, 2005).

To find specimens which were different within the *P. difficilis* group, possibly *P. bullatus* whose identity (i.e. catalog number) we did not know, we generated a hierarchical cluster dendrogram (Euclidian distance, average linkage; Kettenring, 2006) in R (R Core Team, 2018) for the joint sexes, since sexual dimorphism is absent in both species (González-Ruíz & Álvarez-Castañeda, 2005; Fernández *et al.*, 2010). For the dendrogram, we used the BL, BW, OL-BL and LX measurements; reported by González-Ruíz *et al.* (2005) as key characters to differentiate *P. bullatus* from *P. difficilis*. We used hierarchical cluster analysis because it is based on groups' similarities/dissimilarities and is not necessary to know a priori the number of groups nor the identity of their elements (Kettenring, 2006).

According to the cluster dendrogram, four specimens were different from the remaining sample (IIB-UV catalog number: 1052, 1053, 1673, 1695) at a linkage distance > 1.5 (Fig. 1). After a thorough inspection, we identified these four specimens as *P. bullatus* since they showed, on average, smaller somatic (TL, CL, HL) and cranial (OL, NL, LX, BC, PC) values and larger ear (EL) and bulla measurements (BL, BW, OL-BL ratio) than *P. difficilis* (Table 1). These specimens were corroborated as *P. bullatus* by González-Ruíz from the Universidad Autónoma Metropolitana and were re-cataloged in the IIB-UV mammal collection. The four specimens identified as *P. bullatus* constituted the first ones for the IIB-UV mammal collection and were added up to those already deposited in other collections.

For both *P. bullatus* and *P. difficilis* our measurements were lower than those recorded by González-Ruíz *et al*. (2005), likely due to variation of the studied populations or to inter-measurer variation (Santos-Moreno *et al*., 1998). Regardless of these differences, the data and description of species provided by González-Ruíz *et al*. (2005) greatly helped us to discriminate specimens' species. While the BL, BW, and LX measurements were valuable for discriminating specimens through hierarchical cluster analysis, we found that the OL-BL ratio was the most important measurement for species identification. In *P. bullatus* specimens OL-BL ratio exceeded the average value for *P. difficilis*, corroborating the findings reported by González-Ruíz *et al*. (2005). Furthermore, we did not find overlapping average ± SD values for the OL-BL ratio between species. While average ± SD values for *P. bullatus* cranial measurements should be interpreted cautiously due to our $n = 4$, the showed tendency by morphometric data for this species was clear and agreed with the findings of González-Ruíz *et al*. (2005). However, contrary to descriptions by Osgood (1909) and conclusions by González-Ruíz *et al*. (2005), the HL-EL ratio was not useful to us since all the studied specimens presented values within the same range, possibly due to HL values were taken by several measurers.
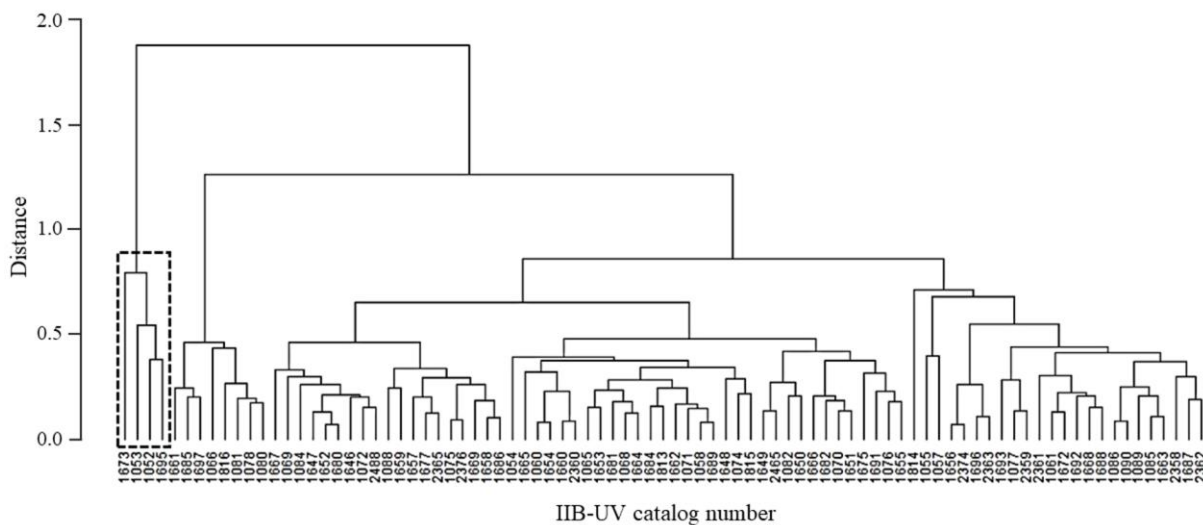

Figure 1. Cluster dendrogram for the joint sexes. Dashed box indicates the specimens different on average from the rest of the group, which was re-cataloged as *P. bullatus* in the IIB-UV mammal collection.

Table 1. Descriptive statistics of measurements (mm) for retrieved *Peromyscus bullatus* and *Peromyscus difficilis*. *n*: number of measured specimens, SD: standard deviation. Refer to text for the use of measures acronyms.

Variable	*Peromyscus bullatus*		*Peromyscus difficilis*	
	n	**Average ± SD**	*n*	**Average ±SD**
TL	4	190.00 ± 26.26	105	213.69 ± 16.37
CL	4	93.00 ± 24.45	105	111.83 ± 11.23
HL	4	23.75 ± 0.50	105	24.22 ± 1.76
EL	4	25.75 ± 0.96	105	25.40 ± 1.65
OL	4	27.80 ± 0.69	85	29.58 ± 0.82
NL	4	10.31 ± 0.38	85	11.37 ± 0.45
LX	4	4.30 ± 0.08	105	4.67 ± 0.15
BC	4	13.03 ± 0.09	103	13.58 ± 0.29
PC	4	4.33 ± 0.09	105	4.53 ± 0.16
BL	4	6.01 ± 0.21	102	5.86 ± 0.20
BW	4	4.87 ± 0.03	102	4.76 ± 0.16
OL–BL	4	21.60 ± 0.43	83	19.81 ± 0.53
HL–EL	4	1.08 ± 0.06	105	1.06 ± 0.14

The contribution by González-Ruíz *et al*. (2005), together with cluster analysis, were valuable for discriminating species when craniums were studied, nevertheless, for identification of *P. bullatus* in the field, besides total length and coloration (slightly smaller and lighter than *P. difficilis*), the habitat type where the specimens are captured is a valuable criterion due to the specific requirements of the species, (Hoffmeister, 1951; González-Ruíz *et al*., 2005). Since only 3.67% of the studied specimens turned out to be *P. bullatus*, whose rarity and small populations in the field are corroborated, our findings support the conservation efforts for this species, which has been left with a reported 6.3% of remnant habitat (Sánchez-Cordero *et al*., 2005).

Since the scientific collections are a valuable bank of information, an accurate assignment of species to specimens is fundamental. Especially for underrepresented species, which in turn are often endemic or poorly studied ones. Additionally, erroneous identification can be detrimental for systematic, taxonomic, morphometric, and conservation studies of species. Even more, due to information deposited in collections has a greater scope and availability through digital platforms. Because of this, a routine review of the specimens to update or correct the information has been widely recommended (Lorenzo *et al*., 2012). Given our findings and the importance of having accurately designated specimens, we support a further search for *P. bullatus* among the *P. difficilis* specimens in collections, when they have been collected from the Oriental basin region in Mexico. The use of the bulla variables, particularly the OL-BL ratio, is useful for performing such task.

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