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Body and skull morphometric variations between two shovel-headed species of Amphisbaenia (Reptilia: Squamata) with morphofunctional inferences on burrowing

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Background. Morphological descriptions comparing *Leposternon microcephalum* and *L. scutigerum* are known. However, these taxa lack of a formal quantitative morphological characterization and comparison between their morphometric patterns. Studies suggest that morphology and burrowing performance seem to be related. For example, the robustness of the head in *L. microcephalum* is positively associated to the digging force. The excavatory movements of this species were described in detail. However, there is a lack of studies comparing locomotor patterns and/or performance among different amphisbaenids sharing the same skull pattern. Likewise, morphofunctional interpretations of the morphological variations are scarce. This paper presents the first study of comparative morphometric variations between two adaptively close amphisbaenid species,*L. microcephalum* and *L. scutigerum,* with functional inferences of fossorial locomotion efficiency.

Methods. Inter-specific morphometric variations were verified through statistical analyses of body and cranial measures of *L. microcephalum* and *L. scutigerum* specimens. Their burrowing activity was assessed through X-ray videoflouroscopy and then compared. The influence of morphological variations on the speed of the digging performance was tested among *Leposternon* individuals.

Results. *Leposternon microcephalum* and *L. scutigerum* are morphometrically distinct species. The first is shorter and robust with a wider head while the other is more elongated and slim with a narrower head. They seem to share the same excavatory movements. However, *L. scutigerum* presented higher averages for speed, travel distance and frequency of excavatory cycles, and lower values for cycle duration in relation to that found for *L. microcephalum*. The animals analyzed reached relatively high speeds, but individuals with narrower skulls dig faster. A negative correlation between the speed and the width of skull was determined, but not with total length or diameter of the body.

Discussion. The morphometric differences between *L. microcephalum* and *L. scutigerum* are in accord with morphological variations previously described. Since these species performed the same excavation pattern, we may infer that adaptively close amphisbaenids with the same skull type would exhibit the same excavatory pattern. The locomotor performance values suggested that *L. scutigerum* is a faster digger than *L. microcephalum*. Such differences are supported by morphometric and morphological features. The negatively correlation between head width and excavation speed is also observed in others fossorial squamates. The robustness of the skull is also related to compression force in *L. microcephalum*. Individuals with wider heads are stronger. Thus, we suggested trade-offs between excavation speed and

compression force during burrowing activity for this species.

Body and skull morphometric variations between two shovel-headed species of Amphisbaenia (Reptilia: Squamata) with morphofunctional inferences on burrowing.

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ABSTRACT

Background. Morphological descriptions comparing *Leposternon microcephalum* and *L. scutigerum* are known. However, these taxa lack of a formal quantitative morphological characterization and comparison between their morphometric patterns. Studies suggest that morphology and burrowing performance seem to be related. For example, the robustness of the head in *L. microcephalum* is positively associated to the digging force. The excavatory movements of this species were described in detail. However, there is a lack of studies comparing locomotor patterns and/or performance among different amphisbaenids sharing the same skull pattern. Likewise, morphofunctional interpretations of the morphological variations are scarce. This paper presents the first study of comparative morphometric variations between two adaptively close amphisbaenid species, *L. microcephalum* and *L. scutigerum,* with functional inferences of fossorial locomotion efficiency.

Methods. Inter-specific morphometric variations were verified through statistical analyses of body and cranial measures of *L. microcephalum* and *L. scutigerum* specimens. Their burrowing activity was assessed through X-ray videoflouroscopy and then compared. The influence of morphological variations on the speed of the digging performance was tested among *Leposternon* individuals.

Results. *Leposternon microcephalum* and *L. scutigerum* are morphometrically distinct species. The first is shorter and robust with a wider head while the other is more elongated and slim with a narrower head. They seem to share the same excavatory movements. However, *L. scutigerum* presented higher averages for speed, travel distance and frequency of excavatory cycles, and lower values for cycle duration in relation to that found for *L. microcephalum*. The animals analyzed reached relatively high speeds, but individuals with narrower skulls dig faster. A negative correlation between the speed and the width of skull was determined, but not with total length or diameter of the body.

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Keywords: Amphisbaenidae, fossorial locomotion, *Leposternon* species, locomotor performance, morphological variation

INTRODUCTION

Amphisbaenians are fossorial reptiles with an elongated and cylindrical body shape (Gans, 1969) and, except the three *Bipes* species, all of them (nearly 200 species, Uetz et al., 2016) are limbless. They are a monophyletic group within Squamata, sub-order Amphisbaenia (Gans, 1969; Kearney, 2003; Kearney & Stuart, 2004).

A strongly ossified and compacted skull is conspicuous in all amphisbaenids. However, their heads may show four different morphological patterns: spade-head, keel-head, round-head, and shovel-head (Kearney, 2003). The "shovel" type, shared by the genera *Rhineura* (Rhineuridae, North America), *Dalophia* and *Monopeltis* (Amphisbaenidae, Africa), and *Leposternon* (Amphisbaenidae, South America), is considered the most specialized for digging (Gans, 1974, 2005; Kearney, 2003; Hohl et al, 2014). This means that these animals are able to penetrate more easily into highly compacted soils reaching greater depths.

Recently, the genus *Leposternon* was synonymized as *Amphisbaena* (see Mott & Vietes, 2009). However, Ribeiro et al. (2011) presented relevant reasons for not adopting this arrangement, including the fact that Mott and Vietes (2009) based their argument only on molecular data, not considering the relevance of morphological traits of major phylogenetic (Ribeiro et al., 2011).

Currently, there are ten recognized *Leposternon* species (Ribeiro et al., 2011; Ribeiro et al., 2015). The species *Leposternon microcephalum* has a widespread distribution, occurring in different regions of Brazil, as well as in Bolivia, Paraguay, Argentina, and Uruguay (Perez & Ribeiro, 2008; Ribeiro et al., 2011). The widespread distribution of *L. microcephalum* throughout South America is associated with recognized geographic morphological variations (Barros-Filho, 2000; Gans & Montero, 2008), including Paraguayan individuals that were suggested as being a different species by Barros-Filho (2000). In the State of Rio de Janeiro, Brazil, *L. microcephalum* may occupy different soil types such as cambi-, lato-, plano-, and organicsoils, normally with herbaceous and shrub vegetation, poor drainage, B textural, and high depth (Gonçalves-Dias & Barros-Filho, 1992). On the other hand, *L. scutigerum* has a more restricted distribution, endemic to the State of Rio de Janeiro, Brazil (Barros-Filho, 1994; Rocha et al., 2009), and occupies yellow podzolic soil with sparse herbaceous vegetation, good drainage, absent B textural, and relatively shallow depth (Gonçalves-Dias & Barros-Filho, 1992). Also, it is included as Endangered (status EN) in IUCN's Red List of Threatened Species (Colli et al., 2016), and in Brazil's National Red List (see [Portaria MMA nº 444, 17 December, 2014]).

Morphological differences between individuals of *L. microcephalum* and *L. scutigerum* from the State of Rio de Janeiro are evidenced by the number of annuli and vertebrae, snout-vent length, cephalic and pectoral shields configuration (Gans, 1971), and skull descriptive morphology (Barros-Filho, 2000; Montero & Gans, 2008). For example, according to Gans (1971), *L. microcephalum* has about 203 annuli; 122-494 mm in snout-vent length; the dorsal head is divided in five rows of shields (Fig. 1A); and the pectoral region is composed of an elongated pair of segments on the midline, and additional pairs elongated or enlarged, may slant toward these median segments (Fig. 1B), whereas *L. scutigerum* has about 269 annuli; 292-457 mm in snout-vent length; the dorsal head is covered by three rows of scales, with a large heavily keratinized shield (azygous) (Fig. 1C), and the pectoral region composed of three pairs of greatly enlarged segments in an hourglass-shaped pattern (Fig. 1D) (Gans, 1971).

Likewise, there are evident differences on the osteological features of *L. microcephalum* and *L. scutigerum*. Vertebrae numbers are 93-103 for *L. microcephalum* and 119-123 for *L. scutigerum* (Gans, 1971). Barros-Filho (2000) described the general anatomy of the skulls of these species including features that we consider as more correlated to the excavation process. The skull of *L. microcephalum* can be distinguished from *L. scutigerum* by the lozenge-shaped outline of the anterior view of the facial region; angulations between facial and medial skull regions tending to less than 120º (mean value = 118.8º); the largest width of the facial region is conspicuously shorter than the width of the occipital region; the prefrontal bones don't have any conspicuous lateral expansions; facial region is not specially expanded laterally at the transversal crest line (between facial and medial regions) (Barros-Filho, 2000). On the other hand, in *L. scutigerum*, the anterior view of the facial region has a triangular-shaped outline; the average angulation between facial and medial skull regions is 121.6º; the largest width of the facial region is very close to the width of the occipital region; the prefrontal bones present characteristic lateral expansions; and there is a characteristic lateral expansion at the transversal crest line (Barros-Filho, 2000).

Despite the good qualitative description of morphological variations present in the literature, there are no body and/or skull morphometric differences supported by statistical approaches for these species. Furthermore, morphofunctional consequences of the morphological variations in *L. microcephalum* and *L. scutigerum* is scarce.

Fossorial animals have to be able to travel along an existing tunnel, burrowing or extending existing tunnel systems, and leave the surface by penetrating the soil (Gans, 1978). The high degree of specialization of the amphisbaenians is well expressed in the speed and effectiveness with which they burrow tunnel systems, and penetrate with all their body in the substrate, even in fairly hard soils (Gans, 1978).

The excavation patterns of the Amphisbaenia range from random movements of the head and lateral movements to "shovel" and "screw" movements, which vary according to the four head types previously cited (Gans, 1974). Shovel-headed amphisbaenians excavate through "shovel" movements of the head, which is considered the more specialized digging pattern (Gans, 1974). In fact, morphological features and underground locomotor performance seem to be related. For instance, body and head size and strength directly affect the ability of fossorial squamates to penetrate the substrate and move inside their galleries (Gans, 1974; Navas et al., 2004). Navas et al. (2004), without any statistical approach, postulated that *L. microcephalum* individuals with narrower heads dig faster than those with a wider head or more robust body. Gans (1978) mentioned that the osteological reinforcement of the transversal crest, together with the discreet lateral highlight of the otic capsules is important to improve the excavation efficiency. Furthermore, Vanzolini (1951) and Barros-Filho (1994) associated the fusion of cephalic and pectoral shields with the reduction of friction with the substrate, facilitating the underground displacement of the animal.

In the last 60 years, the locomotor tunneling behavior of *L. microcephalum* has been incidentally studied through visual observations (Kaiser, 1955), motion pictures of the external body (Navas et al., 2004), and the videofluoroscopy technique (Barros-Filho et al., 2008; Hohl et al., 2014). The more recent description of the excavatory cycle of *L. microcephalum* is presented as: (1) initial static position with the gular and anterior body regions lying over the tunnel floor; (2) retreating and downward bending of the head, with tip of snout touching the floor substrate; (3) a continuous upward and forward head movement, which compacts the substrate granules against the tunnel roof, while the pectoral region compresses the tunnel floor. This is followed by the dropping of the head, returning to the initial static position (Barros-Filho et al., 2008; Hohl et al., 2014).

Nevertheless, all those locomotor behavioral studies are limited to the use of small samples of live animals. Some authors such as Navas et al. (2004) and Navega-Gonçalves (2004) stressed the difficulty to obtain these animals for studies. It is more difficult for a species such as *L. scutigerum*, which has a small distribution and is listed as endangered.

Not only are there few studies of the underground locomotion of *L. microcephalum,* but all of them faced the problem of limited samples (Kaiser, 1955; Navas et al., 2004; Barros-Filho et al., 2008; Hohl et al., 2014). In fact, there are no studies of other species of *Leposternon*. Furthermore, there is a lack of studies comparing locomotor patterns and/or performance among different amphisbaenid species, neglecting the correlations of the functionality of different skull shapes. Similarly, the review of literature shows that quantitative studies inferring the influence of morphological variations on the fossorial locomotor performance of amphisbaenids are scarce (e. g. López et al., 1997; Navas et al., 2004).

Given that there are notable morphological variations between *L. microcephalum* and *L. scutigerum*, we hypothesized that body and skull morphometric variations are also evident. Therefore, such variations could be influencing dissimilarities in locomotor performance. Considering that *L. microcephalum* and *L. scutigerum* share the same general shovel-headed pattern, they tend to have similar excavatory movements. Furthermore, based on the observations of Navas et al. (2004) that individuals of *L. microcephalum* with narrower heads exert less force but dig faster than those with a wider head or more robust body, we believe that *Leposternon* individuals with narrower skulls and bodies, in fact, dig faster.

The main objective of the present study was to characterize the morphometric variations in body and skull between *L. microcephalum* and *L. scutigerum*, providing morphofunctional inferences on burrowing. Thus, we also described the excavatory pattern and performance of *L. scutigerum*, comparing its locomotor traits with *L. microcephalum*; and we verified the relation between the length and robustness of the body and skull with the burrowing speed among *Leposternon* individuals.

MATERIAL AND METHODS

Morphometric analysis

A total of 26 adult *Leposternon* specimens were measured (*L. microcephalum* Wagler, 1824 *n* = 14; *L. scutigerum* (Hemprich, 1820) *n* = 12). The sample included eight live individuals used in the locomotion analyses and 18 specimens from scientific collections deposited at National Museum and Zoology Department of Federal University of Rio de Janeiro (UFRJ). The specimens from scientific collections were prepared, after we took the body measures, through exposure to *Dermestes* sp (Coleoptera, Insecta) larvae or maceration in hydrogen peroxide (H_2O_2)

(see Barros-Filho, 2000). Individuals with more than 150 mm are considered adults (Gans, 1971), and no sexual dimorphism was found for the variables analyzed. *Leposternon microcephalum* has some morphological variations along its geographic distribution in South America. Our analysis included only specimens from Rio de Janeiro, Brazil, therefore, we expected that minor differences could exist considering particular ecotypes of this species. Identification of all specimens can be found in Table 1.

Body measurements were taken using measuring tape (in mm scale) whereas skull measurements used a digital caliper (model Pro-Max, Fowler-NSK scale 0.01mm). The skull measurements were: condilo-basal length (CBL); maximum width (MW), situated at the occipital region covering the optical capsules; the transversal crest width (TCW); and maximum width of the rostral region (MWR) (Fig. 2). The body measurements included: the total body length (TL) and diameter (D) in the middle of the body. Cranial structures terminology followed Gans & Montero (2008). The skull measurements of the live individuals were taken using X-ray records through the software Tracker v. 4.96. The distance between lead pieces in the wall of the terrarium was used as scale (5 cm). For this sample, it was not possible to measure the TCW and MWR.

The frequency distributions of the morphometric variables were tested for normality (Shapiro-Wilk's T) and homoscedasticity (Levene), as well as, skewness and kurtosis. Nonparametric Wilcoxon-Mann-Whitney *U*-Test was used to verify intra- and inter-specific morphometric variations among *L. scutigerum* and *L. microcephalum* specimens by comparing body and skull measurements. Comparisons between groups were performed through Principal Component Analysis (PCA) as a data exploratory method, over the standardized values of the original variables considered (TL, D, CBL and MW), to verify their trends of variation and the group distribution in their multivariate morphological space. Statistical analyses were performed with Statistica v.8.0 (Statsoft, 2008).

Locomotion analysis

Eight adult individuals of the genus *Leposternon* from Rio de Janeiro City were analyzed (*L. microcephalum n* = 7; *L. scutigerum n* = 1). *Leposternon scutigerum* is an elusive species, even considering amphisbaenids as a whole. The difficulty in obtaining live individuals of amphisbaena species in the field is well known and reported in the literature (see Navas et al., 2004; Navega-Gonçalves, 2004; Barros-Filho et al., 2008). The individuals of *L. microcephalum* were collected, recorded and analyzed by Hohl et al. (2014). Field collections were aproved by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), environmental agency of the Brazilian government, with a permanent license number for collecting zoological material (15337), since May 28th, 2008. This locomotor behavior study was approved by the Comissão de Ética para o Cuidado e Uso de Animais Experimentais and Instituto de Biologia Roberto Alcantara Gomes (CEUA/IBRAG/015/2017). The individuals are identified by number of specimen/year of recording in Table 1.

They were recorded using the videofluoroscopy technique to study the locomotor behavior. This technique, based on X-ray recording, has been used in behavioral studies of fossorial species of amphisbaenians (Barros-Filho et al., 2008; Hohl et al., 2014) and caecilians (Summers and O'Reilly, 1997; Measey and Herrel, 2006, Herrel and Measey 2010, 2012).

Before recording, *L. scutigerum* was maintained in the laboratory environment at Vertebrate Zoology Laboratory -Tetrapoda (LAZOVERTE), University of the State of Rio de Janeiro (UERJ) under the same conditions presented by Hohl et al. (2014), i. e., kept in a plastic box containing humid humus-rich soil, and feed with earthworms once a week. The X-rays were performed at University Hospital Pedro Ernesto (HUPE/UERJ), Rio de Janeiro, with a Toshiba videofluoroscopy machine (Toshiba Corporation) that films at 30 frames per second with calibration of 200 mA and 40 kV. In addition, following the same procedures used by Hohl et al. (2014) to film individuals of *L. microcephalum*, *L. scutigerum* was kept in a glass terrarium filled with dry/loose semolina, and with lead markers placed on the outer face of the wall terrarium. More details about the recording procedure can be found in Hohl et al. (2014). After filming, the animal was released back into the wild.

A total recording time of 7 min and 40 s containing 32 motion sequences of *L. scutigerum* was analyzed frame by frame using the Toshiba Daicom Viewer software. The excavatory pattern was analyzed taking into account the details of its body postures and movements performed. The locomotor performance was evaluated through the distance covered, duration of the movement, speed and frequency of cycles performed, according to Hohl et al. (2014).

Despite all *Leposternon* individuals sharing the same skull type, "shovel", the sample contained two species. Thus, the excavatory pattern of *L. scutigerum* (n = 37 cycles) was determined and compared inter-specifically with the three-step gait pattern exhibited by *L. microcephalum* (n = 132 cycles), according to Barros-Filho et al. (2008) and Hohl et al. (2014).

The degree of tunneling specialization is expressed by the speed (Gans, 1978). Thus, after checked the proper normality and homogeneity of variance assumptions, the nonparametric Kruskal-Wallis Test was used to verify, individual-by-individual, variations in excavatory cycle speeds among the eight *Leposternon* individuals: *L. microcephalum* (1/2012 n = 7 cycles; 3/2012 n = 10; 4/2012 n = 12; 5/2012 n = 14; 8/2012 n = 9; 9/2012 n = 13; 10/2012 n = 18); and *L. scutigerum* (1/2017 n = 35). Excavatory cycles considered outliers were removed. Statistical analyses were performed with Statistica v.8.0 (Statsoft, 2008).

The small sample of individuals hindered more comprehensive inter-specific statistical comparisons. Therefore, we evaluated the degree of variation observed in the footages among the individuals recorded as a starting point to estimate possible differences between locomotor performances of *L. scutigerum* and *L. microcephalum*. The three fastest excavatory cycles (i.e. in which the animals reached highest speeds) performed by each individual (n = 7 *L. microcephalum* and n = 21 excavatory cycles; n = 1 *L. scutigerum* and n = 3 excavatory cycles) were quantified according to the locomotory parameters proposed, and then they were tabulated and statistically described. We obtained the Coefficient of Variation (CV) of species' locomotory parameters in order to estimate the homogeneity of the locomotor patterns among the footages of them. Higher coefficients of variation would reflect marked intra-specific differences in the excavatory pattern. Low coefficients of variation would indicate more constant performances of the individuals along the intra-specific footages. Descriptive statistical values were obtained with Statistica v.8.0 (Statsoft, 2008).

Test of the relation between morphometric variables and burrowing speed

The influence of morphological variations on locomotor performance was assessed through Simple Linear Regressions. The speed that expressed the degree of tunneling specialization (Gans, 1978) was used as the dependent variable to be regressed against body and skull measurements considered important for excavation ability according to Gans (1974) and Navas et al. (2004): total body length and diameter, and maximum width of skull (independent variables). The analyses were performed with Statistica v.8.0 (Statsoft, 2008) first adding only *L. microcephalum* individuals, and then inserting the one individual of *L. scutigerum*.

RESULTS

Morphological variations

Inter-specific variations between *L. microcephalum* and *L. scutigerum* were observed on total length (TL) ($U = 33.5$, $p < 0.01$), diameter of the body (D) ($U = 17.5$, $p < 0.001$), condilo-basal length (CBL) ($U = 34.5$, $p < 0.01$), and maximum width of the skull (MW) ($U = 27.0$, $p < 0.01$). However, there were no statistical differences in the transversal crest width (TCW) ($U = 25.5$, $p > 0.05$) or maximum width of the rostral region of the skull (MWR) ($U = 30.0$, $p > 0.05$). Statistics in Table 2.

The PCA analysis indicated two main axes of group differentiation: PC1 and PC2 explained, respectively, 60.63% and 25.03% of variation, totaling 85.66%. According to the PCA scatterplot (Fig. 3), *L. scutigerum* specimens occupied higher positive scores on PC1 and negative scores on PC2, tending to exhibit a greater elongated and slim bodyshape with a narrower head. On the other hand, *L. microcephalum* specimens occupied greater negative scores on PC1 and positive scores on PC2, featuring a shorter and robust bodyshape with a wider head.

Locomotor variations

A detailed analysis of the movement, through visual observation of X-ray images, revealed that the three-step excavatory cycle performed in horizontal and descendant directions by *L. scutigerum* seemed to be the same as *L. microcephalum*. Barros-Filho (2008) and Hohl et al. (2014) described this movement as: (1) initial static position with the gular and anterior body regions lying over the tunnel floor; (2) retreating and downward bending of the head, with tip of snout touching the floor substrate; (3) a continuous upward and forward head movement, which compacts the substrate granules against the tunnel roof, while the pectoral region compresses the tunnel floor. This is followed by the dropping of the head, returning to the initial static position.

According to the locomotor performance data, *L. scutigerum* constructed galleries with an average speed of 0.465 cm s^{-1} (± 0.2), travelling 0.58 cm (± 0.15) in 1.26 s (± 0.43) seconds, and performed almost a complete excavatory cycle each second (0.8 ± 0.3 Hz). Based on the three fastest excavatory cycles of *L. scutigerum* showed that it constructs galleries with an average speed of 0.757 cm s^{-1} (± 0.06), travelling 0.73 cm (± 0.11) in 0.83 s (± 0.23) seconds, and performed a complete excavatory cycle each second (1.06 ± 0.17 Hz). During the three fastest excavatory cycles, *L. microcephalum* was able to reach an average speed of 0.350 cm s^{-1} (± 0.09), travelling 0.59 cm (± 0.18) in 1.77 s (± 0.59), and performed about a half complete excavatory cycle each second (0.61 ± 0.15 Hz).

The mean values and SDs of the locomotor performance variables suggest that some inter-specific differences may exist between the species. *Leposternon scutigerum* presented higher averages for speed, travel distance and frequency of excavatory cycles, and lower values for cycle duration in relation to those found for *L. microcephalum*. However, the lack of a substantial sample of individuals prevent the use of a significance test. On the other hand, the values of the coefficients of variation of the locomotor variables indicated some constancy in the species' performances along the intra-specific footages. Therefore, despite the sample size limitation, based on this functional approach to the excavatory performance of the species, we hypothesized that *L. scutigerum* could be a better excavator than *L. microcephalum,* in the sense of speed of soil penetration and gallery construction. Descriptive statistics values are presented in Table 3.

The Kruskal-Wallis Test showed statistical differences on the speeds of excavatory cycles among individuals of *Leposternon* genus (H = 46.64; $p < 0.0001$). The variations were observed between the *L. microcephalum* individuals 3 and 9/2012 ($p = 0.027$), 3 and 10/2012 ($p = 0.046$), 5 and 9/2012 ($p = 0.005$), and 5 and 10/2012 ($p = 0.028$). Likewise, the speeds of almost all *L. microcephalum* individuals were statistically different from the unique *L. scutigerum* 1/2017 ($p <$ 0.01 to $p < 0.0001$), except the individual 5/2012 ($p = 0.07$). The individuals 3 (0.304 ± 0.123 cm s^{-1}) and 5/2012 (0.362 ± 0.187 cm s^{-1}) were faster than individuals 9 (0.179 ± 0.1 cm s^{-1}) and 10/2012 (0.218 ± 0.09 cm s^{-1}). The individual 1/2017 also presented the highest mean value of speed (0.456 ± 0.145 cm s^{-1}) in relation to all other individuals (Fig.4).

Test of the relation between morphometric variables and burrowing speed

When the analysis was restricted to *L. microcephalum* individuals, Simple Linear Regression demonstrated negative correlation between the speed (dependent variable) and the width of skull (independent variable) ($r^2 = 0.613$; $p = 0.037$) (Fig.5A), but not with total length ($r^2 = 0.001$; $p = 0.944$) or diameter of the body ($r^2 = 0.08$; $p = 0.54$). When inserting the unique specimen of *L. scutigerum,* the analysis revealed a more strong and significant negative correlation between speed and width of skull ($r^2 = 0.798$; $p = 0.003$) (Fig.5B), and also not with total length ($r^2 = 0.30$; $p = 0.153$) or diameter of the body ($r^2 = 0.42$; $p = 0.079$).

DISCUSSION

Inter-specific morphometric variations

Anatomical differences between *L. microcephalum* and *L. scutigerum* from Rio de Janeiro State are well known (Gans, 1971; Barros-Filho, 2000; Gans & Montero, 2008). The initial hypothesis that body and skull morphometric exists was corroborated with significant differences between these two species in total length and diameter of the body, and maximum width of skull.

As expected by the great number of vertebrae, *L. microcephalum* with 93-103 vertebrae and *L. scutigerum* with 119-123 (Gans, 1971), *L. scutigerum* presented a more elongated body. According to Barros-Filho (2000), the largest width of the facial region also covering the transversal crest of *L. scutigerum* is very close to its maximum width of the skull at the occipital region. This configuration is different for *L. microcephalum*, in which the largest width of the facial region is shorter than the width of the occipital region. Based on this difference, the author concluded that *L. scutigerum* "has a very characteristic skull, specially the lateral expansion of the facial region, at the transversal crest line". However, our results indicated statistical differences between these species for maximum width of skull (occipital region), but not for transversal crest width or maximum width of the rostral region (facial region). Thus, the allometrical relation between facial and occipital regions of these species proposed by Barros-Filho (2000) is more related to occipital differences instead of facial ones.

Inter-specific comparisons of excavatory behavior

Barros-Filho et al. (2008) discussed the efficiency of videofluoroscopy as a methodology for analyzing locomotor behavior in fossorial squamates. They concluded that among the methodologies previously used (e. g. visual observations and motion pictures of the external body), videofluoroscopy was the most efficient in analyzing excavatory cycles. Considering that the present study also used videofluoroscopy as the methodology to access the fossorial locomotion of *L. scutigerum*, and replicated the movie set used by Barros-Filho et al. (2008) and Hohl et al. (2014) (i. e. the same glass terrarium marked with lead pieces and filled with dry/loose semolina), we could conclude that the comparison results of locomotor pattern and performance between *L. scutigerum* and *L. microcephalum* were not influenced by methodological factors.

In locomotion studies with limited samples (even with a single individual) some authors consider the number of locomotor cycles analyzed. Thus, the descriptions of the locomotor pattern of a single individual may represent the pattern exhibited by its species, making the data relevant to inter-specific comparisons (see Rocha-Barbosa, 1996; Santori et al., 2005; Barros-Filho et al., 2008). Likewise, important studies on Amphisbaenia locomotion were based on a unique individual (e. g. Kaiser, 1955; Gans, 1960; Barros-Filho et al., 2008). Therefore, we believe that the descriptions of the excavatory movements based on a single individual of *L. scutigerum* do not limit the comparisons with *L. microcephalum* species.

Leposternon scutigerum performed, in horizontal and descendant directions, the same three-step excavatory cycle first described by Barros-Filho et al. (2008) for *L. microcephalum*, reinforced by Hohl et al. (2014), as a retreating and downward bending of the head from an initial static position followed by an upward and forward head movement. This result supports part of the initial hypothesis, that adaptively close amphisbaenids with the same skull type would exhibit the same excavatory pattern. The results also supported the assumption of Barros-Filho et al. (2008) that, in spite of specific differences among the shovel-headed amphisbaenid species, the excavatory pattern was the same for all of those species, based on the fact that the shovel-head is, apparently, a convergence among the group (cf. Gans, 1974).

However, despite sharing the same skull type and performing three-step excavatory pattern, our quantitative approach of the locomotor performance of *L. scutigerum* and *L. microcephalum* suggested differences in all parameters analyzed (specimens' traveling distance, and excavatory cycle duration, speed and frequency). According to Hohl et al. (2014), *L. microcephalum* was able to build a gallery of 12 cm in length in 1 min. Our results showed that this species was able to build a gallery of 21.6 cm in length in 1 min (mean values based on the three fastest cycles), whereas *L. scutigerum* is able to build a gallery of 46.4 cm in length in 1 min, i.e. more than twice faster, on average. Despite the lack of a formal statistical significance test, these values suggest that *L. scutigerum* is a faster digger than *L. microcephalum*. The results were adaptively meaningful, since *L. microcephalum* is considered a specialized digger, even among other amphisbaenids (cf. Gans, 1974; Navas et al., 2004; Hohl et al., 2014). We expected that further analyses of more individuals of *L. scutigerum* corroborate to the differences on locomotor performance between these two species.

Morphological features and locomotor performance

Gans (1974) and Navas et al. (2004) postulated that body, head size and strength directly affect the speed of fossorial squamates to penetrate the substrate and move inside their galleries. However, they only reported it qualitatively. Navas et al. (2004), although relating the robustness of the head with the compression force, did not obtain the speed with which the animals excavated to associate with head width. According to our quantitative approach these previous statements seem partially true. Body dimensions (total length or diameter) seem to be not related to the increased speed of excavation. However, regression analysis showed that the width of skull is related to the speed of excavation (individuals with slender skulls seem to be faster). For example, among *L. microcephalum*, the individuals 3 and 5/2012 which have slender skulls (both with 9 mm) in relation to individuals 9 and 10/2012 (13.7 and 10 mm, respectively), were considered statistically faster. The finding, that head width is negatively correlated with excavation speed, is in agreement with previous studies. López et al. (1997) showed that individuals of *Blanus cinereus* with narrower, longer heads burrow faster. Vanhooydonck et al. (2011) showed that in burrowing skinks *Acontias percivali*, individuals with narrow heads were able to dig faster than broader-headed ones. Similarly to our results, Vanhooydonck et al. (2011) also concluded that the speed in soil penetration is only predicted by the head width, curiouslly getting similar statistical values (*L. microcephalum* - $r^2 = 0.613$, $p = 0.037$; *Acontias percivali* - $r^2 = 0.64$, $p = 0.03$).

The robustness of the skull has been demonstrated to be an important characteristic to improve soil penetration. Beyond being associated with excavation speed, as here presented, Navas et al. (2004) also demonstrated that the robustness of the skull is also related to compression force among *L. microcephalum*. Individuals with wider heads produced greater compression forces (about 25 N). Likewise, Vanhooydonck et al. (2011) and Baeckens et al. (2016) showed a strong positive relation between head size and bite force in *A. percivali* and *Trogonophis wiemanni* sepecies, respectively. According to these authors, bite performance also increased with head size in these species.

Besides individuals of the *L. microcephalum* species, our sample contained a unique *L. scutigerum* specimen that presented the narrowest skull (6.5 mm), and, according to our results, it showed the highest speed mean value in relation to the *L. microcephalum* individuals. Comparing inter-specifically, the morphological features of *L. scutigerum* supported the results of the locomotor performance analysis obtained, and the differences in relation to *L. microcephalum*. For instance, according to Vanzolini (1951) and Barros-Filho (1994), the fusion of cephalic shields reduces the friction with the substrate, and this would be an adaptive advantage favoring the positive selection of individuals with this kind of fusion. *Leposternon scutigerum* (here considered a faster digger) has a unique large cephalic shield placed on the top of its head, the azygous (Fig. 1C), that occupies the corresponding place of several other head shields in *L. microcephalum* (cf. Gans, 1971) (Fig. 1A). The fusion pattern is present in the pectoral shields of these two species (as well as in other shovel-headed amphisbaenid species), but with different and apparently specific and specialized designs (Figure 1B and D; note that *L. scutigerum* has a bigger comparative smooth area). In the less specialized amphisbaenian species (e. g. *Amphisbaena*) there is no pectoral shield fusion (cf. Gans, 1974).

Gans (1978) highlighted that the osteological reinforcement of the transversal crest, together with the slight lateral protrusion of the otic capsules is important to improve the excavation efficiency, here corroborated by the negative correlation between the width of skull and speed through regression analysis. According to our morphological results based on a larger sample, the species *L. scutigerum* presented a narrower skull (maximum width situated at the occipital region covering the otic capsules) when compared to *L. microcephalum* which possesses a wider skull. The preliminary results on the locomotor performance exhibited by the unique individual of *L. scutigerum* seemed to demonstrate some superior morphological specialization for high speeds during digging in relation to *L. microcephalum*, as previously pointed out by Barros-Filho (1994). However, future studies with a larger sample of *L. scutigerum* filmed are needed for a better conclusion.

Likewise, future studies on compression force and bite performance are needed for these species. Although *L. microcephalum* exhibited low speed during digging in relation to *L. scutigerum*, according to Navas et al. (2004), Vanhooydonck et al. (2011), and Baeckens et al. (2016), it might be able to exert greater compression and bite forces. Even small variations in head width may present a large impact on burrowing compression force (Navas et al., 2004) and speed (Vanhooydonck et al., 2011). Thus, our present data on speed, associated with the data from Navas et al. (2004) on compression forces suggest a performance trade-off between compression forces and burrowing speeds in the shovel-headed *Leposternon* (i. e. individuals with narrow heads excavate rapidly but exert less push force, while individuals with robust heads excavate slowly but exert more push force). This relation may be associated with the ecology and geographic distribution differences among *L. microcephalum* (larger geographic distribution occupying different soil types) and *L. scutigerum* (limited distribution occupying a restricted soil type).

The present study addressed the question of whether there are relations between body and skull dimensions and excavatory speed among *Leposternon* species, and if there are notable morphometric and locomotor performance differences between adaptively close species (*L. microcephalum* and *L. scutigerum*) with similar skull pattern (shovel). Our results showed that, indeed, the width of skull is related to speed, and a set of morphological adaptations in the head and body (e. g. scale pattern) may influence the greater locomotor performance differences between *L. microcephalum* and *L. scutigerum*. This seems to be valid even among close related species that share skull similarities.

The methodology we used to assess the amphisbaenid excavatory functionality proved to be an important tool that could shed some light on the relation between head types and digging specialization among amphisbaenids. Future analyses considering generic and familiar levels may provide a broad understanding about the evolution of adaptative digging strategies of this group.

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Table 1: Morphometric data of *L. microcephalum* (*n* = 14) and *L. scutigerum* (*n* = 12) specimens. All measures are in millimeters. Legend: Body measures: TL = total length; D = diameter; Skull measures: CBL = condilo-basal length; MW = maximum width; TCW = tranversal crest; MWR = maximum width of the rostral region; Scientific Collections: MNRJ = Museu Nacional da Universidade Federal do Rio de Janeiro; ZUFRJ = Zoologia, Universidade Federal do Rio de Janeiro. The first nine animals were recorded and released back into the wild and are identified by the number of specimen/year of recording.

Specimens	Species	TL	D	CBL	MW	TCW	MWR
1/2012	*L. microcephalum*	360	13.7	20.8	12.5		
3/2012	*L. microcephalum*	333	15	22.3	9		
4/2012	*L. microcephalum*	294	16.2	16.2	12.5		
5/2012	*L. microcephalum*	354	16	18.7	9		
8/2012	*L. microcephalum*	370	17.5	22.3	12.3		
9/2012	*L. microcephalum*	364	20	20.5	13.7		
10/2012	*L. microcephalum*	298	16.2	18.5	10		
1/2017	*L. scutigerum*	428	11.7	14.8	6.5		
ZUFRJ240	*L. microcephalum*	260	13.1	14.3	8.2	5.1	5.5
ZUFRJ249	*L. microcephalum*	430	18.4	16.8	9.6	7	7.3
ZUFRJ285	*L. microcephalum*	463	20.6	18.9	11.1	7.3	8
ZUFRJ467	*L. microcephalum*	466	19.1	19.1	10.7	7.3	8.1
ZUFRJ468	*L. microcephalum*	326	10.5	14.2	7.9	4.9	5.2
ZUFRJ1320	*L. microcephalum*	490	16.4	20	12.7	7.5	8
ZUFRJ1321	*L. microcephalum*	425	17.6	18.8	10.2	7.2	7.7
MNRJ4036	*L. scutigerum*	481	12.4	17.5	9.8	8	8.5
MNRJ4037	*L. scutigerum*	489	15.3	17.9	9.4	7.8	8.1
MNRJ4038	*L. scutigerum*	408	10.4	14.7	7.4	5.9	5.9
MNRJ4458	*L. scutigerum*	490	12.5	17.8	9.7	8.2	8.3
MNRJ4490	*L. scutigerum*	390	10.7	14.1	7.4	5.6	5.6
MNRJ4791	*L. scutigerum*	443	10.7	16.8	8.8	7.3	7.3
ZUFRJ550	*L. scutigerum*	375	10.7	14.5	7.3	5.8	6.3
ZUFRJ1399	*L. scutigerum*	413	11.8	14.8	7.8	6	6.5
ZUFRJ1401	*L. scutigerum*	467	13.7	18	9.4	8.3	8.4
ZUFRJ1417	*L. scutigerum*	462	14.9	17.6	9.7	7.6	8
ZUFRJ1511	*L. scutigerum*	514	12.8	16.7	8.8	7.2	7.8

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Table 2: Comparative analyses of body and skull morphometric data between *L. scutigerum* and *L. microcephalum*. Legend: C. V. = Coefficient of Variation; Lm = *Leposternon microcephalum*; Ls = *Leposternon scutigerum*. Values are presented by means and standard deviation, in millimeters.

	L. microcephalum	*L. scutigerum*	C. V. (%) Lm/Ls	*U*-Test *P* value
TL	371.1 ± 69.1 (*N* = 15)	446.6 ± 44.1 (*N* = 12)	18.6 / 9.9	<0.01 **
D	16.45 ± 2.77 (*N* = 14)	12.31 ± 1.65 (*N* = 12)	16.8 / 13.4	<0.001 **
CBL	18.67 ± 2.56 (*N* = 14)	16.27 ± 1.55 (*N* = 12)	13.7 / 9.5	<0.01 **
MW	10.67 ± 1.84 (*N* = 14)	8.5 ± 1.16 (*N* = 12)	17.2 / 13.6	<0.01 **
TCW	6.61 ± 1.11 (*N* = 7)	7.06 ± 1.04 (*N* = 11)	16.8 / 14.7	>0.05 ns
MWR	7.11 ± 1.24 (*N* = 7)	7.34 ± 1.07 (*N* = 11)	17.4 / 14.6	>0.05 ns

Table 3: Descriptive statistical values of the fastest excavatory cycles performed by *L. scutigerum* (three cycles of one individual) and *L. microcephalum* (21 cycles of seven individuals). Legend: C. V. = Coefficient of Variation; Lm = *Leposternon microcephalum*; Ls = *Leposternon scutigerum*. Values are presented by means and standard deviation.

	L. microcephalum	*L. scutigerum*	C. V. (%) Lm/Ls
Speed (cm s^{-1})	0.350 ± 0.09	0.757 ± 0.06	25.8 / 8
Distance (cm)	0.59 ± 0.18	0.73 ± 0.11	30.6 / 15.4
Time (s)	1.77 ± 0.59	0.83 ± 0.23	33.3 / 28
Frequency (Hz)	0.61 ± 0.15	1.06 ± 0.17	24.2 / 16.6

Legends of Figures:

Figure 1: Photography showing the cephalic and pectoral shields of *Leposternon* species. Legend: (A, B) Cephalic and pectoral shields of *L. microcephalum.* (C, D) Cephalic and pectoral shields of *L. scutigerum*. The azygous shield is indicated by the black arrow.

Figure 2: Photography of the dorsal view of a *Leposternon scutigerum* skull showing the measures taken. Legend: CBL = condilo-basal length; MW = maximum width; TCW = transversal crest width; MWR = maximum width of the rostral region.

Figure 3: Principal Component Analysis scatterplot showing the distribution of *L. microcephalum* and *L. scutigerum* specimens on the multivariate space through the two main axis of variation (PC1 and PC2). Legend: Arrows indicate the direction of the correlation of each variable with the different PCs.

Figure 4: Graphic showing the speed values (means and SDs) of *Leposternon* individuals.

Figure 5: Excavatory cycle' speed as a function of width of skull in *Leposternon* individuals. Legend: (A) Analysis restricted to *L. microcephalum*. (B) Analysis after insertion of *L. scutigerum*, represented by the star.

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Figure 1

Photography showing the cephalic and pectoral shields of *Leposternon* species.

(A, B) Cephalic and pectoral shields of *L. microcephalum.* (C, D) Cephalic and pectoral shields of *L. scutigerum*. The azygous shield is indicated by the black arrow.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

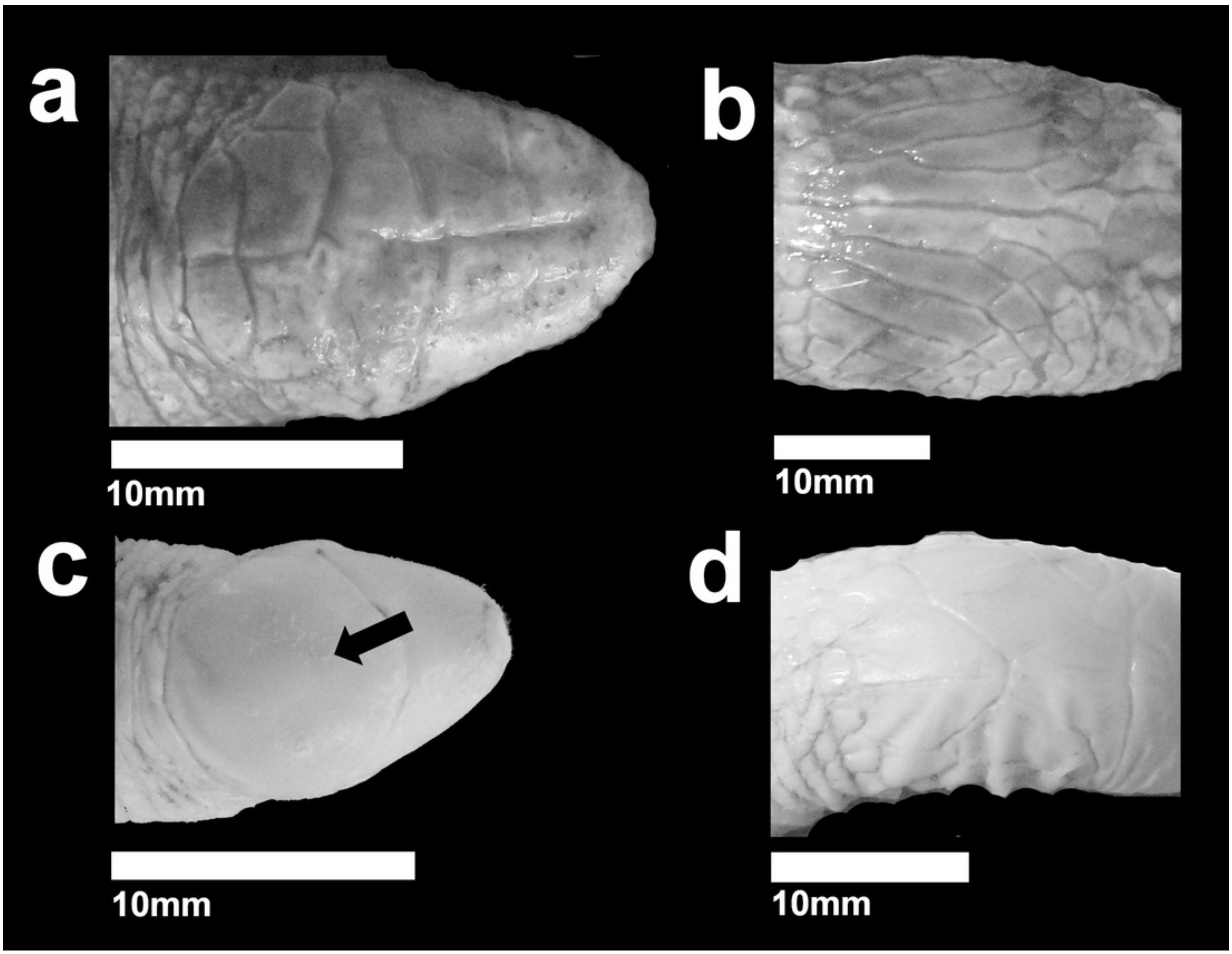

Figure 2

Photography of the dorsal view of a *Leposternon scutigerum* skull showing the measures taken.

CBL = condilo-basal length; MW = maximum width; TCW = transversal crest width; MWR = maximum width of the rostral region.

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

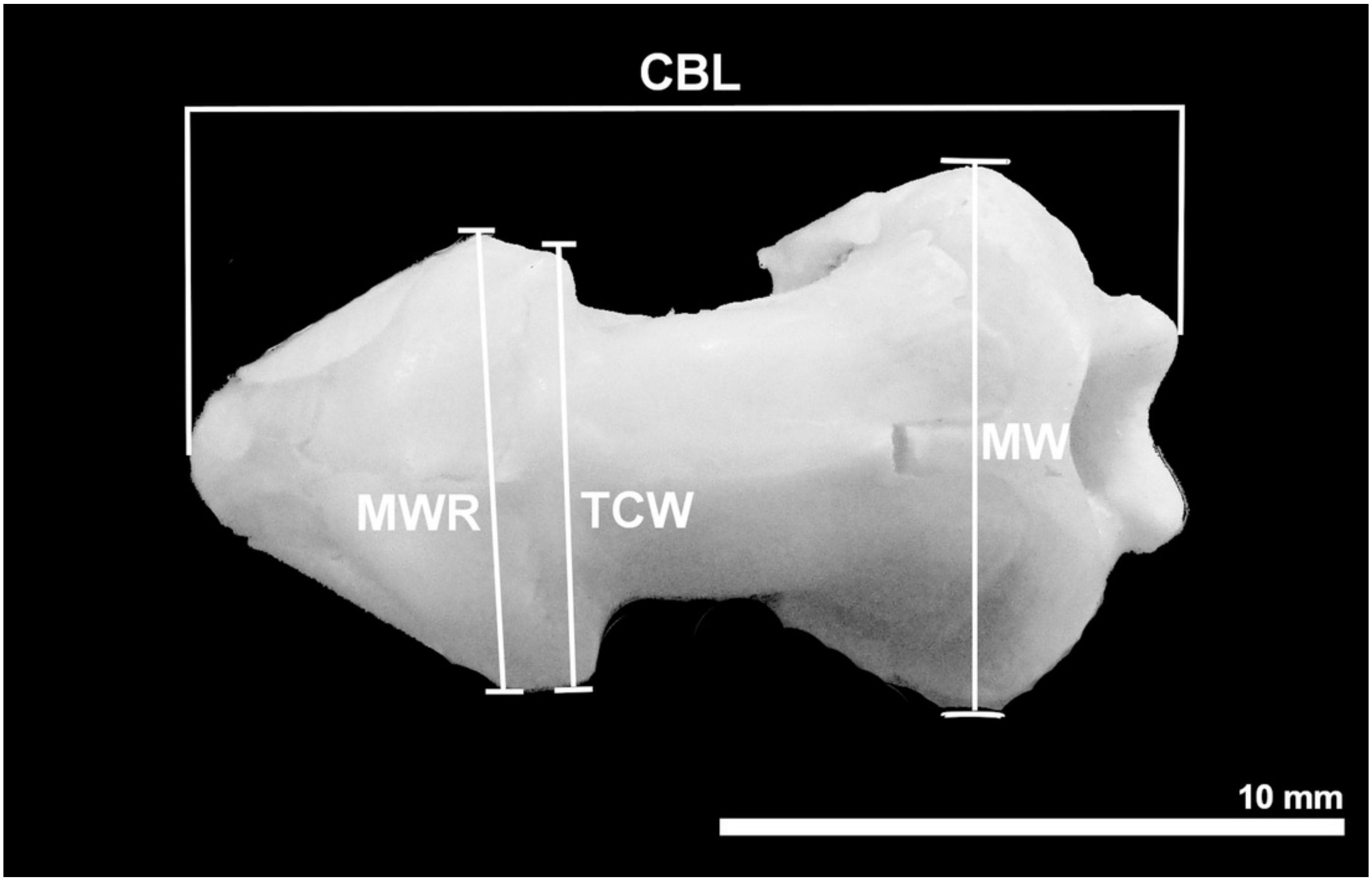

Figure 3

Principal Component Analysis scatterplot showing the distribution of *L. microcephalum* and *L. scutigerum* specimens on the multivariate space through the two main axis of variation (PC1 and PC2).

Arrows indicate the direction of the correlation of each variable with the different PCs.

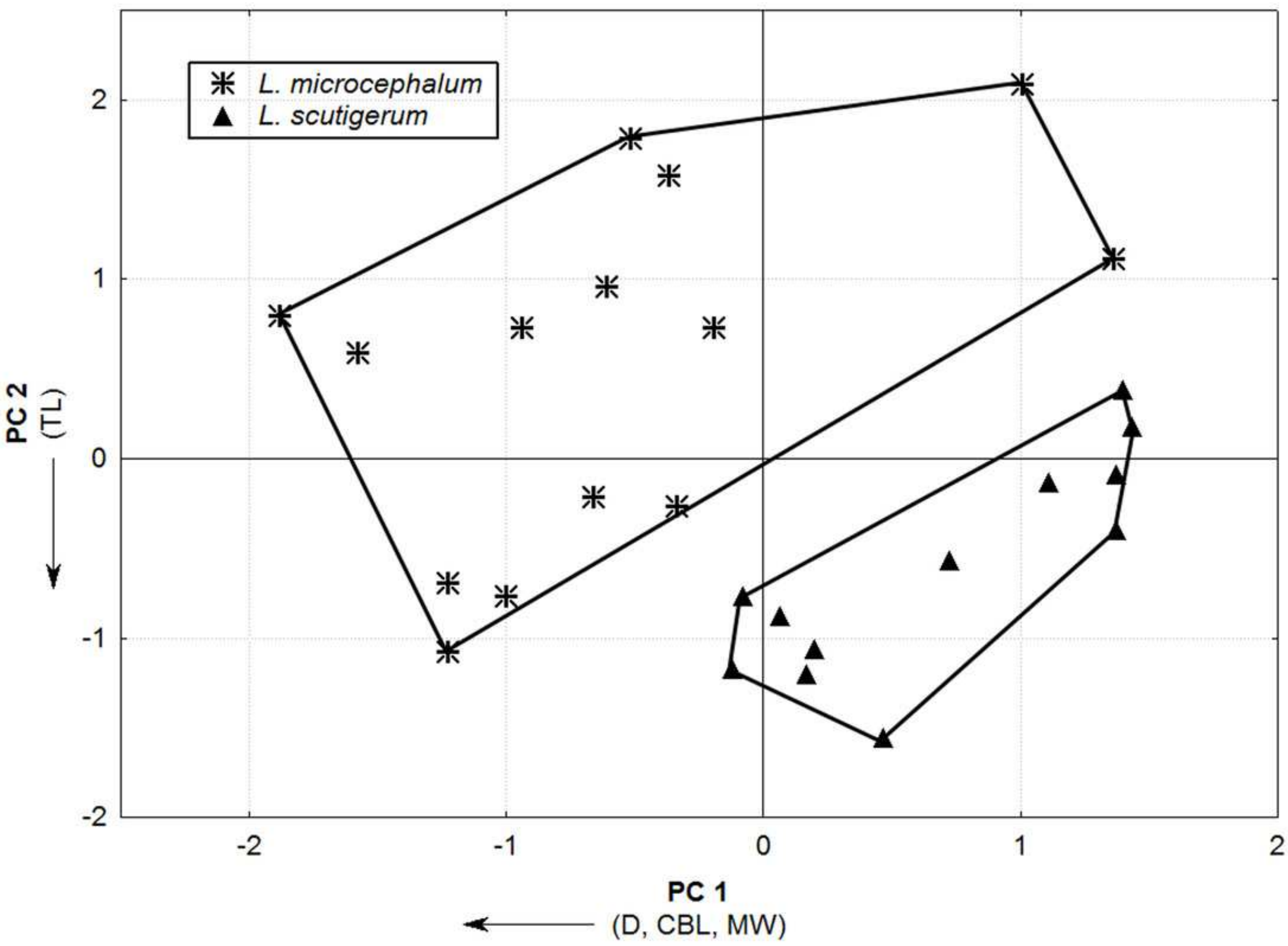

Figure 4

Graphic showing the speed values (means and SDs) of *Leposternon* individuals.

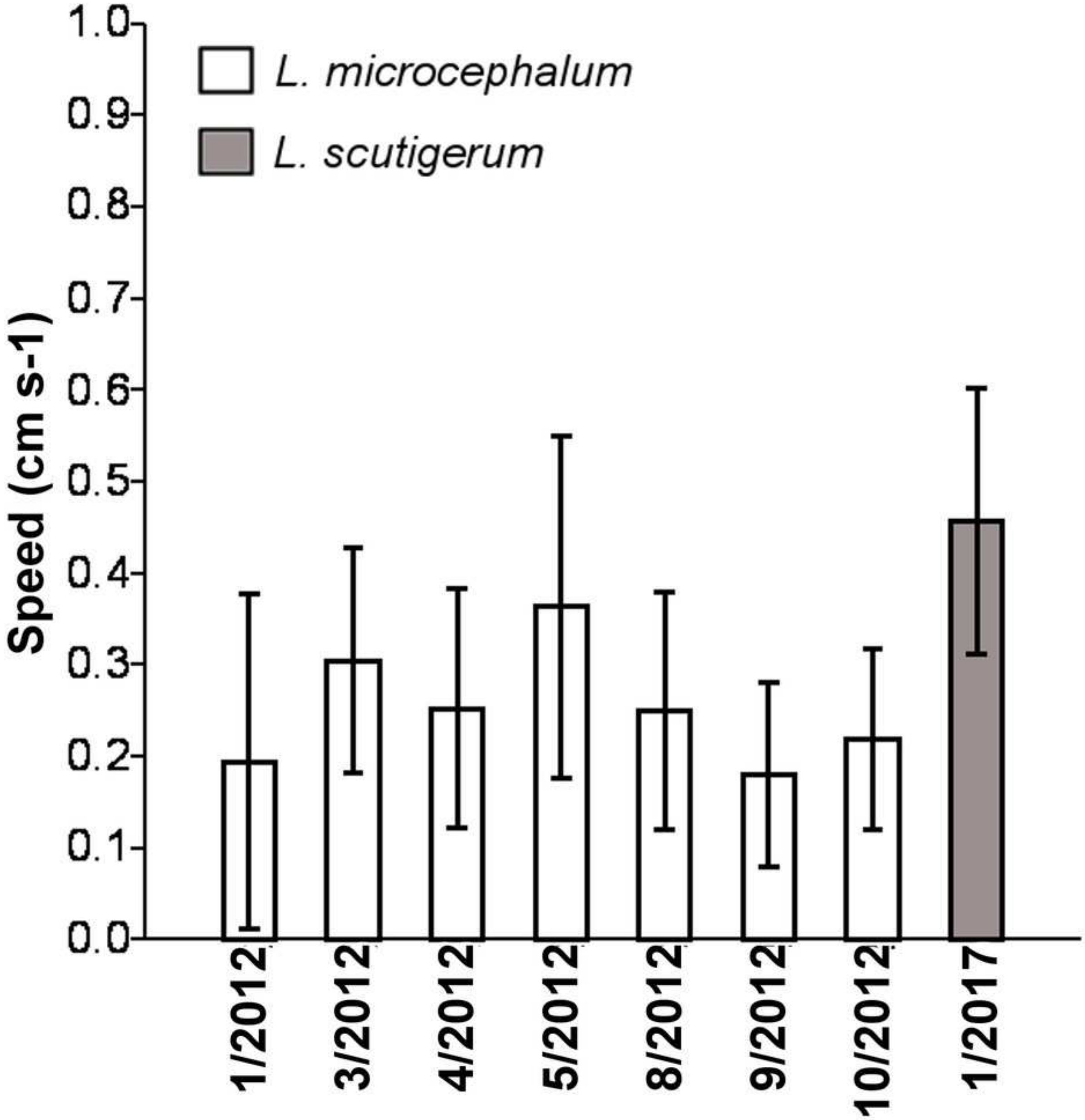

Figure 5

Excavatory cycle' speed as a function of width of skull in *Leposternon* individuals.

(A) Analysis restricted to *L. microcephalum*. (B) Analysis after insertion of *L. scutigerum*, represented by the star.

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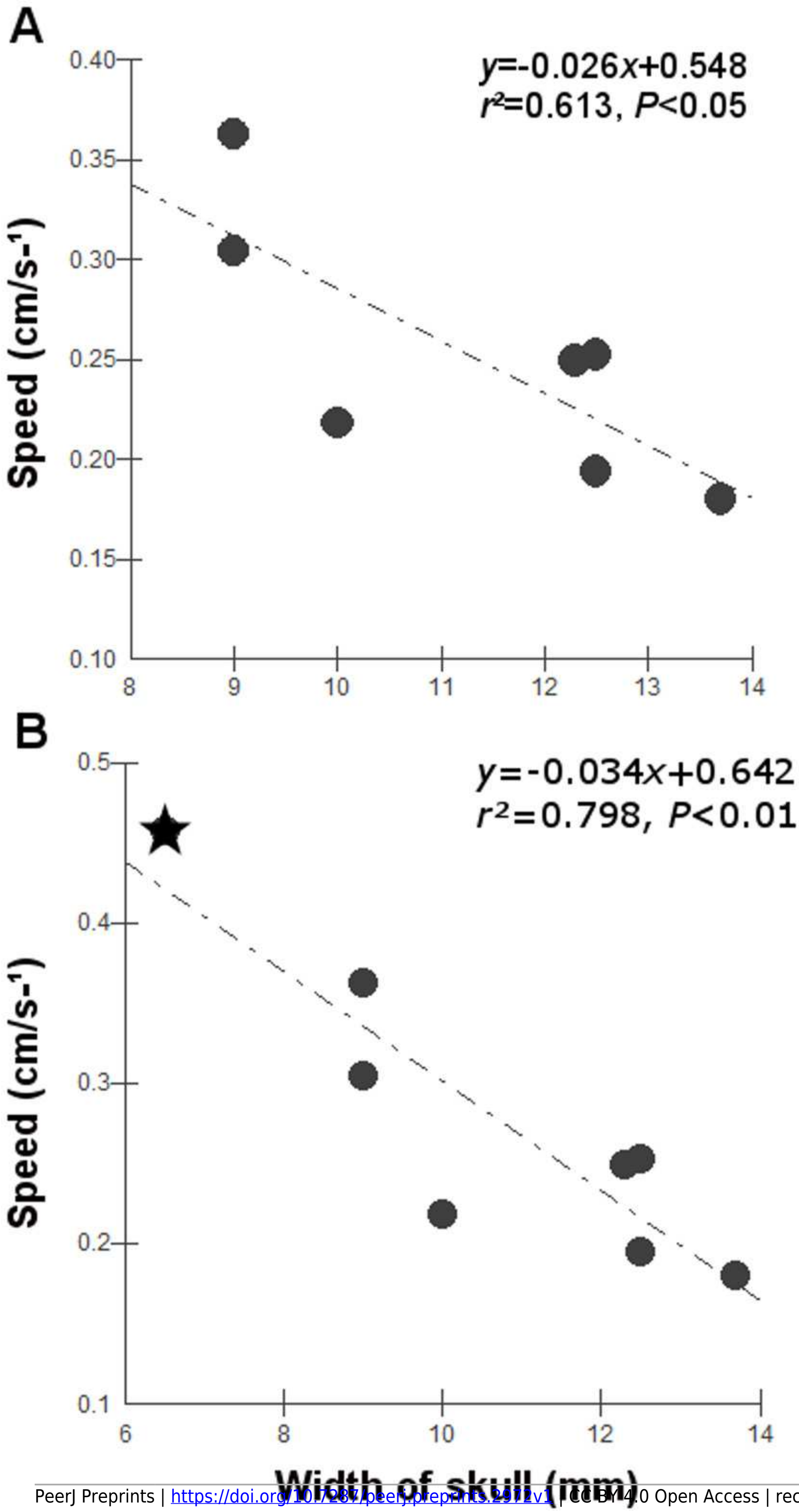
A
y=-0.026x+0.548
r^2=0.613, P<0.05
Speed (cm/s-1)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
8
9
10
11
12
13
14
B
y=-0.034x+0.642
r^2=0.798, P<0.01
Speed (cm/s-1)
0.5
0.4
0.3
0.2
0.1
6
8
10
12
14
Width of skull (mm)