"Isometric variation in the brown-throated sloth *Bradypus variegatus* (Schinz, 1825) mandible"

Original Research Article

ISOMETRIC VARIATION IN THE BROWN-THROATED SLOTH *Bradypus variegatus* (Schinz, 1825) MANDIBLE

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ABSTRACT

Several studies have shown unique morphological traits of sloths, affecting varied phenotypic traits from skeletal parts to soft tissues. Here we present the first descriptive allometric pattern of mandible form among the brown-throated sloth *Bradypus variegatus* (Schinz, 1825), based on the study of 25 specimens by means of geometric morphometric methods. The results obtained indicate that the species has a non-allometric slope, and therefore shape variation could hardly be explained by size. An established "lever index", which can characterize the mandibular mechanical advantage, was maintained independently of the mandibular size. This non-geometric scaling of jaw form in sloth represents another uniqueness of this animal taxon.

Key words: allometry, *Choloepus*, three-toed sloth, two-toed sloth, *Xenarthra*

INTRODUCTION

Sloths are peculiar placental mammals showing unique morphological traits involving both skeletal parts and soft tissues, such as the variation in their cervical vertebrae number [1], the specific sequence of cranial suture closure [2], the inner ear [3], or a late ossification of the sternum and an early ossification of the phalanges and pubis [1]. Moreover, sloths present some of their vertebrae with at least two articulations with adjacent vertebrae [4], sacral vertebrae fused to pelvis [5] and a lack of dental enamel [6].

Extant sloths are distributed in two genera: *Bradypus* and *Choloepus,* in the superorder *Xenarthra*. They differ in the number of fingers (they are sometimes referred to as three-fingered and two-fingered sloths, respectively), although differences comprise much more than merely number of hand digits [7]. The genus *Bradypus* comprises four extant species: *B. tridactylus* Linnaeus (1758), *B. torquatus* Illiger (1811), *B. variegatus* Schintz (1825), and *B. pygmaeus* Anderson and Handley (2001) [8,9].

Three-fingered sloths can be diagnosed by cranial characteristics, including a short rostrum, dorso-ventrally flattened skull, tympanic bullae, mandible with conspicuous coronoid processes, a jugal ending posteriorly in long, flared ventral and dorsal processes, and a condyloid process that is longer than it is wide[10]. Additionally, the pterygoids do not meet at the midline [10]. The tip of the sloth mandible is usually spout-shaped and there is a foramen, representing an external opening of the mandibular canal, on the side of the lower jaw. Incisors and canine teeth are absent, and it is not really possible to distinguish between the similar premolars and molars. A set of peg-shaped cheek teeth that are not clearly divided into premolars and molars are thus just referred to as molariforms. They have a single set of high-crowned, open-rooted teeth that grow continuously throughout their life.

The brown-throated sloth, *B. variegatus*, the most common of the four *Bradypus* species, is of similar size and build to most other species of three-fingered sloths, with adults reaching a length of 41.3 to 70 cm and attaining weights between 2.25 and 5.5 kg [11]. The head is rounded, with a blunt nose and inconspicuous ears. There is usually only a single young after a gestation period of 5-6 months, and while the offspring will no longer nurse after about one month, it still relies on its mother for a further five months (Nowak, 1991). *B. variegatus* is found in Colombia, Venezuela, Ecuador, Brazil, eastern Peru, eastern Bolivia and Paraguay. Elsewhere, it is known in Central America as far north as Honduras [12].

The size and shape of the lateral part of the mandible is supposed to be a good model-system for ecomorphological and evolutionary studies due to its biomechanical roles related to foraging use. Considerable research on the scaling of loading patterns in mammalian locomotor systems has not been accompanied by a similarly comprehensive analysis of the interspecific scaling of loading regimes in the mammalian masticatory complex. In general, geometric morphometrics utilizes powerful and comprehensive statistical procedures to analyze shape differences of a morphological feature, using either homologous landmarks or outlines of the structure. By using this approach [13] in *Bradypus variegatus*, we captured variation in lateral mandible size and shape. The purpose was to assess the allometric and

non-allometric components of mandibular shape variation and ultimately to assess whether shape changes are attributable to a mere change in size, or influenced by other sources of variation in that species. Due to the wide range of sizes, the specimens analyzed here can be considered as pertaining to different age class.

MATERIAL AND METHODS

Data collection

We examined 25 specimens of *Bradypus variegatus* archived in the collections of the *Departamento de Biología* of the *Universidad del Valle* in Cali (Colombia) and *Instituto de Ciencias Naturales* of the *Universidad Nacional de Colombia*. Every specimen had been taxonomically identified to the species level, and was initially collected for other studies.

There is no sexual dimorphism present in *B. variegatus*[1], and therefore we performed all our analyses irrespective of gender. All specimens had been collected from different places in Colombia.

Mandible landmarks obtention

Digital images of left lateral hemimandibles were taken with a Nikon D1500 digital camera equipped with an 18-105 mm Nikon DX telephoto lens. Each mandible was placed in the center of the optical field, with body oriented parallel to the image plane. A set of 14 landmarks on the left hemimandible (lateral aspect) were digitized using TpsDig v. 2.16 software [14]. The landmarks chosen were present on all specimens and were considered to sufficiently summarize the morphology of the lateral aspect of hemimandible -alveolus, tips of processes, and point of maximum curvature of structures- (Figure 1). Because the mandible is constituted by a unique dentary bone of relatively simple shape, most of the landmarks taken were of type 2 (e.g. maxima of curvature). Landmarks were always taken by the same person (PMPC).

Statistical analyses

To obtain information on shape with differences related to size, position and orientation removed [15,16,17], the data were first superimposed on Bookstein's shape coordinates by IMP CoordGen8 [18]. Centroid size (CS), the square root of the summed squared distances of each landmark from the centroid of the landmark configuration, was interpreted as a geometric measure of mandibular size. Subsequently, mandibular form of each specimen was represented by CS, and by multidimensional shape vector in linearized Bookstein's shape space.

Tps Small v. 1.29 software[19] was used to assess the correlation between the 2D distances to the Euclidean distances in that tangent space. The correlation was very close to linear for all of the data (r=0.999; slope, b=0.996), suggesting that tangent space was an adequate approximation to Kendall and therefore no specimens deviated appreciably from the linear regression line. Thus, although the lateral aspect of the mandible is not a perfect flat object, authors considered that the two-dimensional approach applied implies a limited loss of information, and we proceeded with the morphometric analyses.

Size and shape variation

To explore variation in mandibular shape among specimens and to visualize its changes, we conducted a Principal Component Analysis (PCA) on the covariance matrix of the shape variables. The PCA analysis was performed using PAST Package v. 2.17c [20].

Analysis of allometry and removing the effect of size

To regress the shape of the collection of specimens (captured as coordinates of landmarks) onto size (CS, log transformed) as independent variable, we used PAST package v. 2.17c [20]. This regression was carried out with 1000 random permutations and the significance level established at 5%.

Mandibular "lever index"

The jaw can be viewed as a lever system for applying force to the food being chewed. A lever consists of a pivot point (the jaw articulation), and two lever arms. The in-lever arm connects the pivot to the point where an "in-force" (Fi) is applied by a muscle. The out-lever arm connects the pivot to the point where an "out-force" (Fo) is applied by the teeth to the food. The relation Fo / Fi can be interpreted as a "lever index" (Figure 2). In the mandible, one can consider the distance 3-7 as Fi and the distance 3-9 as Fo, and therefore the "lever index" is established as distance 3-9/distance 3-7. Linear correlation was established for log CS/log lever index.

RESULTS

The position of the specimens in the morphospace defined by the first two axes obtained by PCA of mandible shape variables are presented in Figure 3, where the first two axes explained 74.18% (PC1+PC2=63.08+11.09%) of the total observed variance. Coordinates with lesser values (from -0.0496 to 0.0103 on PC1) were X6 and Y6, X8 and Y8, Y9, Y10, Y12 and Y13. Minimal variation was observed on the angular process (primary point of attachment for the superficial masseter muscle, coordinates X6 and Y6) and facial notch (coordinates X8 and Y8). These 8 landmarks were deleted for subsequent regression analysis.

The results of multivariate regression of shape variables (reduced to 20 coordinates) on log CS revealed that shape variation could hardly be explained by allometry (R^2=0.104, $F_{20,4}$=1.182, p=0.487) (Figure 4). The "lever index" was maintained independently of mandibular size (r_s=-0.208, p=0.318) (Figure 5).

DISCUSSION

Morphometrics is defined as the quantitative description, analysis and interpretation of shape and variation of structures in biology. In a fundamental area of research, unlike the analytical approaches, the geometric one is aimed at comparison of the shapes.

As an integral component of the craniomandibular apparatus, mandibles play an important role in feeding mechanics. In the *Bradypus variegatus* sample studied, shape did not vary according to bone size, almost all the observed variation being attributable to mere non-allometric shape changes. If the shape is the same throughout the life of animals, mechanical considerations, such the relative engulfment capacity and the specific physical forces at play during the feeding process, would not increase allometrically with body size. Thus, one can suppose that biomechanical performance of the components of the masticatory apparatus is the same independently of postnatal growth. This non-geometric scaling of jaw form in sloth can be seen as another uniqueness of the sloth group.

We can, however, find other benefits from this conclusion. The ability to reconstruct morphometric data for key fossil taxa, and the integration of these data with extant taxa into comparative phylogenetic analyses, is paramount to understand the evolution of body size and specialized feeding modes in sloths. The presence of that clear mandibular isometry of *B. variegatus* could therefore be used to easily estimate the original shape in extant fragmentary museum specimens. Furthermore, if the evolution of morphology is arguably the evolution of allometry, its changes in different sloth species could also contribute to a better understanding of *Xenarthra* evolutionary relationships.

Finally, our finding raises the question of whether this isometry can also be demonstrated in other areas of the sloth phenotype.

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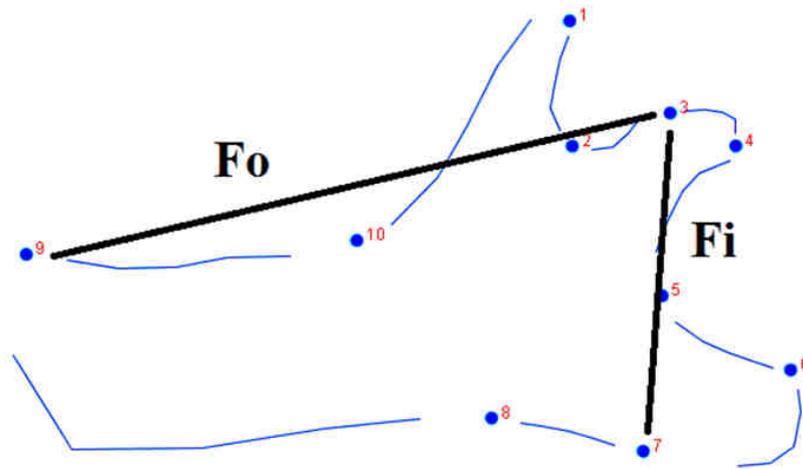
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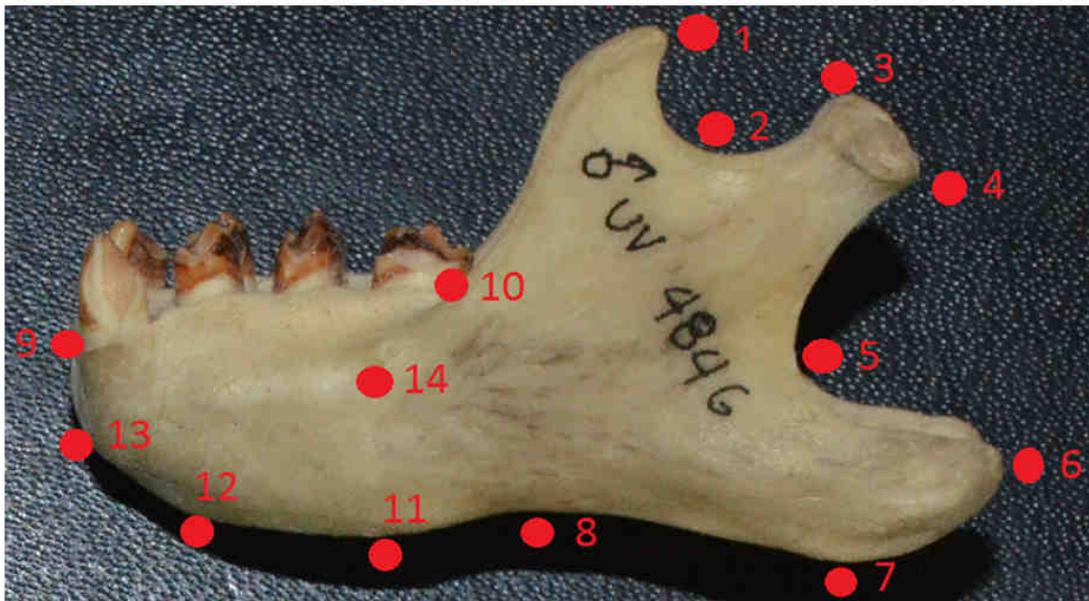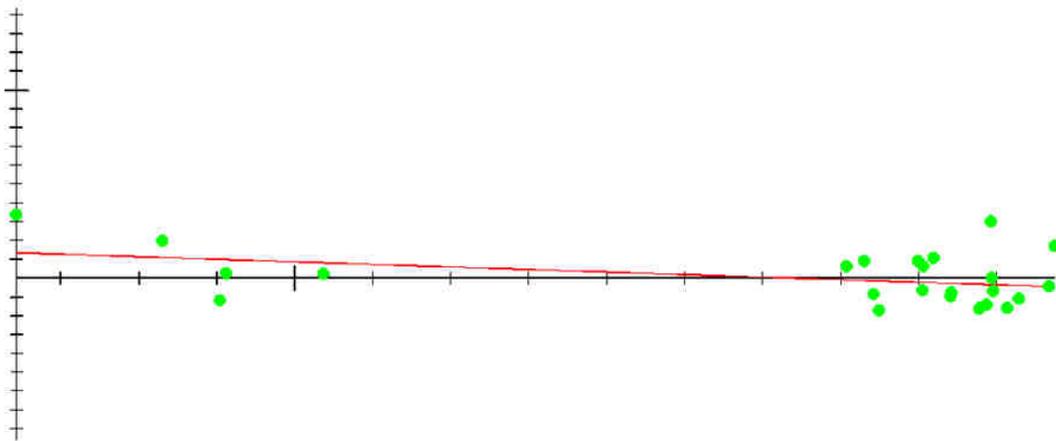
Figure 1. Lateral mandible view of *Bradypus variegatus.* Fourteen landmarks were used to capture mandibular shape. Mandibles were aligned by their tooth row to a stable plane.

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Figure 2. The in-lever arm (Fi, distance 3-7) and the out-lever arm (Fo, distance 3-9). The force multiplying effect of a lever is characterized by the mechanical advantage Fo/Fi. The force can be increased by increasing the out-lever arm or by decreasing the in-lever arm.
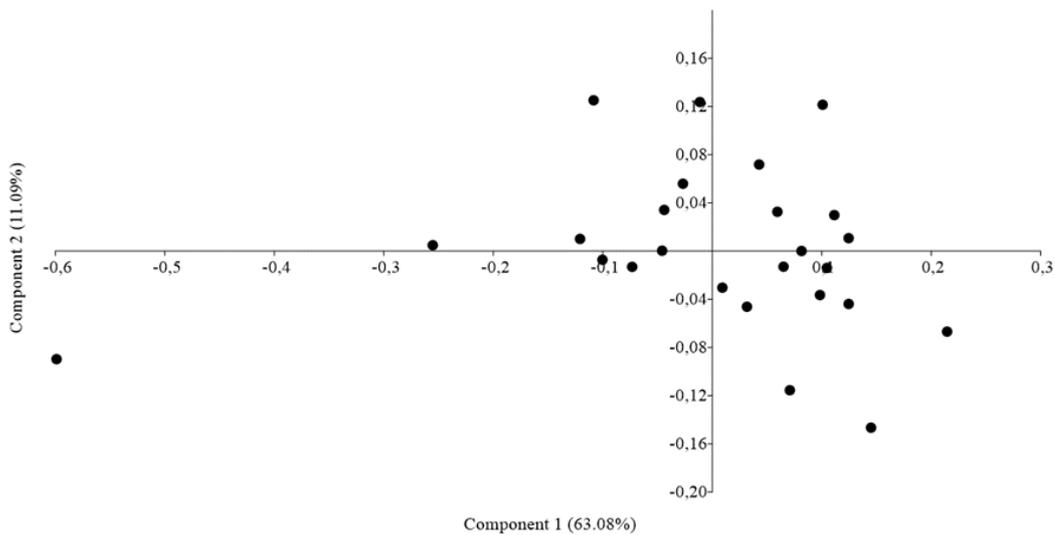
Figure 3. Position of the specimens of *Bradypus variegatus* (n=25) in the morphospace defined by the first two axes obtained by PCA. First two axes explained 74.18% (PC1+PC2=63.08+11.09%) of the total observed variance.

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Figure 4. Multivariate regression of shape variables (28 coordinates, Y-axis) on log CS (X-axis). It revealed that shape variation could hardly be explained by allometry ($R^2=0.104$, $F_{20,4}=1.182$, p=0.487).
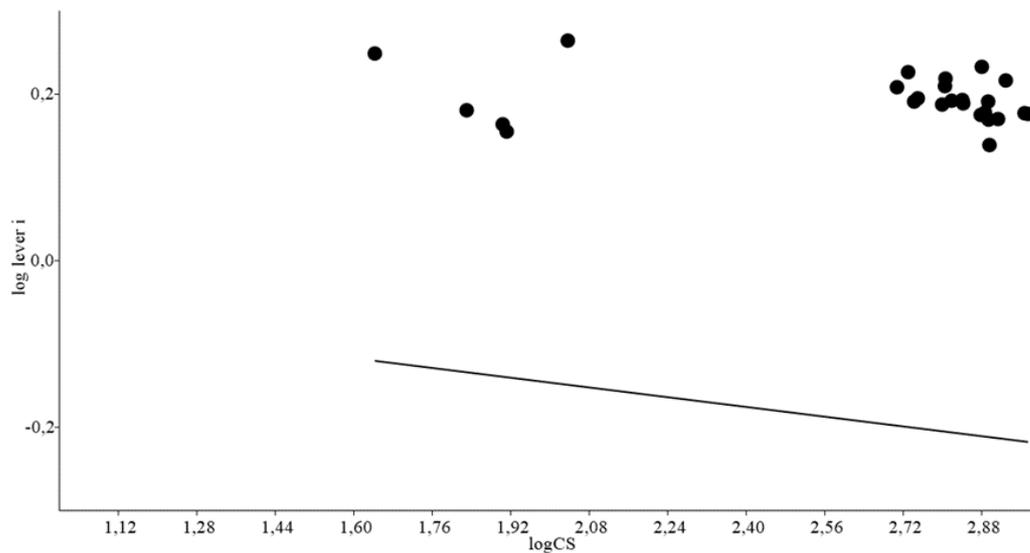
Figure 5. "Lever index" (Fo / Fi) [(distance 3-9)/(distance (3-7)] regressed against mandibular size (both log transformed). The "lever index" was maintained independently of the mandibular size ($r_s=-0.208$, p=0.318).