How much variation is in the shape of fossil pupation chambers? An exploratory geometric morphometric analysis of *Fictovichnus gobiensis* from the Late Eocene of Oaxaca, southern Mexico

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ABSTRACT

Shape is one of the characteristics most often used for the identification of ichnotaxa. Several studies have demonstrated the potential of geometric morphometrics in ichnology, in order to distinguish the nature of the tracers' producers. In this study, we used geometric morphometrics to analyze variation of shape in *Fictovichnus gobiensis*, a fossil pupation chamber produced by Coleoptera. The objective of this study was to analyze diverse clusters of similarly shaped pupal chambers, indicative of several coleopteran producers. The 42 analyzed specimens were collected from late Eocene-aged outcrops in the surrounding areas of Santiago Yolomécatl, northwestern Oaxaca, where these fossil traces are relatively abundant. On the specimens, 2 landmarks and 34 semilandmarks were placed for 2-dimensional Cartesian coordinates. Shape variation was analyzed with Procrustes distances as a measure of similarity. Unweighted Pair Group Method with Arithmetic Mean method was used for cluster analyses, and Principal Component Analysis and Canonical Variates Analysis were used to explore shape variety and differences between the clusters. The elliptical shape of the chambers is variable, but it was possible to group and distinguish 4 main morphs, suggesting 4 potential producers. Morphs recognized in this study represent indirect evidence of beetle diversity that were not directly preserved in the fossil record.

Future analyses in pupal chambers of recent Coleoptera could clarify the nature of the producers. Additional research could establish if more morphs exist in larger samples from Yolomécatl localities.

Keywords: Pupation chambers, shape, *Fictovichnus gobiensis*, Oaxaca, Coleoptera.

RESUMEN

La forma es uno de los criterios más utilizados para la identificación de icnotaxones. Diversos estudios han demostrado el potencial de la morfometría geométrica en icnología, con el fin de distinguir la naturaleza de los productores de las trazas. En este estudio, se analizó la variación de la forma de Fictovichnus gobiensis, *cámaras pupales fosilizadas producidas por coleópteros, por medio de morfometría geométrica. El objetivo del análisis fue analizar diversos conjuntos de cámaras pupales con forma similar, indicativos de varios coleópteros productores. Los 42 ejemplares analizados proceden de afloramientos del Eoceno tardío, en los alrededores del poblado de Santiago Yolomécatl, noroeste de Oaxaca. En los ejemplares se registraron 2 marcas y 34 semimarcas para coordenadas cartesianas en 2 dimensiones. La variación de la forma se analizó con distancias Procrustes como una medida de similitud; el método de agrupamiento de pares con la media aritmética no ponderada se utilizó para el análisis de los agrupamientos, así como un análisis de componentes principales y un análisis de variantes canónicas para explorar la variación de formas y las diferencias entre los grupos. La forma elipsoidal de las cámaras es variable, pero fue posible distinguir 4 morfos principales, lo cual sugiere que existen 4 productores posibles. Los morfos reconocidos en este estudio representan una evidencia indirecta de la diversidad de escarabajos, la cual no está preservada directamente en el registro fósil. Análisis futuros de cámaras pupales de coleópteros recientes permitirán establecer la naturaleza de los productores. Investigaciones adicionales podrán establecer si existen más morfos en muestras más numerosas procedentes de las localidades de Yolomécatl.*

Palabras clave: Cámaras pupales, forma, Fictovichnus gobiensis, Oaxaca, Coleoptera.

ABSTRACT

Geometric morphometrics of *Fictovichnus gobiensis*

1. Introduction

Shape analysis plays an important role in many kinds of biological studies (Zelditch *et al.*, 2004). One such kind of study is ichnology: using shape (besides wall and filling) as a basic characteristic to define insect-trace fossils (Genise, 2017). In recent years, geometric morphometric techniques have been more frequently used in morphological analyses of different fossil taxa, including ostracods (*e.g,* Elewa, 2004), neurosauropods (*e.g,* Bonnan, 2007), brachiopods (*e.g,* Bose, 2013), rodents (*e.g,* Wilson, 2013; Calede and Glusman, 2017; Smith and Wilson, 2017), euprimates (*e.g,* Bennet and Goswami, 2012) and hominins (*e.g,* Bouhallier *et al.*, 2004; Gómez-Robles *et al.*, 2008), however, these techniques are uncommon in ichnological studies.

Geometric morphometric methods have been used in vertebrate trace fossils to infer the potential producer. Regarding dinosaur trackways studies, Rodrigues and Faria dos Santos (2004) characterized variation in shape across various, previously reported Sauropodomorpha ichnotaxa and other dinosaur footprints. Lallensack *et al.* (2016) used these methods in order to distinguish ornithopod and theropod footprints from the Upper Cretaceous in Münchehagen, Germany. Regarding mammals trackways, Ledoux and Boudadi-Maligne (2015) analyzed modern prints from two wolf

subspecies and seven breeds of dogs. These results were compared with presumably dhole tracks documented at Chauvet Cave, France.

On the other hand, *Fictovichnus gobiensis* is a common ichnotaxon reported in the late Eocene outcrops from Santiago Yolomécatl, Oaxaca, southern Mexico (Guerrero-Arenas *et al.*, 2017) (Figure 1a). *F. gobiensis* was originally described by Johnston *et al.* (1996) as being from the Upper Cretaceous Bayan Mandahu Formation of China and the Upper Cretaceous Djadokhta Formation of Mongolia. Their diagnosis was later amended (Alonso-Zarza *et al.*, 2014). This ichnospecies is commonly preserved as hollow structures in paleosols, or as ellipsoid, with smooth casts and lacking a discrete wall (Genise, 2017). They show rounded extremes and a smooth surface in casts detached from the matrix. *F. gobiensis* is very frequent in carbonate rich paleosols. This ichnospecies is attributable to weevil or chafer pupation chambers, as well as other groups of Coleoptera (Johnston *et al.*, 1996; Genise, 2017).

Traditionally, in studies of invertebrate trace fossils, the producers are not identified, given that different tracemakers may produce identical structures when behaving similarly (Bromley, 1996). However, in the case of insect pupation chambers, if the identity of potential producers could be determined, this information might be linked to more specific environmental conditions and

Figure 1 a) *Fictovichnus gobiensis in situ,* from locality Oax-13 La Cruda, Santiago Yolomécatl, Oaxaca, Mexico. Diameter of coin is 21 mm. b) Specimen (UMLP-004) showing marks. Landmarks are represented by black dots; red dots are semi-landmarks; blue dots are semi-landmarks considered as helper points. Scale are equal to one centimeter.
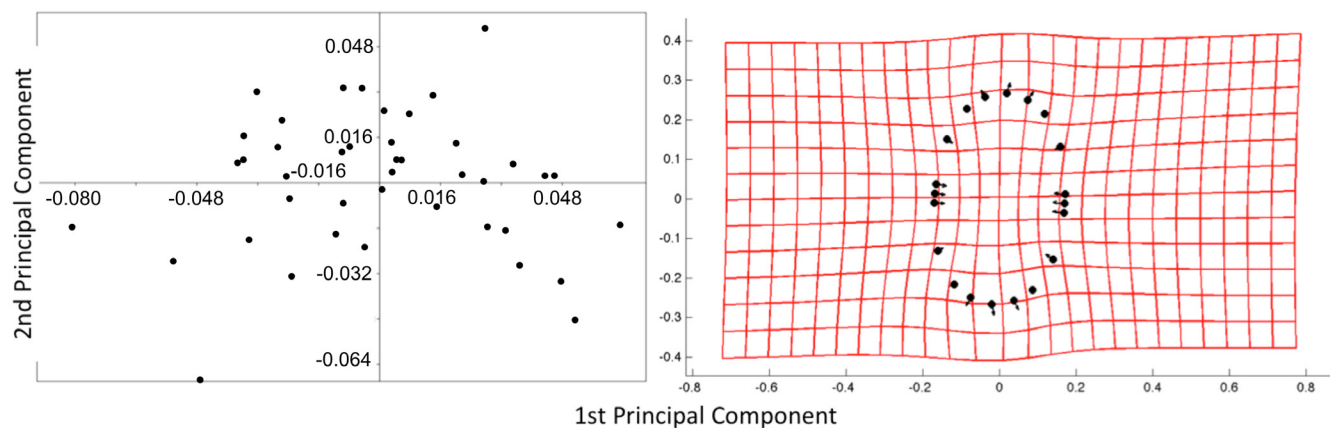
Figure 2 a) Principal Component Analysis of studied specimens. b) Deformation grid; the specimens varied from elongated and thinner to shorter and wider.

even additional paleoecological information such as trophic relations in the paleocommunities could be obtained.

The objective of this study is to evaluate the variation in shape across specimens of *F. gobiensis* collected from a single bed in Santiago Yolomécatl outcrops. Potential producers of the ichnospecies include several taxa (Genise, 2017); thus, we expect that the shape of chambers reflects such different potential producers.

2. Material and methods

Forty-two fossil pupation chambers of *Fictovichnus gobiensis* were examined. The specimens were selected for their completeness and preservation quality. They were collected in outcrops of the Yolomécatl Formation, in southern Mexico (for detailed description of the area and the location of localities, see Jiménez-Hidalgo *et al.*, 2015, 2017). All the specimens come from a single stratigraphic level.

Color images of the specimens were captured using a Kodak EasyShare C195, with the same conditions of fixed focus, camera angle and magnification ratio. The scar marking the exit hole determined the orientation of the specimens; when it was not present, the traces of the scar were used. Two-dimensional (2D) geometric morphometrics

(GM) were used to collect shape data. Two landmarks (Type 3, *sensu* Bookstein, 1991) were positioned in the most exterior points of the rounded extremes of the specimens (point numbers 1 and 19), and 34 semilandmarks (point numbers $2-17$ and $18-36$) were positioned in the ellipse using a drawing fan generated by MakeFan8 (Sheets, 2010a) (Figure 1b). Two-dimensional coordinates of all the marks were digitized using tpsDig2 (Rohlf, 2013). Coordinates were imported into CoordGen 8 (Sheets, 2014a) to proceed to Procrustes superimposition followed by the semilandmark alignment with SemiLand 8 (Sheets, 2010b). The superimposition and alignment eliminated variation that was not due to form (translation, rotation and scaling). In the alignment, sixteen semilandmarks were used as helper points $(4, 6-8, 12-14, 16, 22, 24-26, 30-32,$ and $34)$. Helper points were excluded from subsequent analysis, resulting in the reduction of the final variables number to two landmarks and 18 semi-landmarks.

A Principal Component Analysis (PCA) was performed with PCAGen (Sheets, 2014b) with the aim to explore the shape variation across individuals. Procrustes distances were calculated as a measure of total similarity among shapes; the matrix with pairwise distances was imported into the software PAST (Hammer *et al.*, 2001) for cluster analyses and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and hierarchical
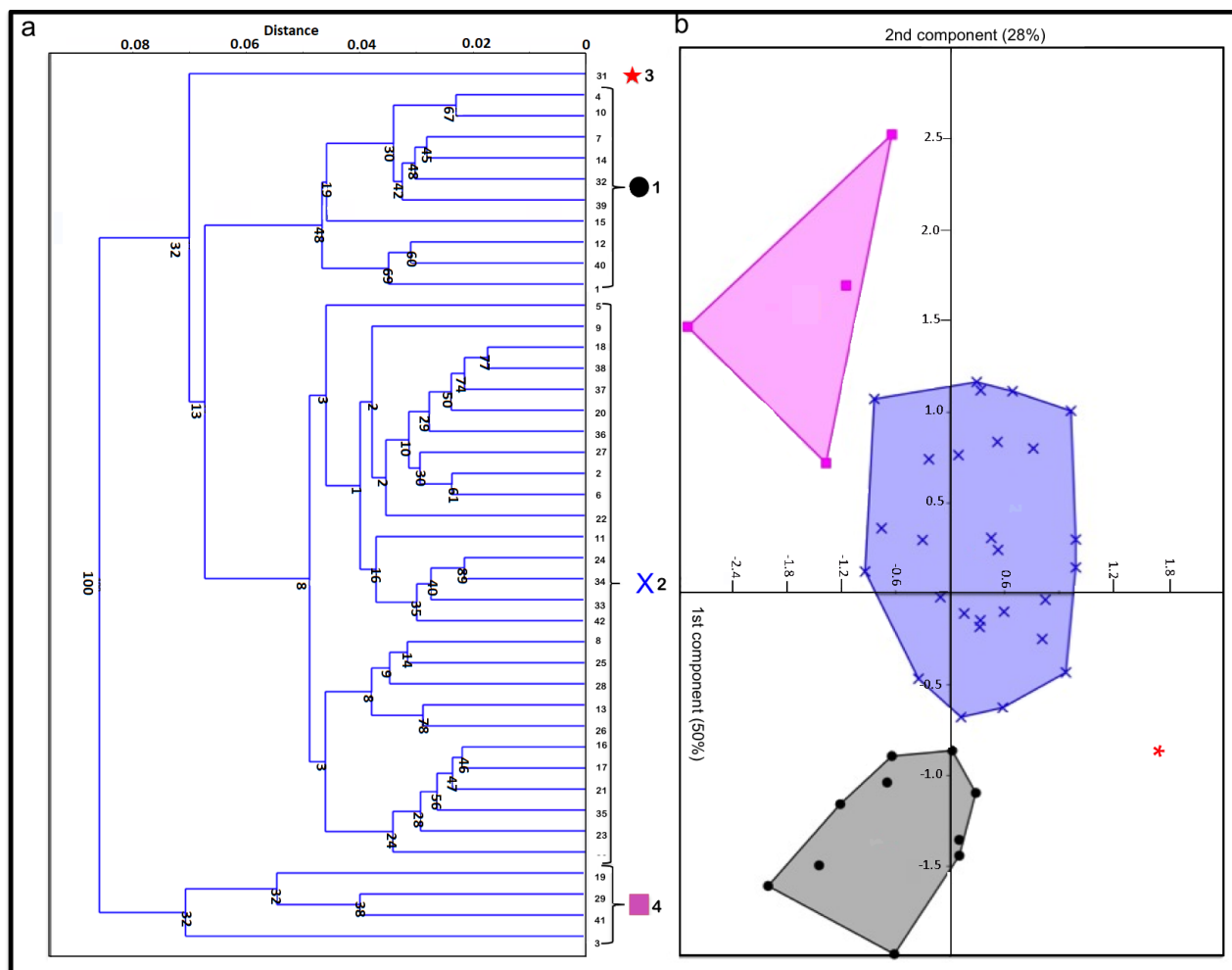
Figure 3 a) Clustering with UPGMA. Each symbol represents a different group of *F. gobiensis* specimens. b) Principal component analysis of *F. gobiensis* specimens.

grouping algorithm was used. Resulting clusters were analyzed with Canonical Variates Analysis (CVA) using CVAGen 8 (Sheets, 2010c) to estimate a MANOVA (Multivariate analysis of variance) for the differences in shape among groups.

3. Results

PCA analysis explains 78% of the variance in the first two axes, 50% in the first principal component and 28% in the second principal component (Figure 2a). The deformation grid over the two principal components shows a deformation along

the ellipse's contour, making some of them elongated and thinner, while others were shorter and wider (Figure 2b).

Cluster analyses with UPGMA formed four groups, three of them were segregated in the first principal component and the fourth one could be seen over the second component represented by only one individual (Figure 3). Although UPGMA analysis suggested four groups, CVA analysis was done with three of them (1, 2 and 4 in Figure 3) because of the lack of variance in the group number 3, composed of only one specimen (Figure 4). In Figure 4, the CVA analyses showed two significant distinct CVA axes on which the three morphs are
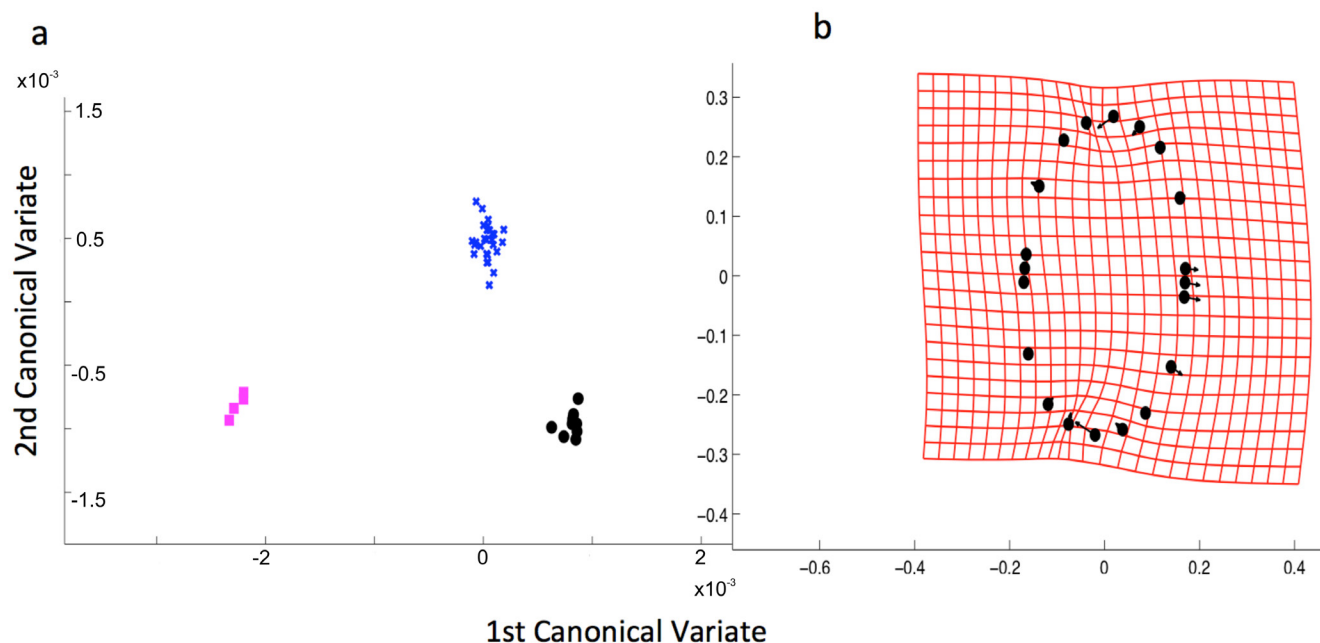
Figure 4 Canonic Variate Analysis of the shape of the recognized morphs. a) CVA of the three detected morphs. b) Deformation grid; the contour near the extremes showed the major variation.

clearly differentiated (Table 1). MANOVA analysis suggests that 78.4% ($p < 0.001$) of the variance is explained by the morph groups' differences (Table 2).

4. Discussion

The four morphs of *Fictovichnus gobiensis* identified in Yolomécatl suggest the presence of at least four potential producers. At present, there is scarce information about traditional morphometrics to infer the nature of insect trace fossil producers. However, several studies suggest the potential to use the shape as criteria for identification of producers. Sánchez *et al.* (2010) analyzed the relative sizes of *Coprinisphaera* specimens of Sarmiento Formation, Argentina, to infer the potential diversity of producers. In this kind of trace fossils, it was demonstrated that size of dung beetle brood balls could be directly related to the size of their producers (*e.g.*, Halffter and Matthews, 1966; Sato and Imamori, 1987).

Producers of *F. gobiensis* could be larvae of soil-dwelling coleopteran groups related to Curculionidae, Tenebrionidae and Scarabaeidae (Johnston *et al.*, 1996; Genise, 2017). Available descriptions of shape or size of similar structures deposited in recent soil produced by these groups do not fit with *Fictovichnus gobiensis* chamber features. There are a few reports of brood masses of dung beetles (Scarabaeidae); Romero-Samper and Martín-Piera (1995) described simple branched brood masses of *Onthophagus stylocerus*. Huerta and García-Hernández (2013) measured volume and weight of brood masses of *Onthophagus incensus*. However, these structures are not comparable with *F. gobiensis*.

Differences in the morphology of pupal fossils could be related with the nature of a specific producer. Further analysis of chamber shape from modern taxa of Curculionidae, Tenebrionidae and Scarabaeidae will clarify the nature of *F. gobiensis* producers, and help us understand if shape variation is linked with different species, sex of pupal specimens, or even some environmental factors.

Table 1. Canonical Variate Analysis.

AXE	EIGENVALUE	LAMBDA	CHI SQUARE	DF	*p*
1	132.111	0.003	169.538	72	$7.672\ e^{-10}$
2	28.339	0.0341	69.2684	35	0.0004906

DF: Degrees of freedom.

Table 2. MANOVA Results.

VARIABLE	SUM OF SQUARES	DF	MS	F
GROUP	0.016045	2	0.008023	11.87
ERROR	0.025675	38	0.000676	
TOTAL	0.04172	40		
P value = 0.0099 based on 100 permutations			Grouping explained 78.377% of total variance	

DF: Degrees of freedom. MS: Mean square.

In our study, we concluded that the four recognized morphs suggest four potential producers. If every morph represents different taxa, we should have a community with at least four kinds of beetles. Vegetation type inferred from ichnofossils, isotopic values of pedogenic carbonates and the mammalian fauna suggest scrubland/woodland paleoenvironments in the Yolomécatl area (Guerrero-Arenas *et al.*, 2017). In modern analogue communities, the diversity of beetles is comparable with our results. In a desert shrubland-grassland ecotone in Socorro County, New Mexico, two species of Curculionidae were collected into patches of creosotebush (Sánchez and Parmenter, 2002). Five species of Tenebrionidae were trapped in a shrub steppe habitat at Cerro Otto, Bariloche, Argentina (Mazía *et al.*, 2006).

The four morphs recognized in this study by means of geometric morphometrics represent indirect evidence of beetle diversity that were not directly preserved in the fossil record.

5. Conclusions

Our geometric morphometric analysis of the *Fictovichnus gobiensis* chamber resulted in the recognition of four morphs. This shape variation in filling

chambers can be related with a particular producer. Although producers of different taxa could be related directly with shape variation, additional studies are required to determinate if other variables (climate, intraspecific variation, phenotypic plasticity, etc.) influence the shape of *F. gobiensis* chambers.

Geometric morphometrics is a potential tool in paleoichnological studies, to obtain additional information about producers of trace fossils. However, at present, it is limited by the lack of information of morphometric data in recent analogues. Knowledge of modern structures is crucial to take advantage of this useful analysis.

Acknowledgements

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