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No Morphometric Distinction between the Host *Constrictotermes cyphergaster* (Silvestri) (Isoptera: Termitidae, Nasutitermitinae) and its Obligatory Termitophile *Corotoca melantho* Schiødte (Coleoptera: Staphylinidae)

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Abstract

Different species may live in termite nests, cohabiting in close association with the host colony or occupying nest cavities without direct contact with the host. The strategy of termitophile organisms to become integrated into termite societies include appeasement through chemical, morphological and/or behavioral mimicry. We investigated the hypothesis that there is a morphological mimicry between the obligate termitophile *Corotoca melantho* (Coleoptera: Staphylinidae) and workers of its termite host *Constrictotermes cyphergaster* (Isoptera: Termitidae). Pictures of thirty-one *C. cyphergaster* workers and *C. melantho* individuals were taken in top and side views and converted into thin-plate splines. Four homologous landmarks and five semilandmarks (reference points) were marked on the head and abdomen of both species and digitized. The body shape of both species are morphometrically similar, so there is no discrimination between specimens of termitophile beetles and worker of termite hosts. Body size of termite hosts is responsible for 20% to 30% of body shape variation, while the body size of termitophiles beetle affects near 50% to 60% body shape. However, termitophiles body shape had a greater variation than worker termites. This is the first study to compare morphological similarity among termites and termitophiles using morphometric geometry. Our results indicated the existence of a morphological mimicry between *C. cyphergaster* and *C. melantho*.

Introduction

The controlled temperature and humidity within termite nests create an internal atmosphere that attracts other animals seeking shelter, protection and food (Noirot & Darlington, 2000). The nest may specially represent a food source for some inquiline termites and termitophile invertebrates (Kistner, 1969). Different species can live in association with termite nests, cohabiting with the host species or occupying nest cavities without direct contact with the host colony. Kistner (1969) termed termitophiles the animals that live at least one phase of their life inside termite nests. In the Brazilian savannah (Cunha & Brandão, 2000; Cunha & Morais, 2010; Lopes & Oliveira, 2005; Costa et al., 2009; Cristaldo et al., 2012) and Brazilian Amazonia (Carrijo et al., 2012) different termitophile groups have been reported in termite nests: Acarina, Anura, Araneae, Blattaria, Chilopoda, Coleoptera, Diplopoda, Haplotaxida, Heteroptera, Hymenoptera, Lepidoptera, Orthoptera, Opiliones and Scorpiones. Strategies of termitophiles integration into the social life of termites can include appeasement by chemical (Wilson, 1971), morphological (Kistner, 1969) or behavioral mimicry (Kistner, 1979). Hydrocarbons are used among social insects in species recognition, colony or castes (Howard & Blomquist, 2005) and are detected by antennal contact. The hydrocarbons of some social insect nest inquilines are very similar to that of their hosts (Howard et al., 1982). Some Staphylinidae (Coleoptera) species maintain mutualistic interactions with their hosts through chemical mimicry (Rosa, 2012) and exocrine glands whose exudate can be licked by the workers, which, in turn, regurgitate stomach content for beetles (Pasteels & Kistner, 1971).

Some Staphylinidae beetles have the ability of overlapping their abdomen on the thorax to reduce body length and develop physogastry (Kistner, 1979; 1982). Physogastry is the development of the reproductive and/or glandular system of termite hosts, in which the glands may produce chemical messages of termitophile acceptance (Krikken, 2008). Most termitophile physogastry beetles

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belong to the family Staphylinidae (Kistner, 1979; 1982). In this family, some species have cuticular hydrocarbons similar to those of their hosts (Rosa, 2012). Sometimes, physogastry is followed by a subsequent secondary sclerotization of some or all of the expanded membrane, as is the case for *Corotoca melantho*, *Spirachtha eurymedusa* and *Termitoiceus* sp. nov. (Seevers, 1957; Kistner, 1982; Jacobson & Pasteels, 1985; Kistner, 1990; Cristaldo et al., 2012). The termitophiles have reduced body size and lateral abdominal projections, and seem to mimic the morphology of worker termites. Kistner (1968, 1969) explained that the mimicry between termitophiles and termites is based on palpation and not on sight. Krikken (2008) described several pronotum trichomes which presumably function in the communication among termitophiles and the termite hosts. In addition, the mentum morphology of both insect groups suggests a trophallaxis relationship.

Corotoca melantho is a species with ovoviviparous development (Liebherr & Kavanaugh, 1985), which may facilitate its survival within the termite nests, once females deposit larvae ready for pupation. The *C. melantho* have a short life cycle, and are thus quickly in touch with worker termites, from which they acquire food by trophallaxis (Costa & Vanin, 2010). Staphylinidae beetles with appendages on the curved and physogastric abdomen are common among species that coexist with ants and termites (Kistner, 1979). The termitophiles may request regurgitated food from the workers (Costa & Vanin, 2010), but may also feed on fecal matter and remains of dead animals (Costa-Lima, 1952).

Considering the interaction between the termite host and termitophile beetle, we investigate the hypothesis of a body shape similarity between the termitophile beetle *Corotoca melantho* Schiødte (Coleoptera: Staphylinidae) and workers of the termite *Constrictotermes cyphergaster* (Silvestri) (Isoptera: Termitidae).

Material and Methods

Examined Material

Twenty-four *C. cyphergaster* nests cohabited by *C. melantho* were collected in an area of Cerrado *sensu stricto*, a savannah biome, at the Parque Estadual da Serra de Caldas (PESCAN). PESCAN is located between Caldas Novas and Rio Quente (17°46’56” S and 48°42’35” W) in the state of Goiás, in the Central region of Brazil. The termite nests were removed from the trees, fragmented into small pieces and a sample of 10% was made to estimate the number of inhabiting individuals. The beetles were identified by comparison with the biological collection of the Zoology Museum of the University of São Paulo (MZUSP). The samples were fixed in 80% ethanol and stored in the laboratory at UnUCET/ UEG.

Morphometric Data

A total of 31 *C. cyphergaster* workers and 31 cohabiting *C. melantho* individuals were collected from 24 nests. Pictures of all specimens were taken in top and side views, with a 3MP digital camera integrated into a stereomicroscope with 10X fixed eyepieces and 30X magnification. The pictures were saved as JPEG images with a 2048x1536 resolution and transformed into TPS (*thin-plate spline*) files using TPSutil version 1.44 (Rohlf, 2009a). Four homologous landmarks represented as Cartesian coordinates (X, Y) were marked on the head of termites and beetles in top and side views (Fig 1), and five semilandmarks (type 3 Bookstein coordinates) were marked on the abdomen of termites and beetles in top and side views (Fig 1). These semilandmarks are not truly homologous because in *C. melantho* the abdomen overrides the thorax. The landmarks and semilandmarks were digitized with the software TPSDig version 2.12 (Rohlf, 2008b).

Fig 1. Distribution of landmarks and semilandmarks in side and top view for *Corotoca melantho* and *Constrictotermes cyphegaster* workers. Four landmarks were marked on the basis of the antennas and the occipital area of the head in top view (a and b). Four landmarks were marked on the basis of the antennas, postoccipital area, the mandible tip and vertex of the head in side view (c and d). Five semilandmarks were marked representing the first and penultimate tergites and the apical tip of last tergite of the abdomen in top view (a and b). Five semilandmarks were marked representing the first, middle and ultimate tergites and the middle sternite of the abdomen in side view (c and d).

The shape variables were obtained overlapping landmarks and semilandmarks through the general Procrustes alignment using the software TPSRelw version 1.46 (Rohlf, 2008c). This method calculates an average configuration (consensus) that minimizes the sum of squares of the distances between the points of each configuration and the reference configuration (landmarks).

The deformation analysis obtained by TPSRelw generates a new set of variables – *partial warps* – calculated as the orthogonal coordinates of each eigenvector. The relative warp method is based on the Principal Component Analysis of partial warp scores, run with $\alpha = 0$ to give the same weight to partial warps at different scales, and results in uniform components. The Thin-plate spline graphs were obtained using the software TPSSplin version 1.20 (Rohlf, 2004d). TPS softwares (Thin-Plate Splines) were obtained free of cost at the Stony Brook University Morphometric Website (http://life.bio.sunysb.edu/morph).

Data Analysis

We used centroid size, which is the square root of the summed square distances between each anatomical landmark/ semilandmark and the shape’s centroid to compare body shapes. The average sizes of the centroid of the species (*C. melantho* and

C. cyphergaster) were compared through a t-test ($P<0.05$). A regression analysis of the shape coordinates (dependent variable) and the centroid size (independent variable) ($P<0.05$) was carried out to evaluated if there is any effect of the size on the shape.

To distinguish shape variables (in top and side views) from termitophile beetles and worker termite hosts a Simple Discriminant Analysis was used, once a single function is needed to distinguish the two clusters. The parity of the means of the two groups was tested using Hotelling's T^2 ($P<0.05$) (Doornik & Hansen, 2008) with 10000 permutations, to meet the multinormality assumption. The percentage of correctly classified individuals was estimated using the Jackknife method to avoid bias in the initially classified group. The Discriminant Analysis carried out uses a linear model to visually confirm or reject the hypothesis that the two species are morphologically distinct (Legendre & Legendre, 2004). All statistical analyses were carried out using R (R Development Core Team 2014).

Results

There were in average 0.002 *C. melantho* individuals for each *C. cyphergaster* worker. A total of two, four or six specimens of termitophile beetles were found for 67% of the *C. melantho* recorded in the sampled nests. However, 18 termitophiles were found in one of the largest termite nests.

The body shape, initially represented by the centroid size of *C. cyphergaster* workers and the termitophiles is similar in side view ($t_{df=60}$= 2.0707; P= 0.0431), but differed in top view ($t_{df=60}$= -1.1847; P= 0.2412). The thin plate splines show deformations in the variations of head and body shape (Fig 2).

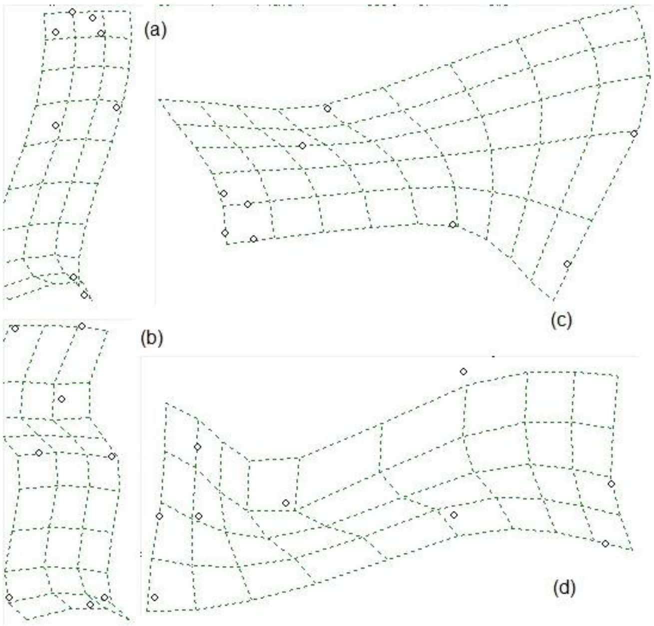

Fig 2. Thin plate spline representation of principal trends (relative warps) in the variation of the body shape. Grid show the deformation of the average shape in opposite directions of the first relative warps for *Corotoca melantho* and *Constrictotermes cyphergaster* in top view (a and b) and in lateral view (c and d).

The termitophile beetles are slightly smaller than worker termites. Still, the difference in body size did not affect body shape for either species. Body size of *C. cyphergaster* has no effect on body shape in top view (r^2= 0.3378; $F_{(gl=14,\ 16)}$= 0.5830; P= 0.8418) or in side view (r^2= 0.2102; $F_{(gl=14,\ 16)}$= 0.3041; P= 0.9847). Body size of *C. melantho* has no effect on body shape in top view (r^2= 0.5469; $F_{(gl=14,\ 16)}$= 1.3790; P= 0.2664) or in side view (r^2= 0.6299; $F_{(gl=14,\ 16)}$= 1.9450; P= 0.1014).

The body shape of both species is similar in side view (T^2= 1.1516; df= 14, 47; P= 0.3317) and top view (T^2= 1.2941; df= 14, 47; P= 0.2712). A total of 70.97% of the specimens were correctly classified in side view and 69.35% of specimens were correctly classified in top view, according to the discriminant analysis (Fig 3). However, after applying the Jackknife method, the accuracy in the classification of individuals into each group dropped to 50% and 56.45%, in top and side views, respectively.

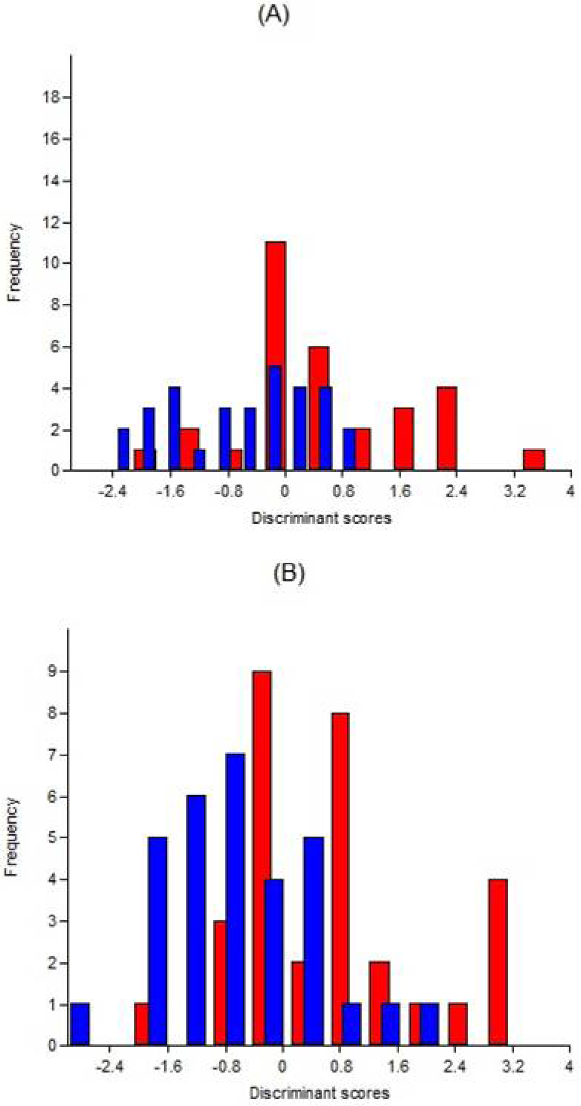

Fig 3. Frequency of discriminant scores for *Corotoca melantho* (red) and *Constrictotermes cyphergaster* (blue) in side view (A) and in top view (B).

Discussion

We found that the *C. melantho* population is higher in larger *C. cyphergaster* termite nests, as already described by Cristaldo et al. 2012. Rosa (2008) reported Staphylinidae beetles as termitophiles in nests of 104 termite species. However, studies regarding termitophile Staphylinidae in Brazil are restricted to the Borgmeier (1923, 1935, 1954, 1959 *apud* Rosa, 2008), Costa-Lima (1952), Cunha & Brandão (2000), Costa et al., (2009) and Cristaldo et al. (2012). In the field, beetles were observed walking inside the nest, near termites, with no antagonistic reaction from its worker termite hosts (personal communication). The diversity of staphylinids observed to be cohabiting termite nests suggests the evolution of a specific interaction between some termite pairs and staphylinid species, which include adaptations such as morphological mimicry (our results) and chemical mimicry (Rosa, 2012).

No difference in *C. cyphergaster* workers and *C. melantho* body shape was observed in the morphometric analysis (see fig 2 and 3). The body shape can be represented by centroid size and shape variables (the partial warps and uniform components). The new shape variables, obtained by overlapping the nine landmarks and semilandmarks by Procrustes alignment, showed that the termitophiles and workers of termite hosts are morphometrically similar. From 20% to 30% of the body shape variation is due to variation on termite host body size. However, body size for termitophiles affects from 50% to 60% of the body shape (see r^2 of the regression between body size and body shape). The Discriminant Analysis confirmed that the specimens of termitophile beetles and termite host worker are indistinguishable (see fig 3). Figure 3 shows higher variation among termitophiles than among the termite hosts. Despite the visual difference on head shape between workers of termite hosts and termitophiles, the average shape is similar in both species (as corroborated by discriminant function).

To our knowledge, morphometric geometric analysis has never been used to compare morphological similarity between termites and termitophiles. Traditional morphometric estimates the variation and covariation of distance measures between pairs of points – length and width of morphological structures (Monteiro & Reis, 1999; Zelditch et al., 2012). According to these authors, geometric morphometry enables analyzing the overall shape of the individual regardless of its size, and locates and describes the regions of shape changes, represented by anatomical points in homologous structures. Although the results indicate that *C. melantho* can mimic the morphology of workers of *C. cyphergaster*, we cannot say that both species co-evolved, because such a statement would require a phylogeny analysis of both species in search of reciprocal adaptations. The interaction between *C. melantho* and *C. cyphergaster* should be investigated by additional analyses, such as behavioral experiments inside the nests and phylogenetic comparisons.

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