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Differences in wings may be sufficient to separate the sexes and two species of *Gynaikothrips* Zimmermann (Thysanoptera: Phlaeothripidae)?

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Abstract. In this study, we use geometric morphometry to discriminate thrips of the species *Gynaikothrips uzeli* (Zimmerman) and *Gynaikothrips ficorum* (Marchal) and also to detect sexual dimorphism in these species. Two hundred individuals, one hundred females and one hundred males, from *G. uzeli* and *G. ficorum*, were used to verify sexual dimorphism. For interspecific differentiation, two hundred females were used, one hundred individuals of each species. It was possible to observe differences in the shape of the wing between sexes in both species. In *G. uzeli*, the first two main components explain 92.5% of the total variation of individuals. The first main component explains 87% and the second 5.5 of the total variation of individuals. For *G. ficorum*, the first two main components explain 78.2% of the total variation of individuals. The first principal component contributed with 62% and the second principal component with 16.2% of the variation of the shape of the wing. Besides, significant interspecific differences were observed in the shape of the wing, where the first two main components were sufficient to explain 86% of the total variation of the individuals. The first principal component explained 76.2% and the second 9.8% of the total variation of the individuals, being possible to verify differences in the shape of the wing of these two species. Geometric morphometry is a viable technique for assessing sexual dimorphism, as well as interspecific differences in the shape of the wings of these species, which are morphologically very similar.

Keywords: Galling insects; *Gynaikothrips ficorum*; *Gynaikothrips uzeli*; morphological analysis and sexual dimorphism.

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The diversity of insects regarding the shape is the result of changes in the size of body parts that may be the result of genetic differences or environmental factors, depending on the phenotypic plasticity degree (Mirth *et al.* 2016). However, it is necessary to use a method that allows the perception and quantification of these variations.

Unlike the traditional morphometry that studies the variation and covariation of linear distance measurements between anatomically homologous points, the geometric morphometrics allows an analysis of the variation of shape and size of a given structure, such as abdomen in Coleoptera (Espinoza-Donoso *et al.* 2020), ovipositor in Hymenoptera (Wang *et al.* 2020), cephalopharyngeal region in Diptera (Sim & Zuha 2019), thorax in Odonata (Ning *et al.* 2019), mandible in Lepidoptera (Millan *et al.* 2018) or legs in Hemiptera (Miller *et al.* 2016) using multivariate statistical methods and digital tools. However, wings of insects are one of the main organs since their two-dimensional structures are suitable for morphometric description (Lorenz *et al.* 2017), for example in the identification of mosquitoes of the subgenus *Culex* (*Culex*) Linnaeus (Diptera: Culicidae) (Simões *et al.* 2020), population structuring in *Triatoma brasiliensis* Neiva (Hemiptera: Triatominae) (Kamimura *et al.* 2020), identification of morphological similarities of two species of Euglossini (Hymenoptera: Apidae) (Grassi-Sella *et al.* 2018). Geometric morphometry presents some advantages, among which are the low cost when compared to studies that use molecular techniques that depend on expensive equipment and reagents to carry out the analyses; possibility to use dry or fresh specimens, besides being an effective technique regarding the classic taxonomy in the identification of species (Lyra *et al.* 2010; Simões *et al.* 2020).

Morphometric variations influenced by environmental or genetic factors can give rise to traces of sexual dimorphism that is evidenced in body size and shape (Virginio *et al.* 2015; Mirth *et al.* 2016; González-Rubio *et al.* 2017). The differences in certain structures are related to functional aspects and characteristics of the life history of insects (Vergara *et al.* 2014). Insects are recurrent models in studies on sexual dimorphism since the differences between males and females can appear in several organs, such as the eyes (Hilbrant *et al.* 2014) and the legs (Miller *et al.* 2016). Other characteristics may also show dimorphism, such as the shape of the body, genitalia (von Groll & Moura 2017), and wings (Benítez & Vargas 2017). The geometric morphometrics technique enabled the identification of sexual dimorphism using wings as in the case studies involving the species, *Aedes aegypti* (Linneaus) (Diptera: Culicidae) (Chaiphongpachara & Laojun 2019), *Centris tarsata* (Smith) (Hymenoptera: Apidae) (Souza *et al.* 2018), *Macaria mirthae* (Vargas) (Lepidoptera: Geometridae) (Benítez & Vargas 2017). In addition to solving questions about sexual dimorphism, geometric morphometrics can be used to separate insect species with complicated taxonomy and served as an argument for taxonomic decisions (Godoy *et al.* 2018; Abd *et al.* 2020; Simões *et* *al*. 2020; Santos *et al*. 2021).

In the present study we used *Gynaikothrips uzeli* (Zimmerman) and *Gynaikothrips ficorum* (Marchal) (Thysanoptera: Phlaeothripidae), because these two species have relation host, respectively, with the plants *Ficus benjamina* (Moraceae) and *Ficus microcarpa* L. (Marchal) (Moraceae) (Tree *et al*. 2015). *G. uzeli* is very similar in structure to *G. ficorum*, the only difference observed between them is the length of the pair of the posteroangular setae of the pronotum (Mound *et al*. 1996; Tree 2012). However, populations of different locations may present considerable variation in the length of the setae since the size is a characteristic affected by a series of environmental conditions (Horne *et al.* 2019), on the other hand, the shape of the structures has evolutionary restrictions (Nunes *et al.* 2013). Mound *et al*. (1996) suggested that *G. ficorum* is, probably, a form of *G. uzeli* that has been disseminated worldwide by horticultural trade. Thus, this study aimed to verify if the geometric morphometrics can be used to differentiate the species *G. uzeli* and *G. ficorum*, as well as, test the effectiveness of this technique in differentiating the wing form between the sexes in these species.

MATERIAL AND METHODS

For the study of the wing difference between the sexes of *G. uzeli* and *G. ficorum*, individuals were collected from galls of trees of *F. benjamina* and *F. microcarpa,* respectively from March to July 2014. The galls were packed in plastic bags, labeled, and transported to the Laboratory of Insect Biology (LABI), Campus Jequié, where the adults were screened and separated for morphometric analysis. 200 individuals, 100 females and 100 males were used for each species. Each individual was dissected with the aid of tweezers and a fine-tipped stylus to ensure the sex of the insect. This procedure was carried out to identify the sexes in both species.

To assemble the slides, the right anterior wings were removed with the help of fine-tipped forceps and fixed on microscope slides with white glue diluted in distilled water. Soon after, the wings were photographed in a Leica S8APO stereomicroscope with digital image capture system, using the program Application suite version 3.4.1.

Then, a file with TPS extension was generated using the Tps Util software, later, the landmarks and semi-landmarks (Figure 1) were inserted in the wings using the TPS Dig2 software (Rohlf 2006). After obtaining the Cartesian coordinates, the Procrustes superimposition, Principal Components Analysis (PCA), discriminating function, and cross-validation were performed using MorphoJ (Klingenberg 2011) and PAST (Paleontological Statistic).

The same methodology for collecting and assembling the slides was used for the analysis of the distinction of the shape of the interspecific wing. In this analysis, statistical analyses of discriminant function and cross-validation. In this study, 100 females of each species were used.

RESULTS AND DISCUSSION

It was possible to observe significant differences in the shape of the wing between the sexes in the two species analyzed ($p<0.001$). For *G. uzeli*, the first main component explains 87% and the second 5.5% of the difference between the wings of males and females (Figure 2). For *G. ficorum*, the PCA also showed differences in the shape of the wing between the sexes, the first two components explained 78.2% of the total variation of the individuals (Figure 3). The first principal component contributed with 62% and the second principal component with 16.2% of the variation of the shape of the wing. Analyzing the shape of the wing, it is possible to verify differences in the wing shape of the sexes it was observed that in males the wing is shorter and wider, while in females the wing shape is more elongated (Figure 4 and 5).

The presence of sexual dimorphism for the shape of the wing was verified from the results obtained, females present greater variation in the shape at the apex of the wing where the established landmarks eight is located, suggesting some type of natural selection in the shape of the wings of these females.

Morphological variations in a given structure can reveal the evolutionary history of a group of organisms and their way of using the environment. According to Hernández *et al* (2015), the modifications in the wings in pterigote insects are not random but are related to environmental factors and geographical causes that influence in the gender, types of metamorphosis, taxa, and reproductive strategies. Thus, the variation in the shape between males and females could play an important role in the flight aerodynamics and in its dispersion process. Although it has a clear function for locomotion, the wings of insects can still exhibit other biological functions, such as protection and defense, foraging, thermoregulation, and sexual differences (Pass,

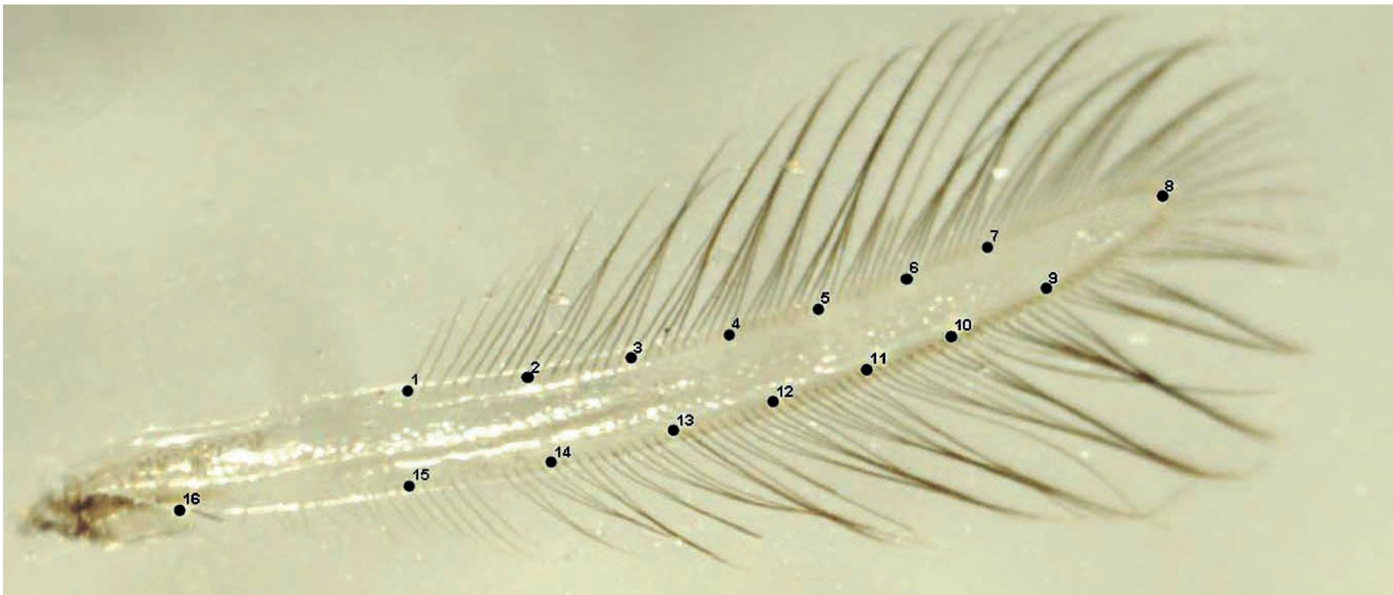

Figure 1. Right anterior wing of females of *Gynaikothrips uzeli* with anatomical marks (1, 8, 16) and semimarks (2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14 and 15). Source: authors.

2018). Females of *G. uzeli* and *G. ficorum* tend to have a more elongated wing than the male (Figure 4 and 5).

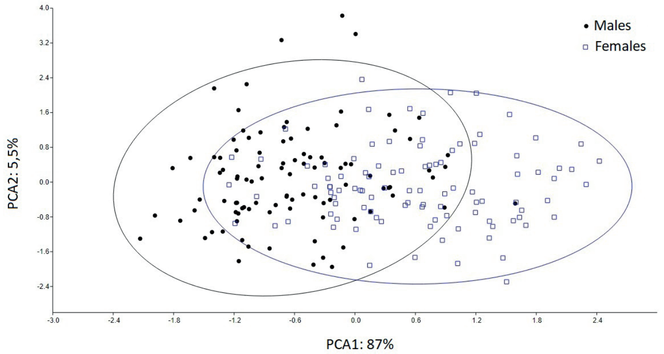

Figure 2. Graph of the principal components showing the separation between males and females of *Gynaikothrips uzeli*. Source: authors.

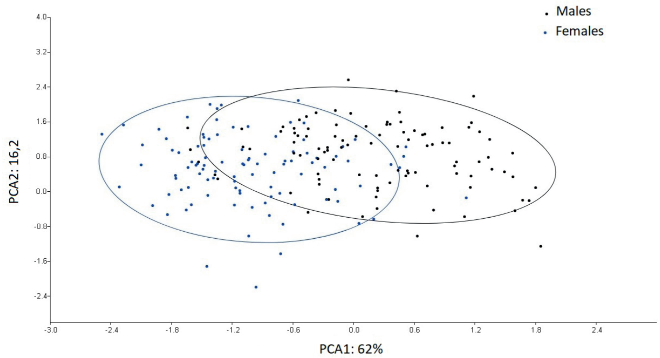

Figure 3. Graph of the main components showing the separation between males and females of *Gynaikothrips ficorum*. Source: authors.

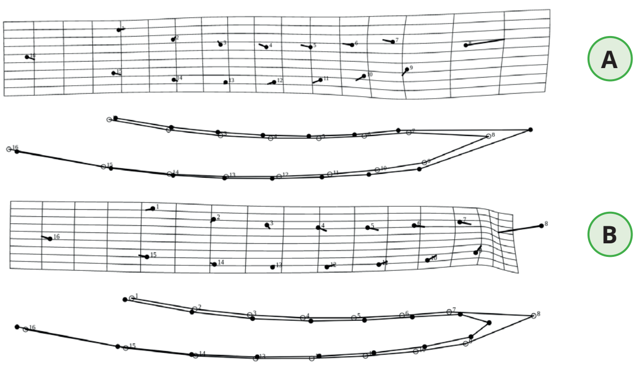

Figure 4. Wing-shaped deformation grids between males (A) and females (B) from *Gynaikothrips uzeli* (Zimmerman). Source: authors.

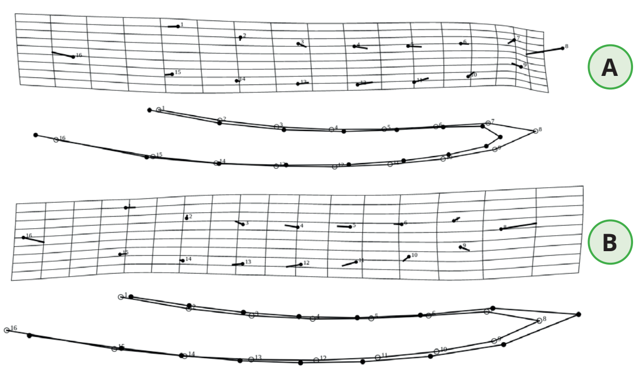

Figure 5. Wing-shaped deformation grids between males (A) and females (B) of *Gynaikothrips ficorum* (Marchal). Source: authors.

For the interspecific analysis, it was possible to notice significant differences (p<0.001), where the first two main components were sufficient to explain 86% of the total variation of the individuals, indicating differences in the shape of the wing of the females between the two species. The first principal component explained 76.2% and the second 9.8% of the total variation of the individuals (Figure 6); this difference can be observed by the thin-plate splines (Figure 7). In the analysis of the cross-validation, we had significant differences (p<0.01) in the separation of the species, and the analyzed individuals were correctly classified in 70% within of your own species (Figure 8). Similar results were found with the analysis of the distance of Procrustes and Mahalanobis with 10,000 permutations.

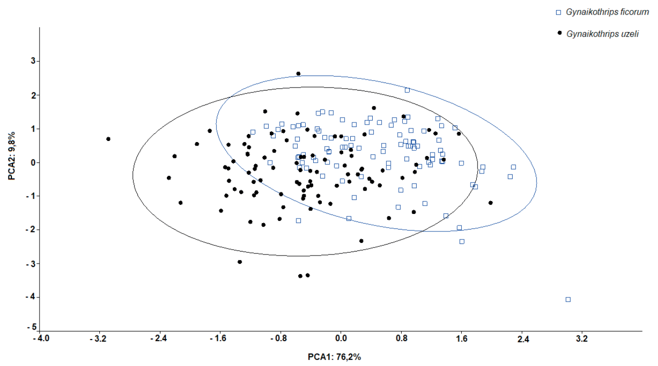

Figure 6. Graph of the main components showing the separation of the species *Gynaikothrips ficorum* (Marchal) and *Gynaikothrips uzeli* (Zimmerman). Source: authors.

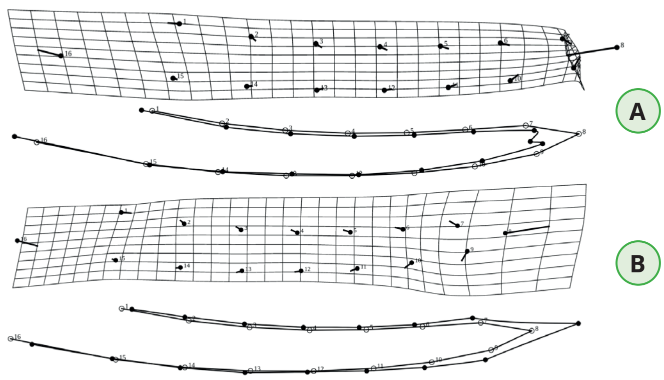

Figure 7. Deformation grids indicating the differences in the shape of the wings of females of *Gynaikothrips ficorum* (Marchal) (A) and *Gynaikothrips uzeli* (Zimmerman) (B). Source: authors.

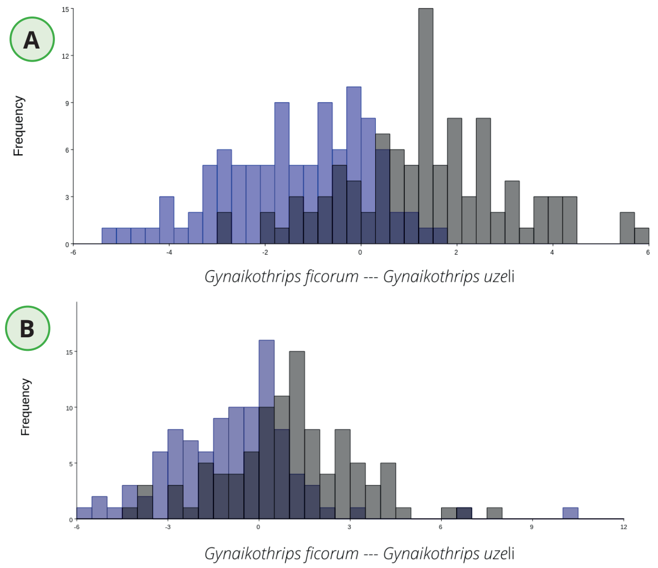

Figure 8. Results of the discriminant analysis (A) and cross-validation (B) peer-to-peer of the species. Blue represents the *Gynaikothrips ficorum* species and gray *Gynaikothrips uzeli*.

When the shape of the wings of the two species is compared, it can be noticed that the wings of *G. uzeli* have a narrower shape near the apex of the wing, forming a triangle in the final portion. As observed in the wing sexing in these species, the landmark that highlighted more was the landmark eight located at the apex of the wing.

These two species are part of a group that is difficult to distinguish by characteristics such as color, size, and morphology (Tree *et al*. 2015). The species have few characters of taxonomic importance that differ them. The main characteristics are the difference in the length of the pair of the posteroangular setae of the pronotum and host plant (Mound *et al.* 1996; Tree 2012). According to Mound *et al.* (1996), these differences would not be enough to separate these closely related species, suggesting that these differences are more ecological and evolutionary. However, Retana-Salazar (2006) found out, using the same species, that they have morphological differences in the posteroangular and epimeral setae and that this morphological variation occurred due to the isolation of these species. Thereby, the wing geometric morphometrics of these species contributes to an approach that makes it possible to highlight the interspecific differences and that the variation in the shape of the wing between the sexes and species may have been determinant for this difference to be established. Other studies that use the geometric morphometrics verified that this technique is a great method to analyze sexual differences in the wings, as in the case of the butterflies of the species *Macaria mirthae* Vargas, Parra & Hausmann (Benítez & Vargas 2017) or to differentiate species that are not easy to identify from the external morphology, as in the case of the subgenus *C*. (*Culex*) species (Simões *et al.* 2020).

The results presented herein allow us to infer that they are two different species. This study showed to be promising for the understanding of these two species that have complex taxonomy. However, in order to accurately state that they are two different evolutionary units it is necessary to include other analyses, such as the genetic and behavioral ones.

There are differences in the shape of the wings of the females and males of *G. uzeli* and *G.ficoum* evidencing the sexual dimorphism in these species and that there are differences in the shape of the wings of females of *G. uzeli* and *G.ficoum*.

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