Evaluating Sexual Dimorphism in the Ambrosia Beetle Xyleborus affinis (Coleoptera: Curculionidae) Using Geometric Morphometrics

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Evaluating sexual dimorphism in the ambrosia beetle *Xyleborus affinis* (Coleoptera: Curculionidae) using geometric morphometrics

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Abstract

Species within genus *Xyleborus* Eichhoff (Curculionidae: Scolytinae) live in a feeding symbiosis with ambrosia fungi and present inbreeding polygyny with highly biased sex ratios and sexual dimorphism in size. Here we evaluate the expression of sexual dimorphism, the allometric effect on shape variation, and the morphological integration of 2 body structures (elytra and pronotum) in *Xyleborus affinis* (Eichhoff) (Curculionidae: Scolytinae) under controlled growth conditions (26 °C, 60% RH, and continuous darkness), using geometric morphometrics. Differences between sexes from statistical multivariate tests indicate the presence of sexual size and shape dimorphism in both structures, with the largest differences and shape variability presented in the pronotum. We found significant developmental integration of both structures (morphological covariation) and no significant differences in allometric trends between sexes. The results suggest a specific allometric pattern in *X. affinis* that could be constrained by genetic or phylogenetic factors. However, quantification of shape and size variation could help to determine the differential effects of environmental stress between sexes during growth in this species. This approach could be valuable for monitoring populations of other *Xyleborus* species living in contrasting environmental conditions, given their wide geographical distributions and the vast amount of host plant species with which they interact.

Key Words: ambrosia fungi; morphological variation; secondary sexual characters; Scolytinae; wood boring beetles

Resumen

Las especies del género *Xyleborus* Eichhoff (Curculionidae: Scolytinae) viven en simbiosis de alimentación con hongos ambrosiales, y que presentan endogamia poligínica, con proporciones sexuales sesgadas y dimorfismo sexual en tamaño. Usando un protocolo de morfometría geométrica evaluamos la expresión del dimorfismo sexual, el efecto alométrico sobre la variación de la forma y la integración morfológica de dos estructuras corporales (élitro y pronoto) en *X. affinis* (Eichhoff) (Curculionidae: Scolytinae) en condiciones de crecimiento controlado (26 °C, 60% HR y oscuridad continua). Los análisis soportan diferencias estadísticas entre los sexos e indican la presencia de dimorfismo sexual en tamaño y forma de ambas estructuras, con mayores diferencias y alta variabilidad de la forma del pronoto. No hubo diferencias significativas en las tendencias alométricas entre los sexos y se encontró una integración significativa (covariación morfológica) en el desarrollo de ambas estructuras. Los resultados sugieren un patrón alométrico específico para *X. affinis* que podría estar limitado por factores genéticos o filogenéticos. La cuantificación de la variación de la forma y el tamaño podría ayudar a explorar los efectos diferenciales del estrés ambiental entre los sexos durante el crecimiento en esta especie. Este enfoque podría ser valioso para monitorear poblaciones de otras especies de *Xyleborus* que viven en condiciones ambientales contrastantes, dada su amplia distribución geográfica, y que crecen en diferentes especies de plantas hospederas.

Palabras Claves: caracter sexual secundario; escarabajos barrenadores; Scolytinae; hongos ambrosiales; variación morfológica

Scolytines represent a diverse group of beetles with worldwide distributions and interesting evolutionary history, including their differences in size, shape, and feeding habits. Within this large group of beetles, ambrosia fungi and phloem feeding are the 2 primary feeding habits (Hulcr et al. 2015). Ambrosia beetles constitute an example of the most common symbioses in any forest ecosystem around the world (Foelker & Hofstetter 2014). These insects create complex networks of tunnels within a large number of woody plant species in which they cultivate a variety of ambrosia fungi from which all developmental stages feed (Beaver et al. 1989; Gebhardt et al. 2004). Typically, fe-

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males initiate their own gallery, where laying eggs and mating between siblings takes place within the galleries (Norris & Chu 1970; Beaver 1976; Kirkendall et al. 1997; Biedermann et al. 2009).

The repeated evolutionary origins of inbreeding and fungus farming in bark and ambrosia beetles (Curculionidae: Scolytinae), including species within *Xyleborus*, promoted specific traits such as highly biased sex ratios, usually characterized by flightless males (haploid) of smaller size than the females (Kirkendall 1983; Johnson et al. 2018). However, the haplodiploidy sex determination system is more common in *Xyleborus* and closely related genera (Kirkendall 1993; Cognato et al. 2011). Females may disperse from their natal galleries, and are exceptionally efficient at colonizing and establishing in new areas by the mating system of daughters with parthenogenetically produced sons (Kirkendall et al. 1997; Gohli et al. 2016), whereas the males could disperse only to nearby galleries searching for mating opportunities (Peer & Taborsky 2004), although this has not been sufficiently documented. Given this mode of dispersal, the galleries are composed of highly related individuals and the social system is determined by their mating system (Atkinson & Equihua-Martínez 1986; Jordal et al. 2000). Due to these reproductive patterns and genetic differences derived from sex determination system, sexual differentiation in size and morphology has been described during development in *Xyleborus* species (Kingsolver & Norris 1977a). However, there is still little information about their sexual differentiation process and expression of dimorphism (Biedermann et al. 2009).

Sexual size dimorphism is determinant in reproductive success and has been studied broadly in insects (Bonduriansky & Rowe 2003; Bonduriansky 2006, 2007; Chazot et al. 2016). In general, sexual size dimorphism in insects is associated with differential selective pressures between sexes, such as social inbreeding, sex-specific developmental regulators, larval diet quality, larval density, and temperature (Badyaev 2002; Bonduriansky 2007; Stillwell et al. 2010). In Scolytinae beetles, expression of sexual size dimorphism has been related to environmental conditions, larval density, and the availability of resources in the branches within which they develop (Richards 1927; Foelker & Hofstetter 2014). Sexual size dimorphism has been measured with traditional morphometric distances related to body size variation in secondary sexual characters (Richards 1927), such as the length and width of the head, elytra, and pronotum (Kingsolver & Norris 1977b; Foelker & Hofstetter 2014). In contrast, patterns of sexual shape dimorphism largely have been neglected despite the importance of shape to ecological functions such as feeding, dispersal, mating, parental care, and other life history aspects (Gidaszewski et al. 2009; Berns & Adams 2010; Worthington et al. 2012).

Sexual size dimorphism has been reported in *Xyleborus* (Norris & Chu 1970; Wood 1982; Kirkendall 1983; Brar et al. 2013) associated with haplodiploid sex determination and high levels of inbreeding (Peer & Taborsky 2007). Body structures such as elytra and pronotum also have been demonstrated as suitable characters for the study of sex differences in other species of the subfamily Scolytinae (Hopkins 1894; Schlyter & Cederholm 1981; Foelker & Hofstetter 2014). Nevertheless, linear distances are correlated highly with size, and pure shape variation could be better studied using a geometric morphometric approach, which allows the independent analysis of shape and size and the extent to which shape is explained by size variation (allometry) (Adams et al. 2004; Worthington et al. 2012; Zelditch et al. 2012), and a more comprehensive quantification of biological shape and, consequently, on the evolutionary processes behind it (Adams & Otálora-Castillo 2013).

Patterns of body shape allometry that could be related to sexual shape dimorphism have not been quantified in species of *Xyleborus*. Considering that sexual selection in haplodiploid systems affect the evolution of exaggerated male traits, to identify the prevalence of such traits and the sexual dimorphism expression could help to describe sexual behavior and evolutionary genetics in haplodiploid systems (De la Filia et al. 2015). Geometric morphometrics could constitute a better approach to understanding sexual selection in somatic characters in this group of species. Quantification of sexual size dimorphism and sexual shape dimorphism in *Xyleborus* species under controlled conditions may provide a starting point to study the expression of sexual dimorphism in this genus. To test whether levels of sexual trait expression are condition-dependent (e.g., on food availability or temperature and humidity), it is important to investigate the response of these traits over a broad range of natural or extreme environmental conditions (Cotton et al. 2004, 2006).

Here, we apply a geometric morphometric protocol to evaluate shape variation and to quantify the sexual size dimorphism and sexual shape dimorphism in 2 morphological structures of *Xyleborus affinis* Eichhoff (Coleoptera: Curculionidae), a cosmopolitan Xyleborini that can be reared easily under experimental conditions (Biederman et al. 2009). Moreover, we determine whether these 2 body structures are covariant, which would indicate their morphological integration. To determine sexual size dimorphism and sexual shape dimorphism in *X. affinis*, we ask the following questions: (1) to what degree is variation of the pronotum and elytra shape explained by size variation in *X. affinis*?; (2) do the 2 morphological structures (elytra and pronotum) of the body shape change in an integrated manner between sexes?; and, therefore, (3) is there sexual dimorphism in the shape and size of both structures? Addressing these questions represents a starting point from which to compare the morphological response of this species under different environmental growth conditions, while contributing to our understanding of these little-known but highly important species for forest and ecosystem regulation of agricultural pests.

Material and Methods

Morphometric data was obtained from 49 individuals (19 males and 30 females), reared under experimental conditions at 26 °C, 60% RH, and continuous darkness, modified from Biedermann et al. (2009). Specimens were reared in the Molecular Entomology laboratory of the Instituto de Ecología, A.C., Xalapa, Mexico. Digital photographs of dorsal view of all samples of *X. affinis* were taken using a Leica Z16 APO camera (Leica Microsystems, Wetzlar, Germany). Two-dimensional coordinates of landmarks and semi-landmarks were digitized from the images of the pronotum and elytra (300 dpi in TIF format) using the software TpsDig 2.12 (Rohlf 2008).

We performed a repeatability test, digitalizing the same set of landmarks on a sample of 20 randomly selected specimens (3× per individual) to select landmark configurations. We estimated the variance ratio between replicates and the total sample in order to evaluate the digitalization error. The digitalization error (0.003) was lower than 0.05, and therefore represents a non-significant proportion of the variance in landmark configurations (Pizzo et al. 2006). In the case of the elytra, we analyzed the average value of the 2 sides of the body, using a configuration of 3 landmarks and 7 semi-landmarks to describe the contour of the elytra (Fig. 1A). For the pronotum, we also analyzed the average of the 2 sides, using a configuration of 3 landmarks and 5 semi-landmarks, while the midline of the body was fixed with 2 landmarks. Semi-landmarks were established using guides drawn in the software Make Fan 8 (Sheets 2014) and then digitized in TpsDig 2.12 (Rohlf 2008).

Shape variation of geometric morphometric data was analyzed using routines in the package geomorph (Adams & Otárola-Castillo 2013)

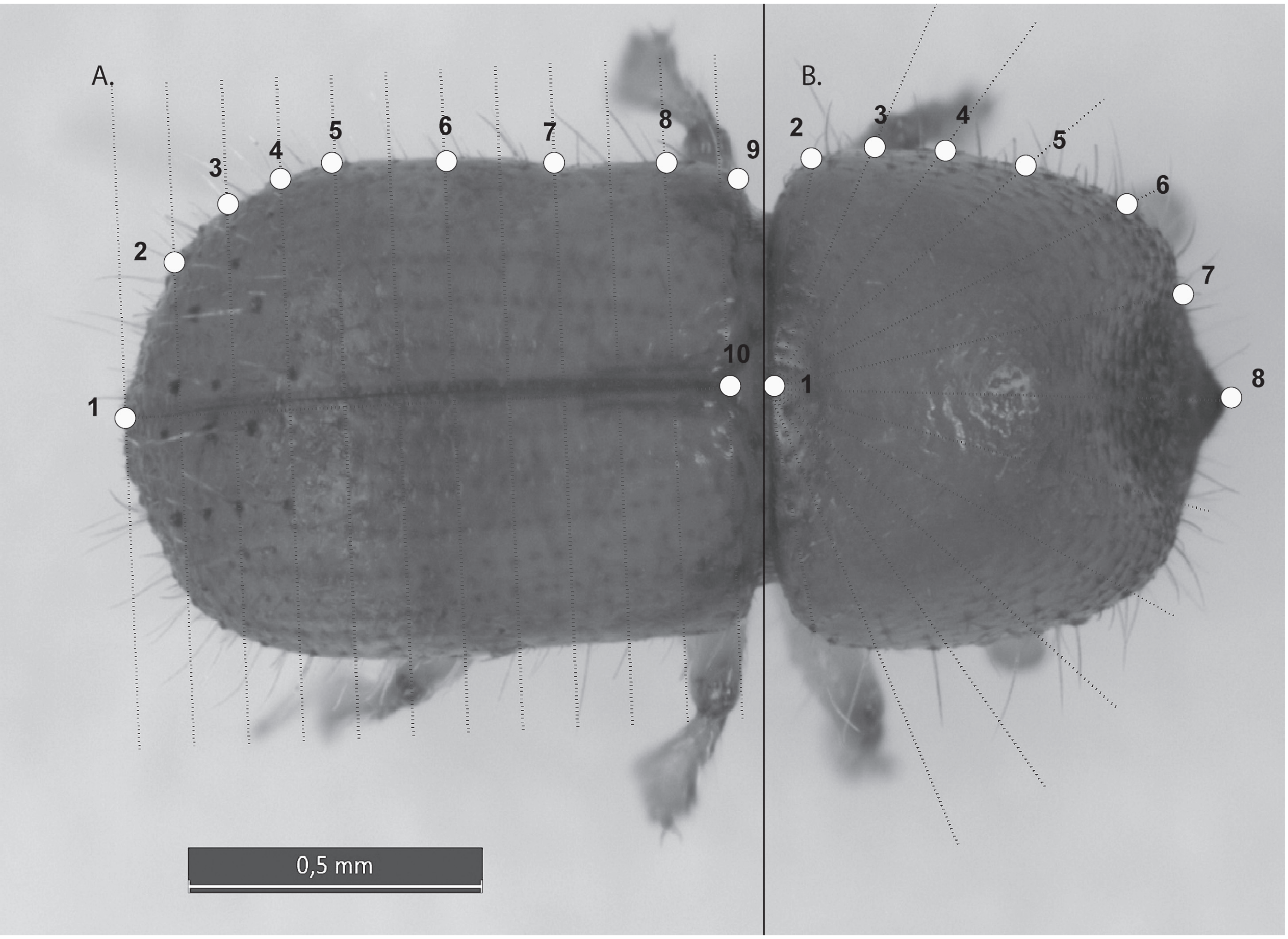

Fig. 1. Configuration of landmarks and semi-landmarks used to register 1 side of the 2 body structures (elytra and pronotum) of *Xyleborus affinis*: (A) configuration of 3 landmarks (1, 9, 10) and 7 semi-landmarks (2–8) describing elytra shape; (B) configuration of 3 landmarks (1, 2, 8) and 5 semi-landmarks (3–7) describing pronotum shape.

of the statistical computing environment R v3.3.3 (R Development Core Team 2017). First, we obtained a size estimator called centroid size, which corresponds to the sum of the Euclidian distances of each landmark to its configuration centroid, or the average coordinate in x and y (Zelditch et al. 2012). Individual differences in landmark configurations resulting from the orientation, position, and size of specimens then were removed by superimposing the configurations using a Generalized Procrustes Analysis. Finally, the semi-landmarks were aligned by sliding them on tangent planes onto the respective surface in order to remove the effects of the arbitrary spacing and to optimize their position with respect to the average shape of the entire sample (Gunz & Mitteroecker 2013). From the projection of these aligned configurations onto a tangent space, we obtained descriptors of shape comprising shape vectors (partial warps) as well as the Procrustes distances between each configuration and the consensus shape (Rohlf & Slice 1990), which was used in all subsequent analyses. All analyses were conducted with package geomorph in R (R Development Core Team 2017).

We evaluated the proportion of shape variation explained by differences in size (allometry) and the interaction between size and sex for each shape configuration. For this, we performed multivariate regressions of shape variables (partial warps) on centroid size, the size estimator of each configuration. In order to estimate the strength of this relationship in each structure, the allometric trends between sexes were described using the predicted shapes by the regression of the first principal component of shape variables on centroid size. The centroid size was log transformed and normally distributed in both structures ($W > 0.93$; $P > 0.05$). We performed a test of homogeneity of the allometric slopes between the sexes for each structure.

Morphological integration between the two body structures (elytra and pronotum) was tested using a partial least squares analysis (Adams & Collyer 2016). This analysis examines the maximal covariation between blocks of shape variables; it calculates singular vectors from the covariance matrices of each shape character and evaluates the correlation between the first singular vectors using a bootstrap resample test with 1,000 replicates to assign the statistical significance of the correlation (Zelditch et al. 2012; Adams & Collyer 2016).

To evaluate the effect of centroid size and sex on shape variation in each (elytra and pronotum) configuration, we performed Procrustes ANOVA analyses and used the *t*-test to evaluate sexual size dimorphism in centroid size. Generalized Goodall's *F* statistics were used to test the contribution of the independent variables (size, sex) and their interaction to shape variation, calculated as a Procrustes distances variance. The significance of this variation was estimated using a permutation test of the residuals of this model (1,000 replicates) and the *P* value was the proportion of random samples that provided a better fit than the original model (Collyer et al. 2015).

We describe sexual shape dimorphism in the elytra and pronotum. We calculated deformation grids from the ordination of configurations using the principal component analyses. The pronotum configuration was reflected in order to visualize the complete structure in deformation grids. We tested the significance of the Procrustes distance between average shapes for each sex and each structure using Goodall's *F* statistic and a Bootstrap resample test with 1,000 replicates. Furthermore, we compared the shape variability between sexes for each structure through morphological disparity (Foote 1993). We used the general morphological disparity as an index of morphological variance in morphospace, estimated as a Procrustes distance variance or the sum of the diagonal elements of the group covariance matrix divided by the number of observations in the group (Zelditch et al. 2012). Group differences in Procrustes variances were evaluated statistically through a permutation test with 1,000 replicates, where the vectors of the residuals are randomized among the groups (Adams & Otárola-Castillo 2013).

Results

A low but significant proportion of variance in pronotum and elytra shape was explained by centroid size variation. The proportion of total shape variation explained by differences in size of the elytra was lower (9%; $F_{16,736} = 4.479$; $P = 0.02$) than that of the pronotum (14%, $F_{21,1128} = 6.917$; $P = 0.01$). While the shape variation in relation to centroid size showed opposite trends between structures in the predicted regression lines, the pronotum changed toward the positive direction of the deformation whenever (centroid) size increased. In contrast, the elytra changed toward the negative direction of the deformation when size increased (Fig. 2). The test of homogeneity of slopes supports parallel allometric slopes between sexes for both structures ($F < 1.32_{45,44}$; $P > 0.13$).

The partial least squares analysis showed a significant correlation between the 2 matrices (pertaining to elytra and pronotum) describing the body shape. The highest morphological integration in the body shape was obtained from the linear regression between the first partial least squares vectors of the pronotum predicting elytra shape ($R = 0.60$; $P < 0.01$) (Fig. 3). Residuals of this linear model were distributed normally ($W = 0.97$; $P = 0.21$).

We found sexual size dimorphism in the elytra and pronotum of *X. affinis*. There were significant differences between sexes in the centroid size of the pronotum ($t_{45.864} = 4.286$; $P < 0.001$) and the elytra ($t_{27.134} = 4.748$; $P < 0.001$). Size differences between sexes were more evident in the pronotum shapes than in those of the elytra (Fig. 4): females were larger and had higher variance ($\sigma^2 = 0.74$) than males in terms of pronotum size ($\sigma^2 = 0.28$), whereas males had higher variance ($\sigma^2 = 0.42$) than females in elytra size ($\sigma^2 = 0.21$).

With different levels of variance explained by sex, sexual shape dimorphism was observed in the elytra and pronotum. The Procrustes ANOVA results showed that a significant proportion of shape variance was explained by centroid size and sex in both structures, with the highest shape variances explained by sex differences (Table 1). For both shape configurations, sex differences were independent of size and, in both cases, the effect of the size/sex interaction was not significant (Table 1).

The ordination of configurations using the principal component analyses showed that extremes of variation were congruent with sex differences, and sexual shape dimorphism was more marked in the pronotum than in the elytra. Deformation grids indicated that females and males changed in opposite directions. In the case of the elytra, differences were observed in the medial landmarks (1, 10), indicating a narrowing of the elytra in females and the opposite pattern in males (Fig. 5). Deformation grids from the pronotum configuration showed the greatest landmark displacement in the posterior (landmarks 2–4) and the anterior (landmarks 7 and 8; Fig. 6) regions. The distances between the average shapes of the sexes were significant for both structures (elytra distance: 0.037; $F_{16,736} = 23.22$; $P = 0.004$; pronotum distance: 0.048; $F_{12,555} = 18.29$; $P = 0.005$). Moreover, the morphological disparity index was highest in males for the pronotum (males: 0.0022; females: 0.0013), whereas greater similarity was shown in the elytra (males: 0.0011; females: 0.001); the morphological disparity was significantly different between sexes only for pronotum shape ($P < 0.01$).

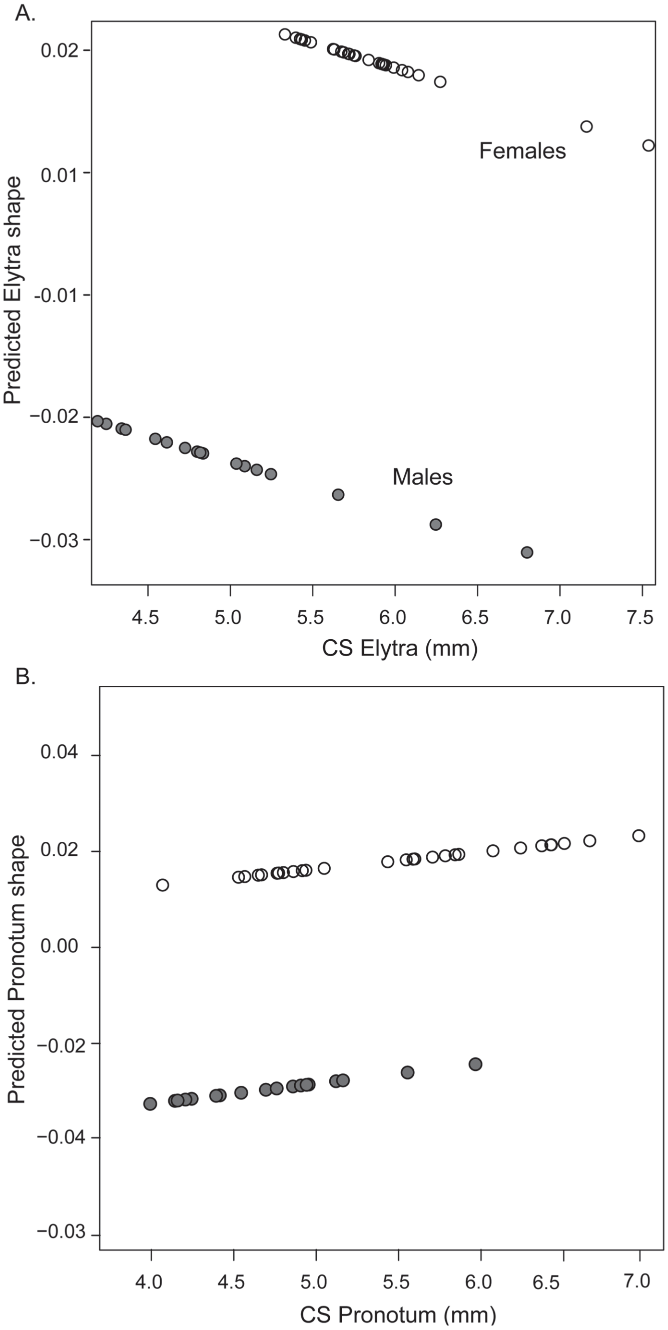

Fig. 2. Allometric regression of shape on centroid size for each sex: (A) predicted elytra shapes (Predline) to each centroid size; (B) predicted pronotum shapes (Predline) to each centroid size.

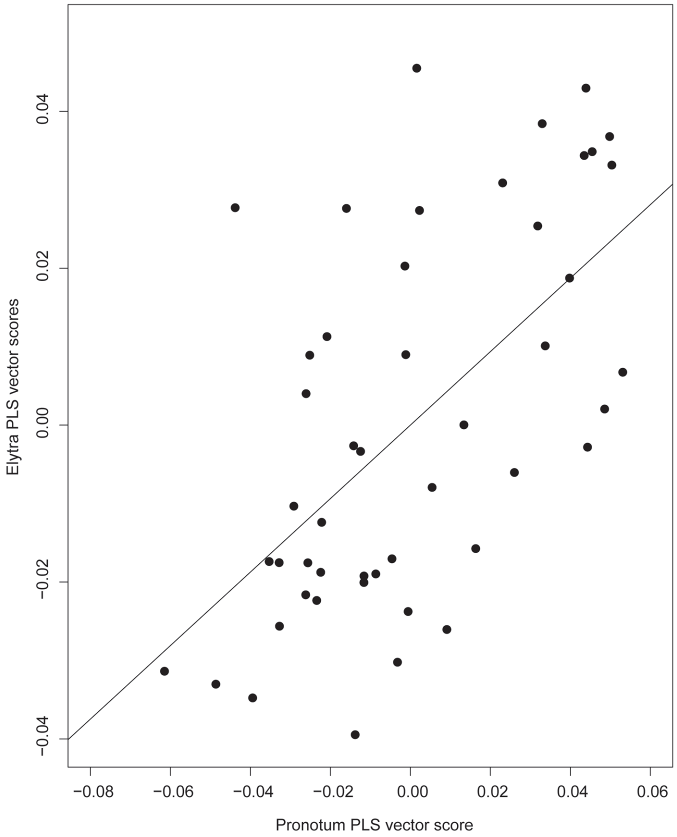

Fig. 3. Linear regression of partial least squares vectors from the pronotum shape matrix and elytra shape matrix.

Discussion

Sexual dimorphism in *Xyleborus* species has been documented widely using size and categorical descriptions (Norris & Chu 1970; Kingsolver & Norris 1977b; Mizuno & Kajimura 2002; Brar et al. 2013; Castro et al. 2019). In this study we quantified the sexual size dimorphism and sexual shape dimorphism of 2 body structures (elytra and pronotum) in *X. affinis* using a geometric morphometrics protocol and found a similar effect of size on the shape variation present in both. While sex differences in the developmental process have been documented in other *Xyleborus* species (Brar et al. 2013), we found that the effect of size on shape variation is not mediated by sex in either structure. Moreover, we found support for the morphological integration of the body elements in *Xyleborus* (Kirkendall 1983) and describe opposite trends in shape variation between both structures, in relation to sex differences.

In *X. affinis*, the amount of shape variation explained by centroid size is similar between sexes and between the 2 body structures analyzed. In general, there is a high correlation between both configurations (pronotum and elytra), suggesting morphological integration during development of body shape in this beetle species. This covariation could be the outcome of developmental interactions between morphological structures, or separate developmental pathways that are equally sensitive to external sources of variation (Klingenberg 2008). Morphological integration of the body structures in this species has not been tested previously but has been suggested from controlled growth experiments (Kingsolver & Norris 1977b). Besides their morphological integration, the shape and size of the elytra and pronotum differ between sexes, and such differences were more pronounced in the pronotum shape than in that of the elytra.

The trends of shape variation in relation to centroid size are opposite in each structure type. For elytra shape, we observed a negative allometric trend from males to females, which is explained by the narrowing of the elytra when the size (centroid) increases. In contrast, the pronotum shape showed a positive allometric trend from males to females, which is explained by the broader and more rounded pronotum shape with increased size. This could be related to the fact that males, as in several *Xyleborus* species, have the anterior slope of the pronotum concave or impressed (Rabaglia et al. 2006). Differences in size are more evident in secondary sexual characters (e.g., horns and mandibles), as has been observed for pronotum shape in other *Xyleborus* species (Kingsolver & Norris 1977a, b; Wood 2007; Foelker & Hofstetter 2014; Roeper et al. 2017) and many other insects, and are dependent strongly on developmental conditions (Richards 1927; Pizzo et al. 2006; Wood 2007; Stillwell et al. 2010).

Such differences in body size in ectothermic organisms are influenced very much by environmental variables such as diet quality and temperature (Cotton et al. 2004, 2006; Stillwell et al. 2007, 2010). For example, in some ectotherms (including insects), it has been found that individuals growing at lower temperatures present larger body sizes (Kingsolver & Huey 2008), and when fed with low-quality diets generally they develop as smaller sizes (Davidowitz et al. 2004, Stern & Emlen 1999). The similarities of allometric trends between sexes and the sexual dimorphism in sexual secondary characters of *X. affinis* suggest a specific phenotypic response under optimal controlled conditions, 26 °C and 60% RH, and continuous darkness (Brar et al. 2013). However, this pattern should change under different breeding conditions (temperature and relative humidity). As has been documented in insects, males in good genetic and body condition are recognized through greater sexual trait size or more vigorous display, or when male quality varies due to environmental growing conditions (Iwasa & Pomiankowski 1999; Cotton et al. 2004, 2006). Considering that haplodiploid sons do not inherit traits from their fathers (De la Filia et al. 2015), the secondary sexual characters could be useful as an indicator of developmental alterations under modified environmental conditions.

It is expected that sexual traits exhibit high variance relative to non-sexual traits, the expression of which is less condition-dependent (Rowe & Houle 1996; Cotton et al. 2004, 2006; Cotton et al. 2006), and our results coincide with this pattern of variation between structures. While the results suggest a morphological integration of the body elements (elytra and pronotum), we found more variation in pronotum shape, with the structure of the body expressing more sexual dimorphism in size and shape. Sexual size dimorphism in the pronotum has been reported previously in *X. affinis*, with the structure presenting greater size in females than males, based on traditional distance measurements of its length and width (Kingsolver & Norris 1977b). The increased pronotum size in the females could be related to the fact that they are the sex that disperses, whereas males are wingless and therefore do not disperse (Biedermann et al. 2011). Also, decreased female pronotum size, perhaps produced by stress conditions, could contribute to a limitation in their capacity for dispersal and colonization considering that dispersal and philopatry are likely rather plastic in this group of species and seems to be a condition-dependent strategy (Biederman et al. 2011).

Body size during development can be influenced by environmental conditions affecting sexual size dimorphism, although the size plasticity of sexual traits depends on the mating system (Stillwell et al. 2010). For example, in species with inbreeding polygyny, phenotypic variations in body size, larval development, and sexual size dimorphism have been observed under different laboratory conditions, as in the genus *Dendroctonus* (Erichson) (Curculionidae: Scolytinae: Hyurgini), which may

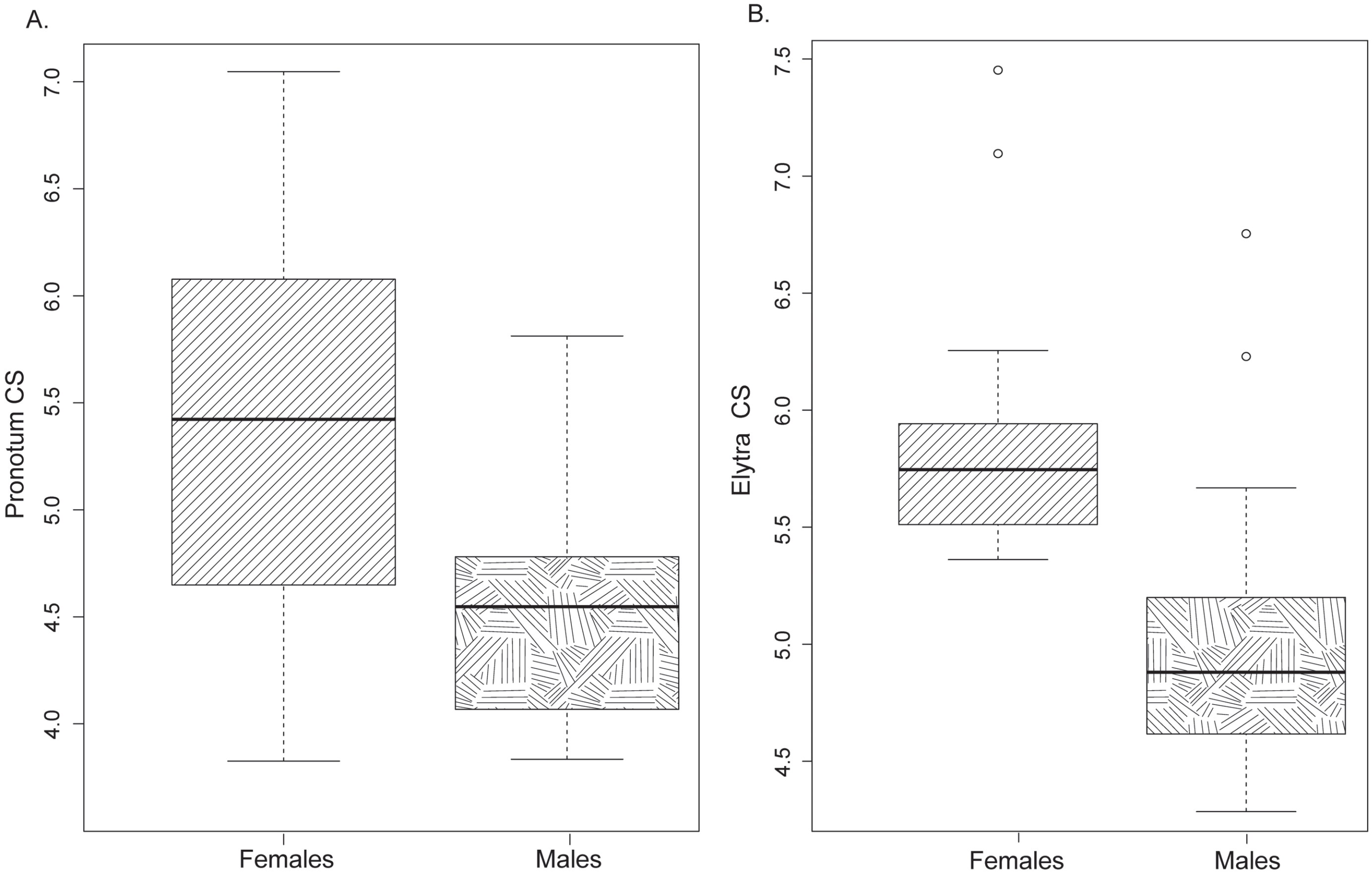

Fig. 4. Boxplots for the body structure centroid size in *Xyleborus affinis*: (A) elytra centroid size; (B) pronotum centroid size. The line within each box represents the median, and the height of each box represents first and third quartiles (75% of all data). The lines correspond to the observed minimum and maximum values and dots are outliers

be mediated by biotic (symbiotic relationships) and environmental variation more than by genotypic variation (Hofstetter et al. 2007). In contrast, species from the genus *Ips* (DeGeer) present lower variability in body length between sexes (Foelker & Hofstetter 2014). However, in both genera, the sex that colonizes first (pioneer sex) displays greater phenotypic size variation than the sex that colonizes second (Hofstetter et al. 2007; Foelker & Hofstetter 2014). Although the concept of pioneer sex does not apply in *Xyleborus*, our results support a greater phenotypic variation in females, the sex that disperses and colonizes (Kirkendall et al. 2015), with higher variability presented in terms of pronotum size and shape.

In the case of sexual shape dimorphism, the morphospace ordination and deformation grids confirm the highest variability in pronotum configurations, as well as the highest morphological disparity between sexes. For the pronotum, we observed a rounded anterior margin and narrow base in the females and a broad base and medial projection in the anterior margin in males. In the case of the elytra, the deformations were smaller and suggested a conserved shape in the external

Table 1. Results from the Procrustes ANOVA analyses testing the effect of centroid size, sex, and their interaction for both elytra and pronotum shape variation. An asterisk (*) indicates significant effects with α of 0.05 to the F statistics.

Elytra shape	Degrees of freedom	Sum of squares	Mean squares	R squared	F	Probability (> F)
Centroid size	1	0.004	0.004	0.086	5.993	0.02*
Sex	1	0.012	0.012	0.253	17.537	0.01*
Centroid size/sex interaction	1	0.001	0.001	0.025	1.732	0.14
Residuals	44	0.031	0.0006			
Total	47	0.048				
Pronotum shape	**Degrees of freedom**	**Sum of squares**	**Mean squares**	**R squared**	**F**	**Probability (> F)**
Centroid size	1	0.009	0.009	0.107	6.784	0.004*
Sex	1	0.014	0.014	0.175	11.098	0.001*
Centroid size/sex interaction	1	0.002	0.002	0.022	1.084	0.221
Residuals	44	0.056	0.001			
Total	47	0.081				

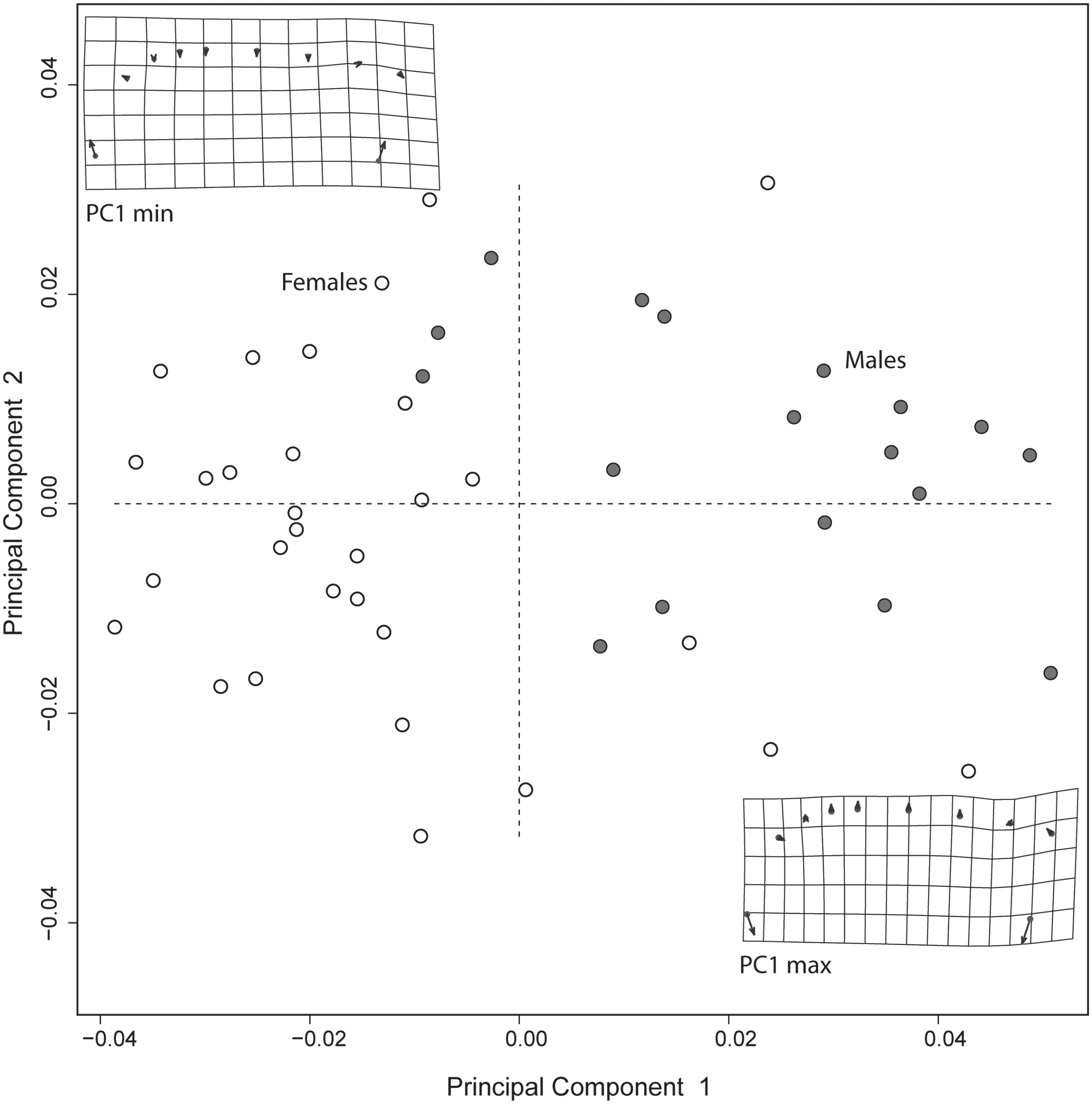

Fig. 5. Ordination plot for principal components 1 and 2 representing elytra shape variation between sexes of *Xyleborus Affinis*: deformation grids describing variation between sexes on the 2 first principal components are presented.

margin accompanied by changes in the medial line, producing narrower elytra in females and broader elytra in males because of body size reduction. These differences could be related to flight capacity between the sexes, possibly due to the fact that only females have flight muscles housed in the pronotum, which represent a significant proportion of the total body mass and thus a greater allocation of energy and resources (Marden 2000). The fitness advantage of wing reduction has been documented in several species, where flightless morphs reach sexual maturity earlier and present higher fecundity (Fujisaki 1992; Zera & Denno 1997), including some scolytine bark beetles, as is the case in the genus *Ips* (Robertson 1998, 2000). In the case of *Xyleborus* species, the energetic cost of building flight muscles and maintaining reproductive effort could be reflected in the differences in body sizes between the sexes, both in the structure that houses the flight muscles (pronotum) and the structure in which the wings are located (elytra).

The multiple origins of mating systems, such as various forms of monogamy and harem polygyny, found in Scolytinae (Kirkendall 1983; Hulcr et al. 2015; Kirkendall et al. 2015; Johnson et al. 2018) have been related mainly to the abundance, quality, and spatial and temporal distribution of essential resources for food and reproduction (Kirkendall

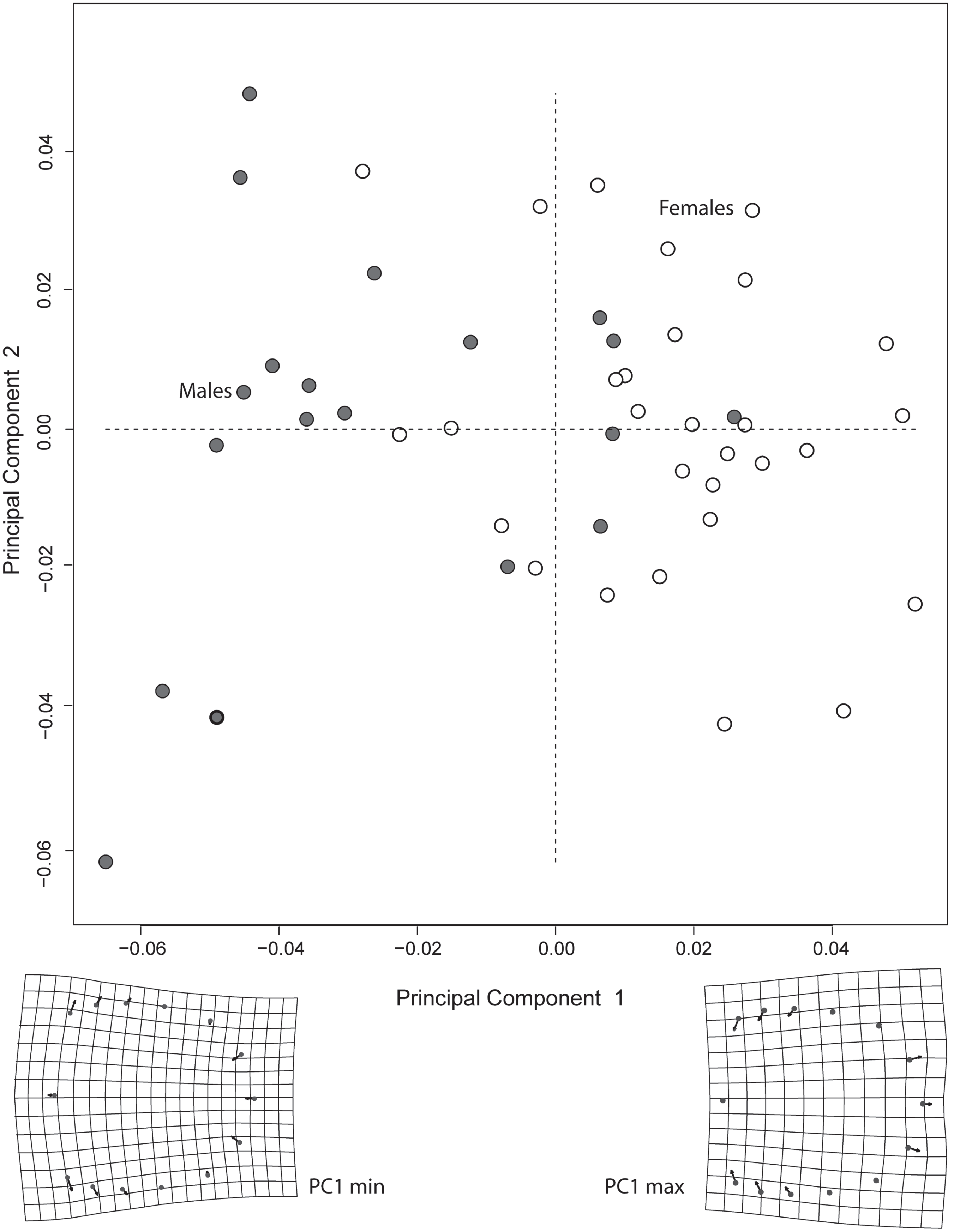

Fig. 6. Ordination plot for principal components 1 and 2 representing pronotum shape variation between sexes of *Xyleborus Affinis*: deformation grids describing variation between sexes on the 2 first principal components are presented.

1983). The greater variability in pronotum shape supports the idea of increased phenotypic response in sexually selected traits (Cotton et al. 2004), particularly in groups of insects where colony growth is mediated by the mating system, as is the case for scolytines (Kirkendall 1983). Our results suggest the importance of the implementation of morphometric studies to quantify size and shape dimorphism in species within the Xyleborini, and represent a step towards understanding the phenotypic response of species that have been demonstrated to be a threat to natural and cultivated vegetation (Carrillo et al. 2014; Lira-Noriega et al. 2018).

Conclusions

We present evidence of morphological integration of the body elements elytra and pronotum, but different allometric trends and expression of sexual dimorphism between these 2 structures in *X. affinis*. The use of a geometric morphometric protocol allowed us to quantify sexual differences in size and shape and describe changes in the pronotum, which could be a good indicator of stress during growth in *Xyleborus* ambrosia beetles and should be examined under different experimental conditions. Moreover, the quantification of sexual dimorphism expression under controlled conditions (e.g., temperature and humidity) could be used to model the distribution and establishment of *Xyleborus* species, many of which are of economic importance.

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