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Quantifying the shape variation of the elytra in Patagonian populations of the ground beetle *Ceroglossus chilensis* (Coleoptera: Carabidae)

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ABSTRACT

Elytra shape variation was analyzed from two forest systems in the Chilean Patagonia, one composed of *Nothofagus dombeyi* and *N. nitida* and a second-growth stand of *N. pumilo*. Geometric morphometrics analysis and multivariate analyses were used on the complete elytra having the shape information (outline) of the trait. The results show several shape variations on the mid bottom of the elytra and confirm the local adaptation of the Patagoninan population at different traits with the results found at the abdominal section of previous studies of the same species, nevertheless, here we found a complete absence of sexual shape dimorphism using the elytra as a trait of sexual differentiation. A positive allometry was found, however, this was not related with sexual dimorphism differentiation but rather with to the forest and second-growth forest.

1. Introduction

Insects classification and biological diversity analyses, have been traditionally based on morphological descriptions and mostly their local adaptation is related with their environment (Daly, 1985; Palmer and Strobeck, 1986). For coleopteran, there are several traits deemed as adaptive (e.g., abdominal sternite, antennae, subelytral cavity, etc.) which differentiate populations (Daly, 1985). These traits have been analyzed using different morphometric techniques (e.g., microscopy, traditional morphometrics and geometric morphometrics) (Benítez et al., 2010a; Benítez et al., 2010b).

Ceroglossus is an endemic genus of *Nothofagus* forests located in South America, it has 8 species from which *C. chilensis* is the most widely distributed from Maule region to the extreme south of Aysen region in Chile and Argentina. *C. chilensis* is a clime tolerant species that has a preference for dry environments (Jiroux, 2006). Some studies conducted with sister species of *Ceroglossus*,evaluate its morphological adaptations regarding to population and hybridization (Alibert et al., 2001; Garnier et al., 2006). For instance, Benítez et al. (2010b), assessed using geometric morphometrics (GM) that the sexual shape dimorphism of *C. chilensis* by means of the abdominal section showing a sex-related difference in shape. In GM, the shape is defined as "at all geometric information that remains when the effects of translation, scaling and rotation are removed from an object (Rohlf and Marcus, 1993; Rohlf and Slice, 1990). Using landmarks is the most common technique in geometric morphometrics. Morphological landmarks are points that can be located precisely on each specimen under study with a clear correspondence in a one-to-one manner from specimen to specimen (Adams et al., 2013; Bookstein, 1991). For *Ceroglossus*, GM studies has been assesed using only the ventral abdominal part of the body, where population differences and sexual dimorphism has been reported in Patagonian population principally by the thickening and thinning of the sternites (Benítez et al., 2011). However, other studies showing sexual dimorphisms are scarce since there exists low competition between males and females as showed by the sex ratio (Benítez et al., 2013a; Benítez et al., 2013b). Aysen region has 4 watersheds named Palena, Cisnes, Aysen and, Baker. The latter is the largest, covering 200 km in length, draining a surface of 26, 487 km^2. The biodiversity present in the region has changed over time because of the deleterious effect of some actions like the establishment of arable and livestock zones (Luebert and Pliscoff, 2006). Over 60 years ago, the native forest was also subject to fires that affected the vegetation and its wildlife. Because of all these events, the landscape and particularly its austral portion were modified, being *C. chilensis* one of the key insects affected. The aim of this article is to evaluate the local adaptation (plasticity) of the elytra shape

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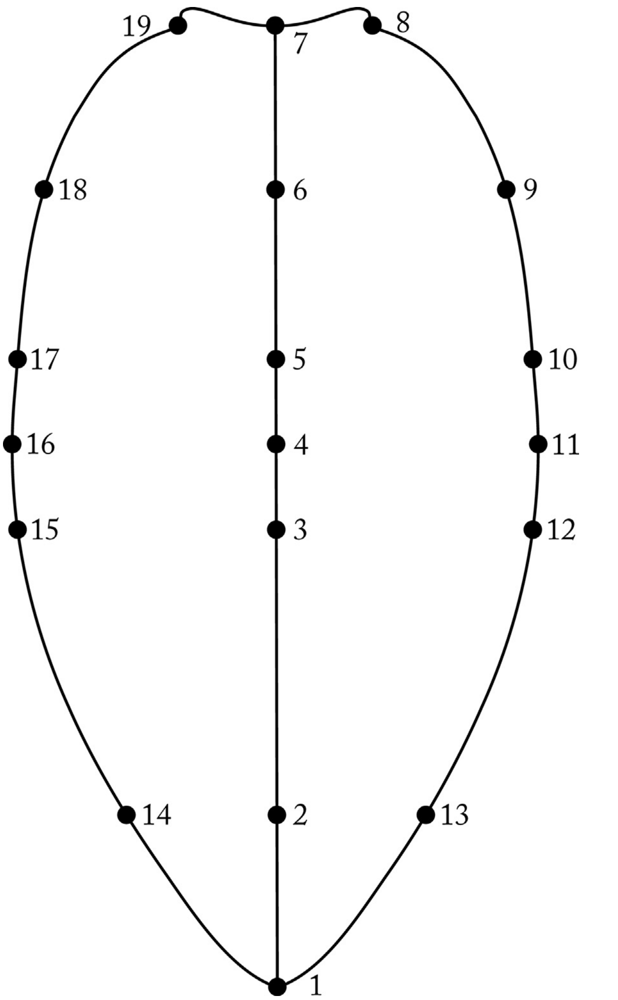

Fig. 1. Representation of the 19 landmarks identified on the elytra of *Ceroglossus chilensis*.

of *C. chilensis*, at the Patagonian population, and also to estimate this trait for sexual shape dimorphism and modifications between Patagonian primary forest and a second-growth forest.

2. Material and methods

Pitfall traps were located in isolated geographic areas of the Baker River watershed, separated in three locations in the forest (F) (F1, F2 and F3) and other three in the second-growth stand (S) (S1, S2 and S3), a reference map can be found at Benítez et al. (2011). In each site 12 traps were installed, separated approximately 5 m from each other, for 3 days and 3 nights. The geometric morphometric analyses were performed with 266 beetles from F and 155 beetles from S. These analyses considered exclusively variations in shape, and was performed using a photograph in dorsal view of males and females with an Olympus X- 715 digital camera and 19 landmarks were digitized in the elytra contour (Fig. 1) using the software TPS Dig 2.26 (Rohlf, 2013). In order to evaluate a possible measurement error, 180 individuals were re-digitized and analyzed using Procustes ANOVA (Arnqvist and Martensson, 1998).

A Procrustes fit was applied to the landmark data using the program MorphoJ (Klingenberg, 2011), a principal component analysis (PCA) was performed to visualize the shape changes and their distribution on the morphospace and a multivariate regression were was calculated to calculate the levels of allometry (size influence on shape) in the elytra data and to confirm previous results found in

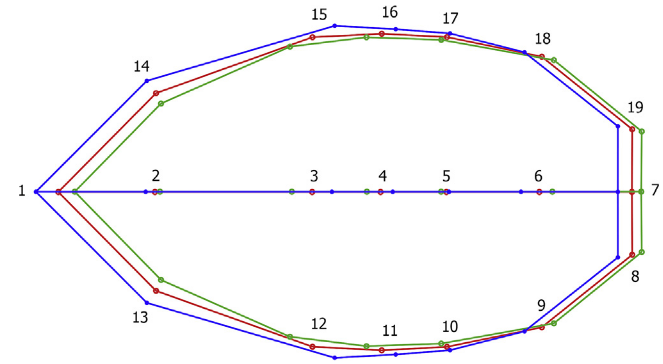

Fig. 2. Wireframe representation of the eltytra shape variation and their corresponding landmarks from Forest F1, F2 and F3 are red, green and blue respectively. *the wireframe were aligned using starting shape. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

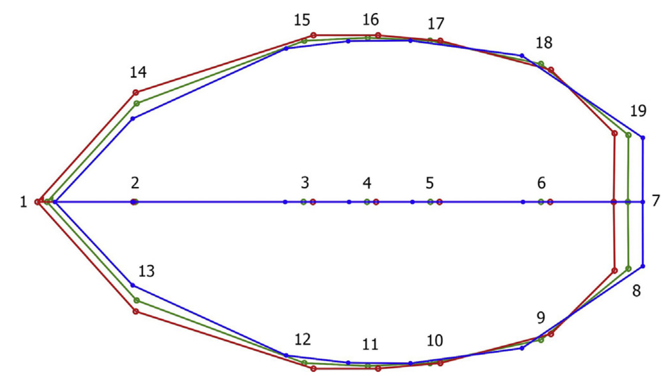

Fig. 3. Wireframe representation of the eltytra shape variation and their corresponding landmarks from Second-growth stands S1,S2 and S3 are blue, green and red respectively. *the wireframe were aligned using starting shape. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Benítez et al., 2011, furthermore a permutation test was performed using 10000 iterations (Klingenberg, 2016; Monteiro, 1999).

3. Results

A measurement error was discarded of our analysis by the results of the Procrustes ANOVA where the error of the elytra showed that the MS for individual variation exceeded the MS of the error (0.0001069051 < 0.0000362014).

The first three principal components (PC) accumulated 74% of the shape variation (PC1 = 30.4%, PC2 = 24.202% and PC3 = 19.63%). The shape variation was clear distinguished between populations by the movement of the landmark 1, this landmark is localized at the end of the elytra suture, also the variation of the mid bottom section of the elytra was noticeable (landmarks 13 and 14) (Figs. 2, Fig. 3), an exclusion of the presence of sexual shape dimorphism was noticeable for the elytra also for the centroid size. A graphical representation of the population variation was expressed by the first two PCís (Fig. 4). The regression analysis shows a significant relationship between size and shape of the elytra (10.34%, P-value:<0.0001), finding a positive allometry where populations of F are smaller than S (Fig. 5).

4. Discussion

The following research describes the shape variation using geometric morphometric for the elytra in *C. chilensis*, confirming the

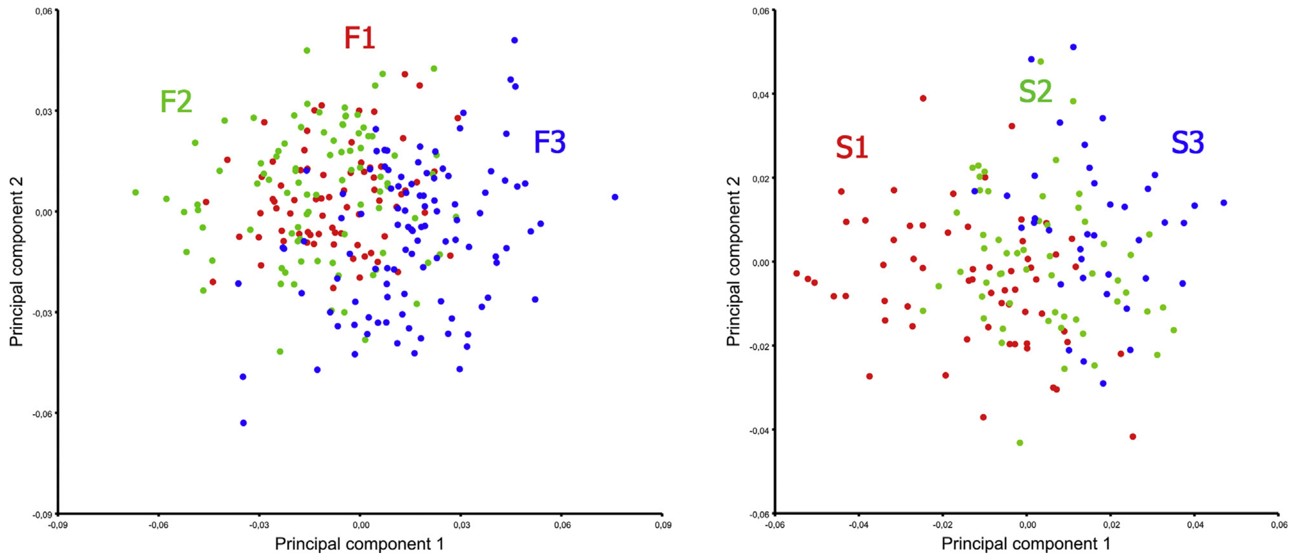

Fig. 4. PCA analysis of the elytra shape variation in *Ceroglossus chilensis*. The graphical visualization represent the shape space in both location F: Forest and S: Second-growth stands. *Each point represents a different shape.

results found by Benítez et al. (2011) for the ventral abdominal section of the body. The main elytra shape variation was found to be wider in S and narrower in F. Nevertheless, the wider shape of the centroid size found in S belonged to bigger specimens collected in F, which could be a possible adaptation to overcome the habitat fragmentation (Alibert et al., 2001; Weller and Ganzhorn, 2004). More studies relating the climate effect and developmental stability are needed for a more definite conclusion related to this phenomenon.

Goczał et al. (2017) describe empirical evidence that elytra protect beetles against predators, being a trait that support desiccation tolerance. Also, it fulfills other functions like minimize the effect of rapid temperature shifts and give protection to the hind wings against damage (Linz et al., 2016). Moreover, it was shown that elytra may play a key role in mimicry (Muñoz-Ramírez et al., 2016). It was noticeable for this study, that the geometric morphometrics analyses also found that sexual shape dimorphism was not a differentiable trait in the elytra, opposite to the results found by Benítez et al. (2011), but confirming the results found for the evolutionary trend where the genera has a poor relationship between size and shape per sex (Benítez et al., 2013a). Similar studies using GM have found sexual differentiation on the elytra in leaf beetles (Adams and Funk, 1997), nevertheless elytra shape variation has been used

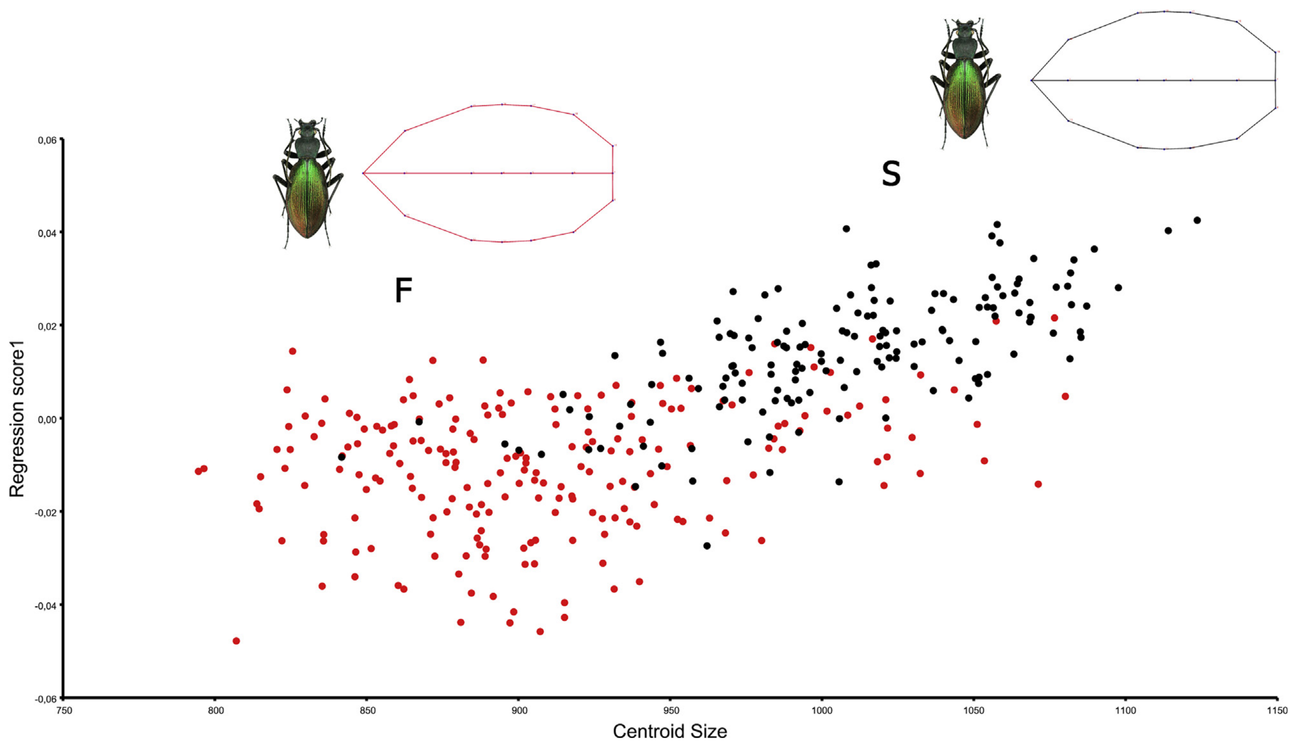

Fig. 5. Multivariate regression of the elytra shape on elytra centroid size. The red points represent the forest individuals and black points represent second-growth stands individual. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more as a taxonomic trait, and only considering one elytron size (Pizzo et al., 2006) and recently using the complete body shape information (two sides) (Cardini, 2016; Hájek and Fikáček, 2010).

Finally, this note confirms that elytra as well as ventral abdominal part of the body in *C. chilensis* can be used as morphological trait to detect shape variation due to environmental condition in forest and their degradation in second-growth stands. Nevertheless, elytra in *C. chilensis* can not be used as a trait to determine sexual dimorphism (Benítez et al., 2013a). Future studies using elytra as model trait are needed to corroborate if these morphological changes could be the expression of developmental instability product of the habitat fragmentation and product of human activities (e.g. deforestation) at Patagonian region.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jcz.2018.02.002.

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