J. Entomol. Res. Soc., 12(2): 87-95, 2010 *ISSN:1302-0250*

Sexual Dimorphism and Morphological Variation in Populations of *Ceroglossus chilensis* (Eschscholtz, 1829)(Coleoptera: Carabidae)

Hugo Benitez De La FUENTE[1*] Marcela VIDAL[2] Raul BRIONES[3]
Viviane JEREZ[1]

[1]Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción. Concepción, CHILE, e-mails: hugobenitez@udec.cl ([*]Correspondence author), vijerez@udec.cl

[2]Biodiversity and Genomics Laboratory, Department of Basic Sciences, Faculty of Science, University of Bío-Bío. Chillán, CHILE, e-mail: marcela.vidal@gmail.com

[3]Doctoral Program in Biodiversity, Biodiversity Iberoamerican Center, University of Alicante, SPAIN, e-mail: rbriones@udec.cl

ABSTRACT

We evaluated differences related to body shape of males and females of *Ceroglossus chilensis* using morphometric geometry. This species is widely distributed in Chile, associated with native forests and exotic plantations. In December 2004 and January 2005 we collected 53 males and 63 females from two plantations of *Pinus radiata* located in the Coast Range and Andes foothills in the Región del Bío-Bío. Individuals were photographed, and 17 landmarks from lateral and ventral views were digitized. We used a multivariate analysis of variance with sex and locality as factors to investigate differences in body form among sexes. Sexual dimorphism was present in both populations; males had a wider proepisternum, while females had a narrower proepisternum and wider abdominal sternites. Differences among populations were also found; the abdomen of both males and females from the Coast Range is more elongated and thicker than in the Andes foothills. These differences raise the question of whether sexual dimorphism may be modulated by natural selection.

Key Words: Coast Range, differences among sexes, geometric morphometry, Andes foothills, Región del Bío-Bío.

INTRODUCTION

Differences between sexes in morphological characters are a common phenomenon in many animal taxa; the most conspicuous one is body size (Gannon & Rácz, 2006). The direction of the difference (whether males or females are larger) is different among groups (Wainwright, 1994; Koehl, 1996). A number of proximal mechanisms have been proposed to explain this dimorphism, such as differential mortality between sexes (Reilly & Wainwright 1994, Ricklefs & Miles, 1994) or differences in growth rate (LaBarbera, 1989). In insects females tend to be larger than males, which gives them certain adaptive advantages such as greater fecundity and better parental care (Forrest, 1987; Andersson, 1994; Moller & Zamora-Muñoz, 1997). However, in some species males are larger in linear dimensions, but smaller in relative mass (e.g. Cepeda-Pizarro *et al.* 1996), which indicates that determination of sexual dimorphism requires more complex measurement techniques related, for example, to volume (Johnson, 1982; Forrest, 1987).

Insects have morphological variation associated with their environment (Daly, 1985; Palmer, 1994; Cepeda-Pizarro *et al.* 1996; Krasnov *et al.* 1996; Williams, 2001, Cepeda-Pizarro *et al.* 2003). There are numerous morphological characters considered adaptive (i.e., length of posterior extremities, curvature of the elytra, form of the subelytral cavity, etc.) which differentiate populations. Reports such as those in coleopterans of the families Lucanidae, Carabidae and Cerambycidae (Shiokawa & Iwahashi, 2000; Moller & Zamora-Muñoz, 1997; Alibert *et al.* 2001; Garnier *et al.* 2006; Bonacci *et al*. 2006; Talarico *et al*. 2007) have distinguished differences in form with linear measurements, even though these are correlated with body size (Lestrel, 1997). In recent years more attention is being paid to morphometric approaches, due to new methods of analysis such as geometric morphology (Bookstein, 1991; Rohlf, 1993a; Rohlf & Marcus, 1993; Adams & Funk, 1997). These methods allow the study of form without the effects of scale, rotation and translation of objects (Rohlf & Slice, 1990; Rohlf *et al.* 1996; Adams & Funk, 1997), thus they have a better biological interpretation and are a graphical tool for the visualization and quantification of morphological variation in different ecological and evolutionary contexts (Adams & Rohlf, 2000; Alibert *et al.* 2001).

Ceroglossus is a genus endemic to *Nothofagus* forests of South America, composed of eight species: *C. chilensis* (Eschscholtz, 1829)*, C. darwini* (Hope, 1837), *C. speciosus* Gerstaecker, 1858*, C. magellanicus* Géhin, 1885, *C. buqueti* (Laporte, 1834), *C. suturalis* (Fabricius, 1775), *C. ochsenii* (Germain, 1895) and *C. guerini* (Germain, 1895). The most widespread species is *C. chilensis*, which has 26 subspecies distributed in Chile from the Region del Maule to the Region de Aysén and in adjacent Argentina in the latter area (Jiroux, 2006); it has great morphological and genetic variability among populations (Okamoto *et al.* 2001; Jiroux, 2006). In the Región del BíoBío, a high abundance of *C. chilensis* has been found in plantations of *Pinus radiata* D. Don, with a disjunction between the Andes Range and the Coast Range (Briones & Jerez, 2007, Benítez *et al.* 2008). It has been reported that the sexual dimorphism in this species is detectable in males by the presence of inconspicuous careens located on the ventral surface of antennal segments five, six and seven, and in females by elytrae apparently more pointed at the apex (Jiroux, 2006). Although these characters are observable upon detailed analysis, other potential characters which are not obvious such as shape of structures might help to discriminate between sexes and discover distribution patterns not detectable with them. The objectives of this report are to determine sexual dimorphism in the lateral and ventral views of *C. chilensis* using morphometric geometry analysis, and to investigate if geographic isolation in this species affects the degree of sexual dimorphism.

MATERIAL AND METHODS

We utilized 116 individuals (53 males and 63 females) of *C. chilensis* collected in December, 2004 and January, 2005 in two plantations located in the Santa Juana area in the Coast Range (37º10'S, 72º57'W) and near San Fabián de Alico in the

Andes Foothills (36º37’ S, 71º50’W), both localities are in the Región del Bío-Bío (Fig 1) (Briones & Jerez, 2007). The sex of individuals was determined based on the presence of antennal careens located from the fifth to ninth segment (Jiroux, 2006), and present “only in males” and observable under a dissecting microscope (Fig. 2).

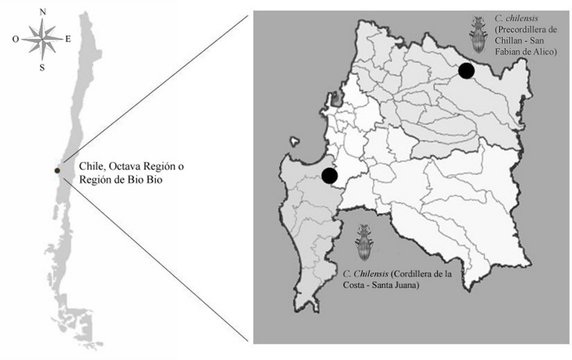

Fig. 1. Map of Chile indicating the Región del Bío-Bío and the study sites for *Ceroglossus chilensis*.

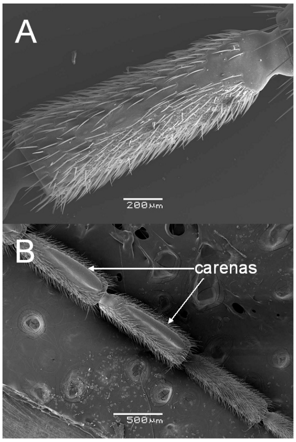

Fig. 2. Morphological differentiation among sex: Fifth antennal segment of (A) female (without careen) and (B) male (with careen) of *Ceroglossus chilensis*.

The geometric analysis, which considered variation attributable exclusively to form, was performed using photographic records of each individual in lateral and ventral views (Bookstein, 1991) with an Olympus X-715 digital camera. A matrix of photographs for each view was constructed using the TpsUtil 1.34 program (Rohlf, 2005). In each view we used 17 homologous landmarks (Fig. 3), which were digitized in the TpsDig 1.31 program (Rohlf, 2001). The X and Y coordinates of the homologous landmarks were aligned and superimposed using the TpsSuper 1.06 program (Rohlf, 2003b), with the least mean squares method based on the generalized procrustes analysis (GPA, Rohlf & Slice, 1990), in order to remove variation not related to form (e.g. rotation, translation and scale). The form variables of the aligned specimens were obtained using the Tps Relw 1.42 program (Rohlf, 2003a), which calculates an interpolation function that projects the data in an Euclidean plane. With this program we also performed a relative warp analysis. Differences in form were analyzed with a multivariate analysis of variance, using sex and locality as factors. To determine if there is an allometric effect in the form we used a linear regression of the first principal component axis (form variable) on the logarithm of centroid size in the TpsSmall program (Rohlf, 2003). All statistical tests were performed with the STATISTICA 7.0 program (Statsoft, 1999).

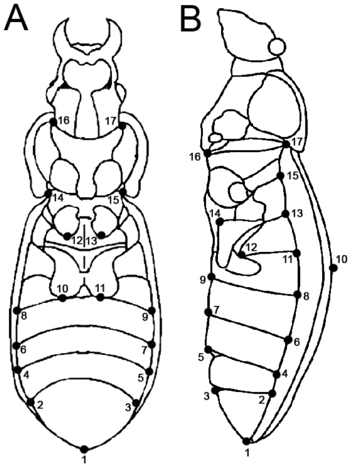

Fig. 3. Localization of the 17 homologous landmarks used in the ventral (a) and lateral (b) views of *Ceroglossus chilensis.*

To minimize measurement errors and avoid bias in the digitalizing of the landmarks, we chose a random subsample of 70 individuals from the insects collected in the two populations. The X and Y coordinates of the homologous landmarks of this subsample were aligned and superimposed with the TpsSuper 1.06 program. The proportional measurement error compared to the raw data was less than 5%, thus error bias had little relevance in the digitalizing.

RESULTS

The multivariate analysis of form found significant differences between sexes for the lateral view (Wilks' lambda = 0,714; $P < 0.001$) and the ventral view (Wilks' λ = 0,502; $P < 0.001$). In this analysis, landmarks 4, 5, and 7 of the lateral view had the greatest relative contributions in distinguishing males from females (0.1780, 0.1578 and 0.1287, respectively); these are related to a wider proepisternum in males and wider abdominal sternites in females. In the ventral view, the landmarks with greatest contribution were numbers 4, 5 and 6 (0.1583, 0.1321 and 0.1067, respectively), indicating that males have a less robust abdomen than females. The first two axes of the principal components analysis explained 79.24% of the variance in the lateral view and 76.71% in the ventral view. This analysis showed a tendency to separate males from females in both views (Fig. 4 and 5).

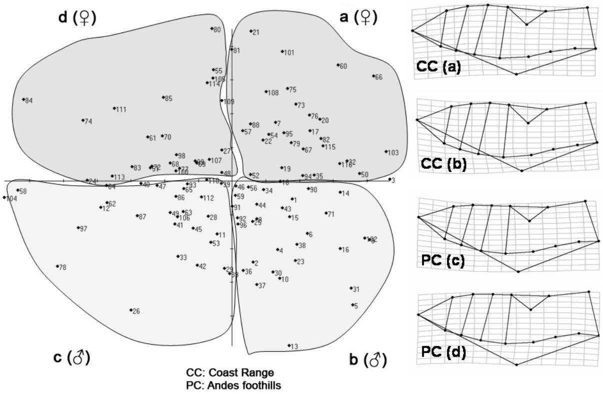

Fig. 4. The first two axes of the principal component analysis of form variables for individuals of *Ceroglossus chilensis* in lateral view, from (a, b) Coast Range and (c, d) Andes foothills. The deformation grid for the sexes is shown for each case.

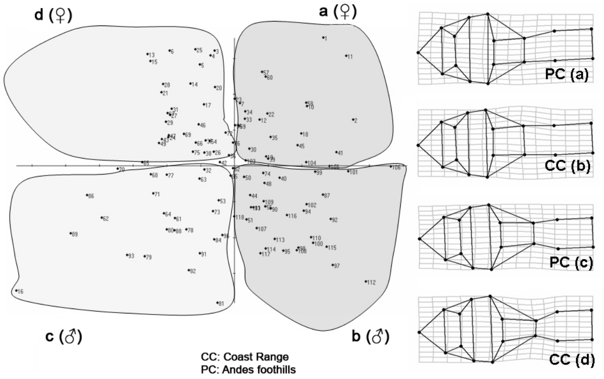

Fig. 5. The first two axes of the principal component analysis of form variables for individuals of *Ceroglossus chilensis* in ventral view, from (a, b) Coast Range and (c, d) Andes foothills. The deformation grid for the sexes is shown for each case.

We did not find differences between localities for the lateral view (Wilks' lambda = 0.948; *P* = 0.119); however, the interaction with sex was significant (Wilks' λ = 0.897; *P* = 0.007). By contrast, for the ventral view we found a significant difference between localities (Wilks' λ = 0.825; *P* < 0.001), but the difference between sexes was not significant (Wilks' λ = 0.974; *P* = 0.412). The landmarks with greatest relative contribution for localities in both views are given in Table 1. In the lateral view, individuals from the Coast Range tended to be more robust in the abdominal area (landmarks 4, 6 and 7), while in the Andes foothills they were more compressed dorsoventrally.

Table 1. Relative contributions (RC) of each landmark to *Ceroglossus chilensis* body shape in lateral and ventral views.

	Coast Range		Andes Foothills	
Landmark	RC in lateral view	RC in ventral view	RC in lateral view	RC in ventral view
1	0.005	0.011	0.005	0.010
2	0.072	0.055	0.069	0.055
3	0.039	0.044	0.039	0.050
4	0.150	0.140	0.150	0.125
5	0.113	0.108	0.115	0.123
6	0.086	0.109	0.087	0.086
7	0.132	0.096	0.132	0.100
8	0.054	0.076	0.052	0.054
9	0.073	0.064	0.071	0.079
10	0.009	0.072	0.011	0.063
11	0.060	0.066	0.062	0.083
12	0.041	0.044	0.040	0.051
13	0.068	0.044	0.065	0.050
14	0.019	0.027	0.020	0.028
15	0.061	0.026	0.061	0.028
16	0.003	0.007	0.003	0.008
17	0.013	0.007	0.016	0.007

DISCUSSION

Our results indicate that *C. chilensis* in the Bio-Bio Region of Chile has sexual dimorphism in form. One of the most notable aspects is the larger abdomen of females. This is a morphologically essential character that allows the transport of more eggs, and therefore gives the female greater fitness (Andersson, 1994; Cepeda-Pizarro *et al.* 1996). In the last few decades, a number of hypotheses have been proposed to explain sexual dimorphism in insects (Wilkund & Forsberg, 1991; Fairbairn, 1997; Walker & Rypstra, 2001; Esperk *et al.* 2007). The best supported working hypothesis has been the connection of sexual selection *versus* natural selection, along with environmental variation, although male-male competition (Saito & Mori, 2005; Emlen, 2008) and the segregation of sexes due to limited resources (Saito & Mori, 2005) have produced notable selective differentiation. The use of landmarks to study bilaterally symmetric animals has been frequently questioned (Mitteroecker, 2009), since these increase the degrees of freedom without adding much additional information (Dryden & Mardia, 1998), which leads to high values in the variance-covariance matrix (Alibert *et al.* 2001). Here we used the entire organism in accordance with the methodology proposed by Alibert *et al.* 2001, which considers the variation in form holistically. This includes the differentiation of sexes, and allows a more realistic view of the variation in form in a deformation network.

The two populations studied only showed differences in the ventral view, which may indicate that there has been little selective pressure on morphology in these two localities. Although these differences are not obvious, individuals of the Coast Range had less thickened bodies than those of the Andes foothills. It has been reported that a climate with high relative humidity and constant temperatures promotes a thinner subelytral cavity, thus this result was expected for the Coast Range. The individuals of the Andes foothills had more visible morphological variations, which may be a consequence of the instability of the environment in this area (more variation in temperature, leading to thicker subelytral cavities). However, in spite of the climatic differences between populations there were not large morphological differences in the sexual dimorphism between populations, from which we may infer that gene flow has not been interrupted between them. However, further investigation will be necessary to examine possible differences in the gene pools of these populations (Alibert *et al.* 2001; Garnier *et al.* 2006) and their relation with morphology.

ACKNOWLEDGEMENTS

This study was financed by Project DIUC Nº 205.113.066–1.0. The authors thank the personnel of the electron microscopy laboratory of the Universidad de Concepción. MAV was financed by FONDECYT Project 3095015.

REFERENCES

Adams, D., Funk D. J., 1997, Morphometric inferences on sibling species and sexual dimorphism in *Neochlamisus bebbianae* leaf beetles: *Multivariate applications of the thin-plate spline. Systematic Biology,* 46(1): 180-194

Adams, D., Rohlf, F. J., 2000, Ecological character displacement in *Pletodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences of the United States of America*, 97: 4106-4111.

Andersson, M., 1994, *Sexual selection*. Princeton: Princeton University Press.

Alibert, P., Moureau, B., Dommergues, J. L., David, B., 2001, Differentiation at a microgeographical scale within two species of ground beetles, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. *Zoologica Scripta,* 30(4): 299-311.

Benítez, H., Briones, R., Jerez, V., 2008, Asimetría Fluctuante en dos poblaciones de *Ceroglossus chilensis* (Coleoptera Carabidae) en el agroecosistema *Pinus radiata*, *R*egión del BioBío. *Gayana,* 72(2): 131-139.

Bonacci, T., Brandmayr, P., Giglio, A., Massolo, A., Mazzei, A., Odoguardi, R., Romeo, M., Talarico, F., Zetto-Brandmayr, T., 2006, Agonistic behaviour of Scarites buparius (Forster, 1771) (Coleoptera: Carabidae) in relation to body size. *Entomologica fennica,* 17(3): 340-344

Bonnet, X., Legarde, F., Henen, B.T., Corbin, J., Nagy, K. A., Naulleau, G., Balhoul, K., Chastel, O., Legrand, A., Cambag, R., 2001, Sexual dimorphism in steppe tortoises (*Testudo horsfieldii*): influence of the environment and sexual selection on body shape and mobility. *Biological Journal of the Linnean Society,* 72(3): 357-372

Bookstein, F. L., 1991, *Morphometric Tool for Landmark Data*. Geometry and Biology Cambidge: Cambridge University Press

Briones, R., Jerez, V., 2007, Efecto de la Edad de la plantación de *Pinus radiata* en la Abundancia *de Ceroglossus chilensis* (Coleoptera: Carabidae) en la Región del Biobío, Chile. *Revista Bosque,* 28(3): 207-214

Cepeda-Pizarro, J., Vásquez, H., Veas, H., Colon, G. O., 1996, Relaciones entre tamaño corporal y biomasa en adultos de Tenebrionidae (Coleoptera) de la estepa costera del margen meridional del desierto chileno. *Revista Chilena de Historia Natural,* 69: 67-76.

Cepeda-Pizarro, J., Vega, S., Vásquez, H., Elgueta, M., 2003, Morfometría y dimorfismo sexual de *Elasmoderus wagenknechti* (Liebermann) (Orthoptera: Tristiridae) en dos eventos de irrupción poblacional. *Revista Chilena de Historia Natural,* 76: 417-435.

Daly, H. V., 1985, Insect morphometrics. *Annual Review of Entomology,* 30: 415-438.

David, B., Dommergues, J. L., Magniez-Jannin, F., 1996, Exploration d'un espace morphologique. Les carabes (Coleoptera) du nord-est de la France. *Comptes Rendus de l'Académie des Sciences*, Paris, 319, 835–842.

Dryden, I. L., Mardia, K. V., 1998, *Statistical Shape Analysis*. Wiley, Chichester pp. 347.

Emlen, D. J., 2008, The evolution of animal weapons *Annual Review of Ecology, Systematics, and Evolution,* 39: 387-413

Esperk, T., Tammaru, T., Nylin, S., Teder, T., 2007, Achieving high sexual size dimorphism in insects: females add instars. *Ecological Entomology,* 32, 243–256

Fairbairn, D. J., 1997, Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics,* 28: 659-687

Forrest, T. G., 1987, Insect size tactics and developmental strategies. *Oecologia,* 73(2): 178-184.

Forslund, P., 2000, Male-male competition and large mating. advantage in European earwigs, *Forficula auricularia*. *Animal Behaviour,* 59:753–762

Gannon, W. L., Rácz, G. R., 2006, Character displacement and ecomorphological analysis of two long-eared *Myotis* (*M. auriculus* and *M. evotis*). *Journal of Mammalogy,* 87(1): 171-179

Garnier, S., Gidaszewski, N., Charlot, M., Rasplus, J., Alibert, P., 2006, Hybridization, developmental stability, and functionality of morphological traits in the ground beetle *Carabus solieri* (Coleoptera, Carabidae). *Biological Journal of the Linnean Society,* 89(1): 151-158.

Jiroux, É., 2006, *Le genre Ceroglossus. Collection Systematique*. Magallanes. Francia.174.

Johnson, L.K., 1982, Sexual selection in a brentid weevil. *Evolution,* 36: 251-262.

Koehl, M., 1996, When does morphology matter? *Annual Review of Ecology and Systematics,* 27: 501-542.

Krasnov, B., Ward, D., Shenbrot, G., 1996, Body length and leg variation in several species of darkling beetles (Coleoptera: Tenebrionidae) along a rainfall and altitudinal gradient in the Negev Desert (Israel). *Journal of Arid Environments,* 34(4): 477-489.

Labarbera, M., 1989, Analyzing body size as a factor in ecology and evolution. *Annual Review of Ecology and Systematics,* 20: 97-117.

Lestrel, P. E., 1997, *Fourier descriptors and their applications in biology.* Cambridge Univ. Press. 466 pp.

Mitteroecker, P., 2009, Advances in geometric morphometrics. *Evolutionary Biology,* 36(2): 235-247.

Moller, A. P., Zamora-Muñoz, C., 1997, Antennal asymmetry and sexual selection in a cerambycid beetle. *Animal Behaviour,* 54: 1509-1515.

Okamoto, M., Nobuo, K., Zhi-Hui, S., Syozo, O., 2001, Sympatric Convergence of the Color Pattern in the Chilean *Ceroglossus* Ground Beetles Inferred from Sequence Comparisons of the Mitochondrial ND5 Gene. *Journal of Molecular Evolution,* 53(4-5): 530–538

Palmer, M., 1994, Ecological factors associated with body size in populations of *Macrothorax morbillosus* (F) (Carabidae, Coleoptera). *Acta Oecologica-International Journal of Ecology,* 15(6): 689-699.

Reilly, S. M., Wainwright, P. C., 1994, *Conclusion: ecological morphology and the power of integration*. *In:* Wainwright PC & SM Reilly (eds) Ecological morphology. Integrative organismal biology: 339-354. University of Chicago Press, Chicago, Illinois, USA. 356 pp.

Ricklefs, R. E., Miles, D. B., 1994, *Ecological inferences from morphology; an ecological perspective. In:* Wainwright PC & SM Reilly (eds) Ecological morphology. Integrative organismal biology: 13-41. University of Chicago Press, Chicago, Illinois, USA.

Rohlf, F. J., 1993, *Relative warp analysis and example of its application to mosquito wings. In:* LF Marcus, I Bello & A García-Valdecasas (Eds) Contributions to Morphometric (pp. 131-159) Madrid: Museo Nacional de Ciencias Naturales.

Rohlf, F. J., 2001, TPSdig, v. 1.31. NY: State University at Stony Brook.

Rohlf, F. J., 2003, TPSsmall, v. 1.2. NY: State University at Stony Brook.

Rohlf, F. J., 2003a, TPSrelwarp, v. 1.35. NY: State University at Stony Brook.

Rohlf, F. J., 2003b, TPSSuper, v. 1.35. NY: State University at Stony Brook.

Rohlf, F. J., 2005, TPSUtil, v. 1.34. NY: State University at Stony Brook.

Rohlf, F. J., Slice, D., 1990, Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology,* 39(1): 40-59.

Rohlf, F. J., Marcus, L. F., 1993, A revolution in morphometrics. T*rends Ecology & Evolution,* 8(4): 129-132.

Rohlf, F. J., Loy, A., Corti, M., 1996, Morphometric analysis of Old World Talpidae (Mammalia, Insectivora) using partial warp scores. *Systematic Biology,* 45(3): 344-362.

Saito, Y., Mori, K., 2005, Where does male-to-male „aggression“ compromise „cooperation“? *Population Ecology,* 47: 159-166.

Shiokawa, T., Iwahashi, O., 2000, Mandible dimorphism in males of a stag beetle, *Prosopocoilus dissimilis okinawanus* (Coleóptera: Lucanidae). *Applied Entomology and Zoology,* 35(4): 487-494.

Statsoft, 1999, *STATISTICA for Windows*. StatSoft Inc, Tulsa.

Talarico, F., Romeo, M., Massolo, A., Brandmayr, P., Zetto, T., 2007, Morphometry and eye Morphology in tree species of Carabus (Coleoptera: carabidae) in relation to Habitat demands. J*ournal of Zoological Systematics & Evolutionary Research,* 45(1): 33-38.

Wainwright, P. C., 1994, *Functional morphology as a tool in ecological research*. *In:* Wainwright PC & SM Reilly (eds) Ecological morphology. Integrative organismal biology: 42-59. University of Chicago Press, Chicago, Illinois, USA.

Walker, S. E., Rypstra, A. L., 2001, Sexual dimorphism in functional response and trophic morphology in *Rabidosa rabida* (Aranae: Lycosidae). *American Midland Naturalist,* 146(1): 161-170.

Wellborn, G., 2000, Selection on a sexually dimorphic trait in ecotypes within the *Hyalella azteca* species complex (Amphipoda: Hyalellidae). *American Midland Naturalist,* 143(1): 212-225

Wiklund, C., Forsberg, J., 1991, Sexual size dimorphism in relation to female polygamy and protandry in butterflies: a comparative study of Swedish Pieridae and Satyridae. *Oikos,* 60(3): 373-381.

Williams, B. L., 2001, Patterns 1356 pp of morphological variation in *Speyeria idalia* (Lepidoptera: Nymphalidae) with implications for taxonomy and conservation. *Annals of the Entomological Society of America,* 94: 239-243.

Received: December 03, 2009 Accepted: March 24, 2010