

Research Article

Carapace shape of some aeglid crabs: plasticity at different levels

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ABSTRACT. Geometric morphometric techniques were applied for a better comprehension of inter- and intra-specific morphological variability of freshwater aeglid crabs. Carapace morphological patterns were used to address hypothesis regarding 1) the simple existence of local adaptations or 2) actual stable evolutionary features within the lineages studied. Two clades were included in this analysis: the former encompassing the closely related species *Aegla castro*, *A. parana*, *A. schmitti*, and the latter including the closely-related species *A. ligulata*, *A. longirostri* and *A. inconspicua*. Overall, distinct carapace shapes were found not only between species but also among different populations of same species. In some cases, species belonging to distinct, distantly related clades were more similar in carapace morphology than to closely related species of the same clade. This meant that there was no stable carapace morphology pattern for each major lineage. Results suggest that carapace of these crabs is plastic within lineages, although has a stable, unchangeable component readily recognizable by the geometric morphometric analysis at the species level, plus a more plastic component that may change according to the environment in which they inhabit.

Keywords: Aeglididae, Crustacea, adaptation, carapace shape, morphometric analysis.

La forma del caparazón de algunos cangrejos aeglídeos: plasticidad en distintos niveles

RESUMEN. Técnicas de morfometría geométrica se aplicaron para una mejor comprensión de la variabilidad morfológica inter e intra-específica de los cangrejos aeglídeos de agua dulce. Se utilizaron patrones morfológicos de los caparazones para hacer frente a la hipótesis sobre 1) la simple existencia de adaptaciones locales o 2) características evolutivas estables dentro de los linajes estudiados. En este análisis, se incluyeron dos clados estrechamente relacionados entre sí: el primero abarca las especies *Aegla castro*, *A. parana*, *A. schmitti*, y el segundo incluyó a *A. ligulata*, *A. longirostri* y *A. inconspicua*. Se encontraron diferencias en la forma del caparazón no solo entre especies, sino también entre las distintas poblaciones de una misma especie. En algunos casos, especies pertenecientes a clados distintos y alejados, fueron más similares en morfología del caparazón que especies estrechamente relacionadas del mismo clado. Esto significó que no hubo un patrón estable de morfología del caparazón para cada linaje. Los resultados sugieren que el caparazón de estos cangrejos exhibe plasticidad dentro de los linajes, aunque tiene un componente estable fácilmente reconocible por el análisis morfométrico geométrico a nivel de especie, más un componente más plástico que puede cambiar de acuerdo con el entorno en que habitan.

Palabras clave: Aeglididae, Crustacea, adaptación, forma de caparazón, análisis morfométrico.

INTRODUCTION

Individuals belonging to the same taxon tend to have similar bauplans due to genetic and developmental mechanisms that maintain a more or less stable macro-evolutionary pattern of phenotypic expression (Carroll *et al.*, 2005). However, environmental conditions may

influence phenotype, causing plastic responses in body allometry or in ontogenetic development (Rongling *et al.*, 2003). Phenotypic plasticity, defined as the capacity of expressing alternative patterns of morphology, physiology and/or behavior in response to environmental pressures, is the source of morphological variability within natural populations (Schlichting, 1986).

In this context, we tested the hypothesis that in different crab lineages would be evolutionary distinct morphological patterns. In other words, although each species has its singular form, a group of related species must share a general morphology due to common evolutionary history.

Anomuran freshwater crabs of the family Aeglididae present just one extant genus, *Aegla* Leach, 1820. Those are benthic forms inhabiting under rocks, roots and leaves on the bottom of limnic systems such as rivers, lakes, water bodies within caves, and fast-flowing rivulets. The family is endemic of South America, occurring in Chile, Brazil, Bolivia, Uruguay, Paraguay and Argentina (Melo, 2003). There are more than 70 described species, and approximately 35 of which are endemic to southern Brazil (Bond-Buckup *et al.*, 2008; Santos *et al.*, 2010, 2012, 2013).

Aeglid crabs display high morphological variability that has been subject of intense investigation. A common conclusion emphasized in most of those studies is the difficulty in establishing a clear-cut distinction among species (Jara, 1986; Martin & Abele, 1986; Giri & Colins, 2004; Giri & Loy, 2008). This fact makes the group an ideal model for morphometric studies.

Geometric morphometries can be defined as a group of techniques based on analysis of the contour of body structures and establishment of anatomical reference points that can be recognized as homologies (Zelditch *et al.*, 2004). It can be characterized as a cutting-edge tool for ecological studies and evolution biology, as it allows the investigation of ecologic or phylogenetic causes of morphologic variation in relation to ontogenetic stages, sexes, taxa and different populations of a same taxon (Reis, 1988).

In the present study, we applied geometric morphometries techniques for Brazilian aeglid crabs aiming a better comprehension of intra and inter-specific variability in carapace morphology, relative to distinct lineages. To accomplish this goal, we employed recent and comprehensive phylogenies of the genus *Aegla* by comparing two clades of closely-related species.

MATERIALS AND METHODS

Species

Six aeglid crab species were used in this study, belonging to two different clades in phylogeny presented by Pérez-Losada *et al.* (2004, 2009). From these, three were closely related species of subclade “C”: *Aegla castro* Schmitt, 1942, *A. parana* Schmitt, 1942 and *A. schmitti* Hobs III, 1979, all with close

geographic distributions. Other three, belonging to subclade “E” of Pérez-Losada *et al.* (2004) were *A. ligulata* Bond-Buckup & Buckup, 1994, *A. longirostri* Bond-Buckup & Buckup, 1994 and *A. inconspicua* Bond-Buckup & Buckup, 1994, which also have close geographic distribution. Individuals of different populations for each species were included in the analyses. The species of subclade “C” were photographed at the Natural History Museum of Capão da Imbuia (Curitiba, Brazil). Pictures of species of subclade “E” were obtained from aeglid crab collection of Federal University of Rio Grande do Sul (Porto Alegre, Brazil). These species were used to compare de carapace shape between clades to assign if shape is evolutionary fixed in the lineages.

Morphological analysis

Cephalothorax dorsal images of close to 30 adult males of each species, belonging to different populations (Table 1), were obtained. All images were captured with same focal distance with the aid of a wooden frame, attached to a plain board, fitted with a mechanism that allowed height adjustment according to the size of the crab. All images were taken with a Samsung ES80 digital camera and grouped with TPSUtil software version 1.44 (Rohlf, 2010).

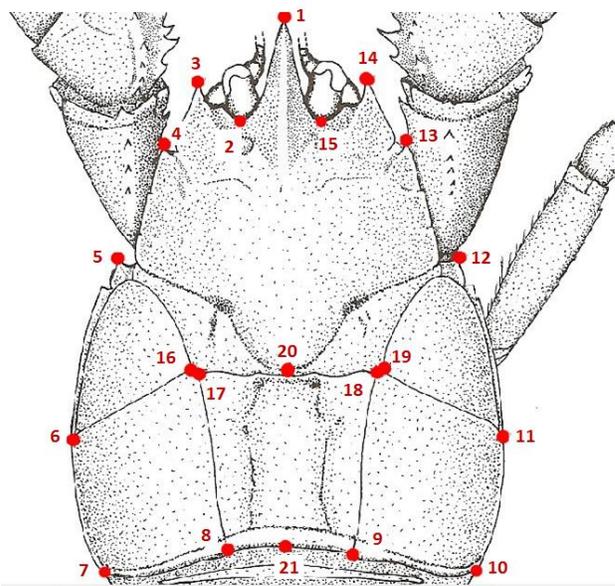
Twenty one symmetric and homologue anatomic landmarks (coordinates) were recognized on aeglid carapaces in each image (Fig. 1) using TPSDig2 version 2.12 (Rohlf, 2008). In addition, each image had their coordinates set in triplicate to reduce the error margin. The average of coordinates was used in subsequent analysis.

The anatomic coordinates for each species and populations were processed using procrustes analysis, to remove the bias introduced by size variability, and photograph position to get standardized information on carapace form (Rohlf & Slice, 1990). This analysis was performed using the Morpho J software version 1.05d (Klingenberg, 2011), and considering object symmetry. Some outliers identified were excluded from subsequent analysis resulting in the number of individuals on Table 1. A canonical variable analysis (CVA) was performed to explore the shape variations of the six species, and to maximize visual discrimination among groups. The Mahalanobis distance was calculated between all pairs of species. Data were then analyzed by discriminant analysis considering species and populations to sort out groups by their carapace shape. For comparisons between populations, those represented by few individuals were excluded from analyzes.

In addition, the Morpho J software was also used to extract the centroid size, defined as square root of the

Table 1. Number of individuals (n), locality and date of collection of each aeglid population obtained from the scientific collections.

Species	Population	n	Locality	Date collected
Clade C				
<i>Aegla parana</i>	1	10	Rio Iguaçu, São Mateus do Sul-PR	02/10/1966
	2	5	Rio Iguaçu, Pinhão-PR	04/10/1987 to 10/10/1987
	3	4	Rio Jordão, Pinhão-PR	20/10/1992
	4	7	Rio Jordão, Reserva do Iguaçu-PR	24/04/1996
<i>Aegla schmitti</i>	1	21	Rio Irai, Quatro Barras-PR	?/11/1998
	2	4	Rio Irai, Pinhais-PR	27/12/1967
	3	5	Rio Maurício, Manduituba-PR	15/12/1972
<i>Aegla castro</i>	1	5	Rio Irai-PR	17/08/2001
	2	8	Parque Estadual Cachambu, Londrina-PR	22/10/1983
	3	7	Rio Quebra Perna, Ponta Grossa-PR	28/05/1984 to 31/05/1984
	4	3	Parque Estadual Cachambu, Londrina-PR	21/10/1983
Clade E				
<i>Aegla ligulata</i>	1	5	Afluente do rio Santana, Antas-RS	28/11/2004
	2	2	Arroio Contendos, Rota do Sol-RS	23/09/1997
	3	14	Bacia Tainhas, Contendas-RS	04/06/1997
	4	9	Rio Baio Branco, Cambará-RS	27/11/2004
<i>Aegla inconspicua</i>	1	3	Rio Lavapés, Canela-RS	11/09/2004
	2	4	Canela-RS	12/09/2004
	3	9	Arroio Cerrito, São Francisco de Paula-RS	09/10/2004
	4	12	Arroio Cerrito, Maquiné-RS	19/10/2001
<i>Aegla longirostri</i>	1	5	?	25/11/2009
	2	3	Novo Treviso-RS	?/?/1982
	3	3	Arroio Afluente do Rio Carneiro, Casca-RS	30/10/2000
	4	5	Arroio dos Ratos, Parte Baixa-RS	03/12/2003 to 04/12/2003

**Anatomic position**

1	Rostral tip
2	Left orbital sinus
3	Tip of the left anterolateral spine
4	Tip of the left hepatic lobe
5	Tip of the left epibranchial tooth
6	Intersection of the left branchial line
7	Distal tip of the left branchial area
8	Posterior tip of the left dorsal longitudinal line
9	Posterior tip of the right dorsal longitudinal line
10	Distal tip of the right branchial area
11	Intersection of the right branchial line
12	Tip of the right epibranchial tooth
13	Tip of the right hepatic lobe
14	Tip of the right anterolateral spine
15	Right orbital sinus
16	Anterior tip of the left "linea aeglica dorsalis"
17	Posterior tip of the left "linea aeglica dorsalis"
18	Anterior tip of the right "linea aeglica dorsalis"
19	Posterior tip of the right "linea aeglica dorsalis"
20	Basis of the cervical groove
21	Tip of the posterior centre of the cephalothorax

Figure 1. Location and description of the 21 symmetric and homologous landmarks set on the digital images of the dorsal surface of the carapace of *Aegla*, after Melo (2003).

sum of squares of the distance of each anatomic coordinate and the centroid, the latter corresponding to the vector of the average of coordinates x and y of all anatomic landmarks marked on each image. This data was obtained for each species and clade and tested in relation to general averages using ANOVA to species comparison and t test to clades.

RESULTS

Morphology of anterior region of aeglid carapace is very stable, except for the rostrum. In contrast, the morphology of posterior region is variable, as evidenced by the overlap of procrustes as depicted in Fig. 2.

The two clades compared did not show morphological difference (pairwise discriminant analysis, $P > 0.05$). All six species compared showed significant differences in carapace shape (pairwise discriminant analysis, $P < 0.05$). Three groups of species were clearly distinguished by canonical analysis (Fig. 3) according to their carapace morphology: i) *A. parana* from clade “C” plus *A. longirostri* from clade “E”, with Mahalanobis distance = 4.2; ii) *A. castro* and *A. schmitti*, both from the clade “C”, with Mahalanobis distance = 2.6; iii) *A. ligulata* and *A. inconspicua* both from the clade “E”, with Mahalanobis distance = 1.9. All the other pairs of species presented Mahalanobis distances superior to 6.3. It was therefore found that two species belonging to distinct clades were more similar in carapace morphology than to the members of same clade.

First canonical axis was positively related to rostrum length and negatively related to width of carapace posterior region. This means that the larger value on axis 1 would translate in a longer rostrum and narrower back end of carapace. Second canonical axis was related to anterolateral spines position and carapace width. In this case, a large value on axis 2 would result in a narrow angle formed by anterolateral spines and a broader carapace at intersection of branchial line (Fig. 3).

Species and clades presented no significant differences regarding centroid size in relation to overall mean, implying that aeglid species studied had similar carapace sizes, according with ANOVA and t -tests performed ($P > 0.05$). This suggests that variability found is related to carapace shape.

All pairwise comparisons of carapace morphology between populations of a given species differed significantly (discriminant analysis, $P < 0.05$ in all cases), with high percentages of correct classifications.

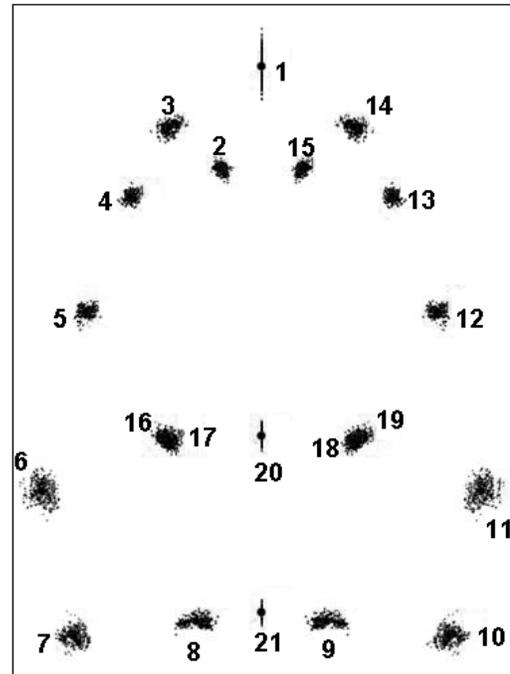


Figure 2. Procrustes overlap showing the regions of high variability in the carapace of the aeglid species studied (for numbers see Fig. 1).

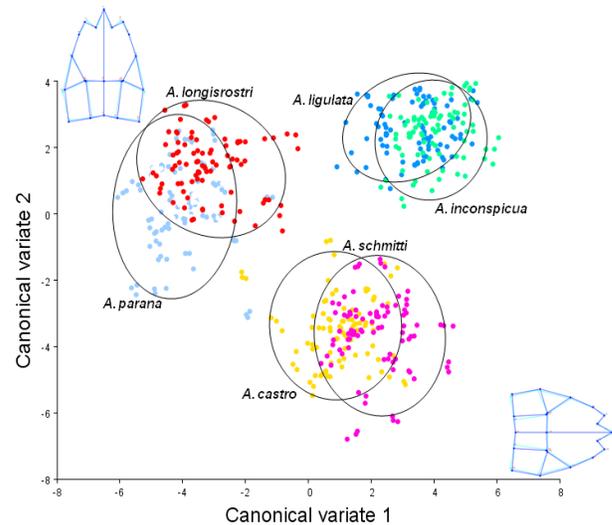


Figure 3. Canonical Variable Analysis (CVA) including all aeglid species, showing the three groups sorted out by similar carapace morphology. Each dot represents a single individual and the colour refers to species. The 90% confidence interval is indicated for each species. The outlines drawn in each axis represent the gradation of form from negative to positive values of CV.

Those results suggest the existence of a morphological pattern in aeglid carapaces.

DISCUSSION

It was demonstrated that crabs studied display carapace shape plasticity. A great diversity of organisms expresses phenotypical plasticity in response to biotic and abiotic environmental factors, resulting in variations of behavior, physiology, morphology and growth (Karban & Baldwin, 1997; Agrawal *et al.*, 1999; Dewitt & Scheiner, 2004).

In this study, significant differences in carapace shape were found between species of distinct clades which were phylogenetically distantly-related. Accordingly, species belonging to distinct lineages can be more similar in carapace morphology than to ones of closely-related species of their own clade. This suggests that carapace morphology is not a stable evolutionary feature within lineages. Rather, carapace shape may reflect the phenotypical variability associated to environment in which each species inhabit. Also, some species can be more plastic than others, depending on habitat characteristics, since environmentally induced phenotypes can be favorable, and hence selected in more dynamic environments (Fernandes & Bichuette, 2013).

However, some stability in specific components of carapace morphology was also identified at the species level, suggesting that at least some parts of the carapace morphology are conserved within each lineage, usually the anterior portion of the carapace excluding the rostrum, and central portions of the carapace. On the other hand, at population level analysis, carapace shape reflects ecological adaptations to environments where each population lived, since populations differed in form. The rostrum and the back end are the more plastic portions of carapaces, and should be more subject to environmental forcing.

The Family Aeglididae displays high morphological variability which precludes clear-cut species recognition. A number of studies on aeglid crab carapace morphology were carried thus far, including: the morphological differentiation of *A. neuquensis* carapace shape for isolated populations and with populations living sympatrically with *A. riolimayana* (Giri & Loy, 2008); description of interspecific variation of carapace morphology of sympatric *A. uruguayana* and *A. platensis* in La Plata River basin (Giri & Collins, 2004); morphologic variation of adult males carapaces of *A. schmitti* in rivers and rivulets located on opposite sides of a mountain range in southern Brazil (Trevisan & Masunari, 2010); phenotypic expressions of carapace morphology of different populations of *A. araucaniensis* along a river basin in relation to territorialism over *A. abtao* and *A. denticulata* in Chile (Barría *et al.*, 2011); morphologic

variation of *A. plana* carapaces in three distinct river basins in southern Brazil and morphological variability of carapace shapes of natural and introduced populations of *A. schmitti* in surface and cave rivulets (Fernandes & Bichuette, 2013).

Intraspecific variations of carapace morphology in different populations of given species inhabiting different environments were reported in all the aforementioned studies, in line with our findings. This suggests that different environmentally-driven phenotypic expression of carapace morphology is a rule for *Aegla* populations. In other words, characteristics of rivers, streams or lakes, like current, depth, bottom type, presence of predators or competitors can shape the form of aeglid crabs as a local ecological adaptation. Same species in a habitat with a different set of characteristics can have different shape. Aeglid crabs have small body sizes and benthic habitats. This causes physical characteristics of rivers to be effective barriers for them, isolating populations even in geographically close regions (Marchiori *et al.*, 2015). Aeglids also have low dispersal potential, missing a larval stage in its ontogenetic development, and strict environmental requirements such as clear and oxygenated water (Melo, 2003). These characteristics further increasing the possibility of isolating the population.

Most differences found in the present study were related to rostrum length and width, and extension of carapace posterior half. It is difficult to establish the relation of these body differences with the prevailing environmental conditions in habitats of aeglid crabs, especially without knowing in detail such conditions. However, as shown by Giri & Loy (2008), studying the carapace shape of *A. neuquensis* in presence and absence of a competitor, it is clear that environment promote character offsets. These authors point that river populations have more variable cephalothorax shape than lake populations, because river represent a more dynamic environment. Probably for this reason, *Aegla* carapace features are rarely used in traditional identification keys (Melo, 2003). This means that the use of geometric morphology tools may be one of the best ways to discriminate species.

Our results suggest that there is not a single pattern in carapace shape for each major lineage, and that local adaptation may respond for a large extent of morphological variation found for investigated aeglid populations. This biogeographic effect has been previously found for other aeglid species, implying that specific micro-basin environmental conditions may shape carapace morphology of different aeglid populations (Hepp *et al.*, 2012). Therefore, it may be concluded that aeglid crabs have a stable part in carapace morphology -that allowed us specific

recognition by the use of geometric morphology techniques-, and a variable plastic component that reflects environmental conditions of the water bodies in which they inhabit.

Genetic studies are required for a better understanding of morphological variability in carapace shapes of aeglid species investigated in the present study. In addition, morphological studies including more species and clades (and maybe with fresh specimens to avoid any possible deformation due to preservative method), as well as detailed environmental descriptions of water bodies where aeglid species can be found should be pursued in order to identify the main environmental factors responsible for distinct morphologies of aeglid crab carapace.

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