Geographic differences in the carapace shape of the crab *Cyrtograpsus affinis* (Decapoda: Varunidae) and its taxonomic implications

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SUMMARY: *Cyrtograpsus* genus was traditionally considered to be composed of three species: *C. angulatus*, *C. altimanus* and *C. affinis*. However, recent studies have found solid evidence suggesting that *C. affinis* and *C. altimanus* belong to a single species and hypothesize that the morphological differences which caused this misclassification could be related to different ecophenotypes or life stages. Here we report a geometric morphometrics study on the carapace shape of *Cyrtograpsus* specimens from the Río de la Plata estuary (36°S) and the Nuevo Gulf (42.75°S), testing for shape differences between different sizes (allometry) in the two environments. We found that previous morphological descriptions of the two species were associated with different sizes of a continuous, statistically significant allometric shape variation, concluding that *C. affinis* is a junior synonym of *C. altimanus*. We also found significant differences in the carapace shape between estuarine and marine environments, suggesting an effect of the environmental variables on carapace shape and a potential adaptive value of this trait.

Keywords: *Cyrtograpsus altimanus*, allometry, geometric morphometrics, Río de la Plata estuary, Nuevo Gulf, local adaptations, contrasting habitats.

RESUMEN: Diferencias geográficas en la forma del caparazón del cangrejo *Cyrtograpsus affinis* (Decapoda: Varunidae) y sus implicaciones taxonómicas. – El género *Cyrtograpsus* fue tradicionalmente considerado como formado por tres especies: *C. angulatus, C. altimanus* y *C. affinis*. Trabajos recientes reportan sólida evidencia que sugiere que *C. altimanus* y *C. affinis* pertenecen a una única especie y proponen que las formas que originaron esta clasificación errónea pueden corresponderse con diferentes ecofenotipos o estadios sucesivos del ciclo de vida de una misma especie. Este trabajo presenta un estudio de morfometría geométrica sobre la forma del caparazón de especímenes del género *Cyrtograpsus* provenientes del estuario del Río de la Plata (36°S) y del golfo Nuevo (42.75°S), en el que se prueban las diferencias en la forma del caparazón relativas al tamaño (alometría) y a los distintos ambientes. Nuestros resultados muestran que las descripciones morfológicas previas de ambas especies están asociadas a diferentes regiones de una variación alométrica continua y estadísticamente significativa, y nos permite concluir que *C. affinis* es un sinónimo juvenil de *C. altimanus*. Además encontramos diferencias significativas en la forma del caparazón entre especímenes provenientes del ambiente estuárico y el marino, lo cual sugiere un efecto de ciertas variables ambientales sobre la forma del caparazón y un potencial valor adaptativo de esta característica.

Palabras clave: *Cyrtograpsus altimanus*, alometría, morfometría geométrica, estuario del Río de la Plata, golfo Nuevo, adaptaciones locales, hábitats contrastantes.

INTRODUCTION

Morphology has played a central role in species definition, and different forms have usually been defined as species in the absence of intermediate linking forms (Mallet 2005). The understanding of morphological variation and its spatial structure has been of crucial importance to answer fundamental questions in evolutionary biology, including the relationship between genotype and phenotype (Hopkins and Thurman 2010). Different selective pressures can generate and maintain different phenotypes, and morphological analyses are also useful to show those adaptive differences. However, some geographic variations acting upon morphology, reproductive patterns, growth rates and mortality are not always consistent with genetic variation. In some situations this variation is related to phenotypic plasticity as a result of different environmental conditions (Orensanz *et al.* 1991, Cadrin 2000). In marine benthic invertebrates, planktonic dispersal is considered to play a key role in homogenizing gene frequencies, and lack of larval exchange is thought to promote differentiation and increase the genetic structure and morphological differences between distant populations (Levin 2006).

Carapace shape has been used as a basic tool in crab systematics at species level (e.g. Rathbun 1918), but has also had ecological (Giri and Loy 2008, Sardà *et al.* 2005) and fishery-related significance (Cadrin 2000, Chang and Hsu 2004). Usually, carapace shape has been estimated through curvatures or pairwise comparison of linear measurements (Brian *et al.* 2006) and only a few studies have focused on the evaluation of its allometric changes (Huber 1985, Spivak and Schubart 2003, Botello and Alvarez 2006, Costa and Soares-Gomes 2008). This is partly because classic morphometrics fails to separate size from shape variation (see Rohlf and Bookstein 1987). Allometry describes how an organism changes in shape and proportion as it grows (Peters 1983). Since the pioneering works of Huxley (1932) and Teissier (1960), it has been extensively reported in decapods and has usually been estimated by analyzing the relationship between the size of a structure (in crabs the chelae or abdomen) and the total size of the specimen (in crabs the carapace width or length). Three different types of size-shape relationship should be distinguished: ontogenetic, static, and evolutionary allometry (Cheverud 1982, Klingenberg 1996). Ontogenic relationships are the type that have been studied most and deal with covariation between characters during growth (Klingenberg 1996). This type of allometry is usually an important source of carapace shape variation and is reflected by the existence of many cases of junior synonymy (Santana and Tavares 2010, Osawa and McLaughlin 2010) resulting from the initial description of juvenile and adult forms as different species.

The field of morphometrics has undergone a revolution in which traditional approaches based on the statistical analysis of sets of inter-landmark distances have been replaced by geometric morphometrics methods (Rohlf and Marcus 1993). Geometric morphometrics methods capture shape using the Cartesian coordinates of homologous landmarks after specimen displacement, orientation and size have been removed from the data. These approaches make it possible to analyze the covariation of shape and other variables (e.g. size) as an integral part of organism biology (Adams 1999). From a methodological viewpoint, Procrustes-based geometric morphometry of landmark data is an ideal tool for analyzing allometry for two reasons (Bookstein 1991): i) landmarks can be used as the carrier of biological hypotheses of different morphogenetic units, and their patterns of variation can be analyzed in isolated as well as pooled data sets; ii) this method is based on the operational separation of size and shape, which provides a surprisingly high analytical resolution in questions of allometry (Bookstein 1991, 1996, Rosas and Bastir 2004). In addition, this method generates a set of shape variables that can be used for statistical hypothesis testing, and provides a way of visually describing patterns of shape differences in the data (Adams 1999). Thus, shape differences between local populations of the same species can be reliably examined by using a global approach to shape analysis through geometric morphometrics (Aldrich 1993). There are several applications of geometric morphometrics methods to the shape of different crab structures, including the analysis of variation between sexes and among allopatric and sympatric populations, and the evaluation of geographic and gender differences and population structure (Giri and Loy 2008, Rufino *et al.* 2006, Silva *et al.* 2010).

Cyrtograpsus (Decapoda, Varunidae; sensu Schubart *et al.* 2000, Schubart *et al.* 2002) is an endemic South American genus (Rathbun 1918) with three previously recognized members that inhabit temperate marine and brackish waters: *C. angulatus* (Dana, 1851), *C. altimanus* (Rathbun, 1914), and *C. affinis* (Dana, 1851). *C. angulatus* is the largest (maximum carapace width, MCW=61 mm; Leal and Barón, pers. comm.) and most intensely studied member of the genus (see Méndez Casariego *et al.* 2008, Daleo *et al.* 2009 and references therein); it has a bioceanic distribution and in the southwestern Atlantic it is found from Río de Janeiro (Brazil) to Santa Cruz province (Argentina) (Spivak 1997). *C. altimanus* (MCW=25 mm; Spivak and Schubart 2003) is reported to occur from Río Grande do Sul (Brazil) to northern Patagonian gulfs (Argentina) (Spivak 1997, Silva 2009). Both species can coexist in a wide range of habitats, from intertidal to shallow subtidal and from soft (muddy) to rocky bottoms in marine or estuarine areas (Spivak 1999). Their feeding habits are omnivorous, with a predominance of benthic microalgae (Martinetto *et al.* 2007, Silva 2009). The smallest (MCW=12.1 mm) and least studied species of the genus, *C. affinis* (Spivak and Schubart 2003), ranges from Cape St. Roque (Bra-

TABLE 1. – Sample composition. n: sample size and CWR: carapace width range (linear measurement in mm between the fourth lateral teeth).

Sampling location	Geographic position	Sampling dates	n	CWR	Labels
Nuevo Gulf	42°45′S, 65°00′W	April 13 to 28, 2005	137	1.51-27.76	"Madryn"
Río de la Plata estuary	35°07′S, 56°02′W	November 19, 1997	39	5.61-11.31	"Spivak 01"
	36°05′S, 56°37′W	March 9, 1998	109	4.26-9.54	"Spivak 05"

zil) to northern Patagonian gulfs, but it has only been found abundantly in sub-littoral environments near the Río de la Plata estuary (Spivak 1997, Spivak and Schubart 2003). These three species also differ in carapace shape (Rathbun 1918, Boschi 1964): roughly hexagonal with prominent anterolateral marginal teeth in *C. angulatus*, rounded with small teeth in *C. altimanus* and approximately square, a little broader than longer, with three smaller lateral teeth in *C. affinis*.

Recent comparisons based on molecular genetics and traditional morphometrics analyses support the hypothesis that *C. affinis* and *C. altimanus* "represent different ecophenotypes or life stages of the same species" (Spivak and Schubart 2003). Furthermore, morphological comparison of the first larval stages (Spivak and Cuesta 2000) and the gastric mill ossicles (Huespe *et al.* 2008) found no differences between the species; hence, at present *C. affinis* and *C. altimanus* are considered to be the same species (*Cyrtograpsus affinis* Dana, 1851). The aim of this paper is to test the suggestions of Spivak and Schubart (2003) on the basis of the allometric and geographic differences in carapace shape in specimens from the Río de la Plata estuary and the Nuevo Gulf. Specifically, the following null hypothesis will be tested: 1) changes in carapace shape are not related to variations in the size of the specimens, and 2) shape variations between geographic locations do not persist once allometric variation is statistically removed.

MATERIALS AND METHODS

Study sites

Nuevo Gulf (42°45′S, 65°W) is an elliptical basin communicated with the southwestern Atlantic ocean through a 17-km-wide strait. Its maximum depth is 184 m and it is more than 110 m deeper than the surrounding continental shelf (Mouzo *et al.* 1978). The bottoms are characterized by large extensions of sandy, gravel and limestone seabeds (Parker *et al.* 1997). The hydrographic conditions are distinguished by seasonal fluctuations in temperature (between 8°C and 18°C) and a constant salinity, near to 34 psu (Rivas and Ripa 1989). The Río de la Plata is an extensive and shallow coastal plain estuary in the western south Atlantic (35°20′S, 56°36′W), between Argentina and Uruguay. The main characteristics of the estuary are the large area that it covers and the occurrence of a quasi-permanent salt wedge, which generates bottom and surface salinity fronts (Giberto *et al.* 2004). Temperature ranges between 10°C and 24°C (Spivak 1997) and salinity ranges between approximately 0 and 30 (Guerrero *et al.* 1997). The average length is 300 km and the width varies between 40 and 200 km. It has an average depth of 5 to 25 m and a tidal range of 1 m, with the bottoms dominated by fine sediment and mud (Giberto *et al.* 2004).

Sample collection

A total of 285 crabs belonging to the genus *Cyrtograpsus* were captured: 137 specimens were collected in Nuevo Gulf on a muddy bottom at 18 m depth. They were initially identified as *C. altimanus* because the adult specimens captured matched the morphological traits reported for this species (see Boschi *et al.* 1992) and the sampling location was south of the distribution range reported for *C. affinis* (see Spivak 1997). Nevertheless, carapace width and shape was highly variable. This sample will be called "Madryn" hereinafter. In addition, 148 specimens identified initially as *C. affinis* were collected at two sites of the Río de la Plata estuary and labeled "Spivak 01" (10 m depth) and "Spivak 05" (13 m depth). The sample information is summarized in Table 1.

Sample processing and image analysis

The pereiopods were removed and the specimen was submerged in water on a Petri dish in order to minimize the light reflection. Carapaces were photographed in dorsal view with a digital camera (Canon Power Shot A510, 3.2 Megapixel), setting the picture plane parallel to its coronal plane (Fig. 1). Among the distortions that can cause artificial changes in shape, parallax distortion is the most frequent. This distortion can lead to a considerable skew in the data when the specimens are set near the borders of the picture's frame. To avoid parallax distortions, all photographs were taken setting the specimens in the centre of the image, with most of the image margins not occupied by the carapaces. Smaller specimens (ranging from 1.5 to 10 mm carapace width) were photographed under a binocular microscope with 6×, 12× and 25× magnification.

Fifteen landmarks and semi-landmarks were digitized using the *tpsDig* and *tpsUtil* software (Rohlf 2004a,c) on the left side of each carapace, assuming bilateral symmetry. Landmarks and semi-landmarks (Bookstein 1991) were chosen considering their homology in all specimens and their ability to capture the general shape of the carapace. Also, these landmarks and semi-landmarks satisfied the statistical
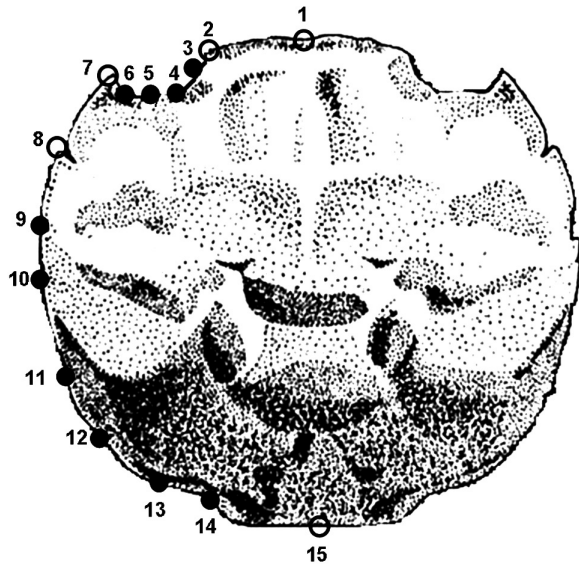
Fig. 1. – Landmarks configurations on the *Cyrtograpsus* carapace. Empty and full circles represent anatomical landmarks and semi-landmarks, respectively. Landmarks 1 and 15 marks the anterior and posterior edge of the carapace, respectively; landmarks 2 and 7 are located on the internal and external border of the ocular depression, respectively; and landmark 8 indicates the first lateral teeth. Semi-landmarks described the general contour of the carapace. Figure adapted from Boschi *et al.*, 1992.

restrictions associated with geometric morphometrics methods (Rufino *et al.* 2006). The coordinates of semi-landmarks were aligned by means of the sliding semi-landmark method implemented in the *tpsRelw* software (Rohlf 2004b) and discussed by Perez *et al.* (2006) and Sheets *et al.* (2006). The semi-landmark points are slid along the outline curve in order to minimize the bending energy of the configurations (Gunz *et al.* 2005). Landmark and sliding semi-landmark configurations (landmark configurations hereinafter) can be seen in Figure 1.

All photos included a grid of known dimensions and were scaled using the scaling procedure implemented in the *tpsDig* software (Rohlf 2004a). On each landmark configuration the centroid was defined as the point in the centre of the configuration that minimizes the sum of squares (Sneath 1967). Centroid size was computed as the square root of the sum of the squares of the distances from all landmark configurations to their centroid, using the *tpsRelw* software (Rohlf 2004b). In order to avoid any deviation of centroid size from linearity and to scale all values in the comparisons, we used the logarithm in base 10 of the centroid size (Log centroid size from now on) that is considered as an unbiased estimator of size (Bookstein 1991). The use of log-transformed centroid size was also suggested by Klingenberg (2008) for ontogenetic studies including very early, small stages in which shape change is often concentrated disproportionately.

Landmark configurations were optimally aligned using a generalized Procrustes analysis to remove the effects of translation, rotation and scale (Rohlf and

Slice 1990, Rohlf 1999). After superimposition, each landmark configuration corresponds to a single point in a non-Euclidean multidimensional space known as Kendall's shape space (Kendall 1981, 1984). This space has $2p - 4$ dimensions, where p is the number of two-dimensional landmarks and semi-landmarks. Because of the difficulty in performing standard multivariate statistics in non-Euclidean space, each data point is then projected into a Euclidean space (also of $2p - 4$ dimensions) tangential to a reference point (usually the mean) in the shape space (Kent 1994, Rohlf 1999). Since shape data derived from Procrustes superimposition lie in a non-linear, hyper-hemispherical shape space (Slice 2001), common statistical methods based on linearity can only be performed when the data is projected onto a Euclidean (linear) tangent space (Rohlf 1996). This approach of utilizing an orthogonal projection from the Procrustes hyper-hemisphere to tangent space is thought to yield the necessary linearization of curved distances in shape space (Bookstein 1991, Dryden and Mardia 1998, Slice 2001). A criterion for the reliability of statistical analysis is provided by a strong correlation between Procrustes and tangent space distances. In the present study, this correlation was computed using the *tpsSmall* software (Rohlf 1998) and was very strong and highly significant (correlation: 1.00; $P<0.001$). Therefore, the coordinates of the superimposed landmarks and semi-landmarks can be used in multivariate statistical analyses to address a wide range of biological questions (e.g. Klingenberg 2010). Multivariate descriptions of the data in the tangent space (the shape variables) can be generated through a variety of methods (Rohlf 1999). Here we use the thin-plate spline approach, which decomposes the data into two components of uniform shape change (known as uniforms scores) and $2p - 6$ components (known as partial warp scores) of non-uniform shape change (Bookstein 1991, 1996, Rohlf 1993). Uniform scores describe differences that affect all parts of the carapace equally (global differences), and partial warp scores describe localized departures from the average carapace map. Shape variation is described as the difference of each individual from a reference, after translation, rotation and scaling of all individuals and all homologous landmarks and semi-landmarks are superimposed. Each individual is represented in this Bookstein metaphor by its partial warp scores, which depict each individual's contribution to the grid deformation defined by them. The fifteen landmarks and semi-landmarks on the carapace led to 24 partial warp scores and 2 uniform scores for each sample.

Statistical analysis

Carapace shape allometry

Multivariate regression of shape (the aligned coordinates) on Log centroid size (carapace size) was performed using the MorphoJ software (Klingenberg 2011). The statistical significance of the regression was
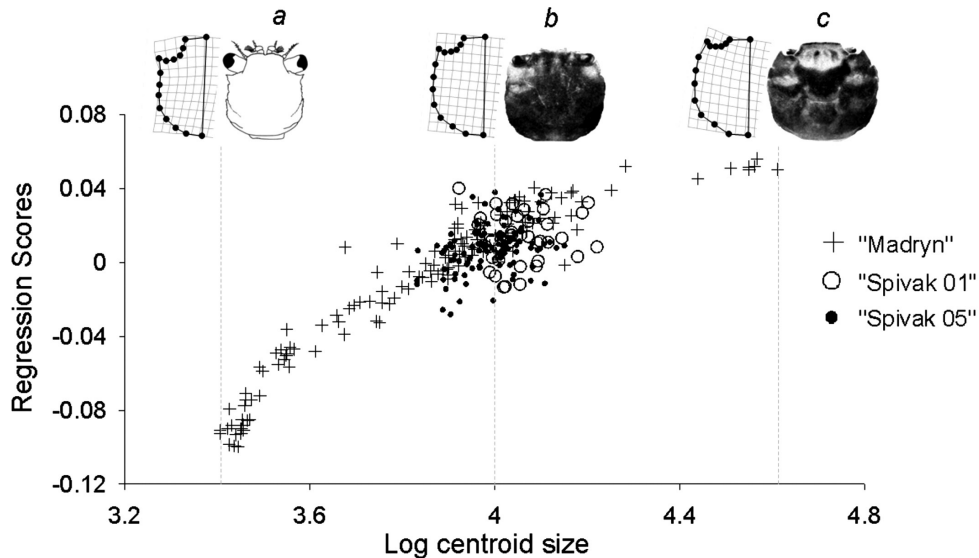
FIG. 2. – Scatterplot of the regression scores (carapace shape) versus the Log centroid size (carapace size). Drawings are those reported in literature describing *C. affinis* first juvenile instar (*a*, Spivak and Cuesta 2000) and adult (*b*, Rathbun, 1918), and *C. altimanus* adult (*c*, Rathbun, 1918). The thin-plate spline deformation plots show the carapace shape corresponding to 3.4, 4 and 4.6 Log centroid size, respectively.

tested with permutation test against the null hypothesis of independence (Good 2000). The regression routine in MorphoJ computes a regression score for each specimen (Drake and Klingenberg 2008). The vectors of regression coefficients from this analysis can be visualized as shape change per unit of size increase. The scatterplot of the regression scores against the Log centroid size was made as it provides a graphical means to examine the strength of association between shape and size (Drake and Klingenberg 2008).

Shape differences between geographic locations

Two different approaches were used to assess geographic differences in shape:

a) Multivariate analysis of variance (MANOVA; Sokal and Rohlf 1995) was used to test the differences in shape variables (partial warp scores and uniform scores) between sampling locations. Post hoc comparisons were tested by means of a Newman-Keuls test. Alternatively, a permutational multivariate analysis of variance (PERMANOVA; Anderson *et al.* 2004) was performed using shape variables as the dependent variable and location as the independent variable. In order to generate a balanced design with 137 specimens per sample, the two estuary samples were pooled, thus achieving a new sample size of 148 individuals. Then, 11 specimens were randomly removed from the pooled sample in order to achieve the sample size of 137 required for a balanced PERMANOVA (Anderson 2001, Tracey *et al.* 2006).

b) In order to generate allometry-free shape variables, a multivariate regression of shape variables on Log centroid size was fitted to a polynomial model (r^2=0.845 and P<0.01). This model was selected because of the lack of any a priori functional relationship

between size and shape. Residuals of the regression model were considered as allometry-free shape variables (Webber and Hunda 2007, Hopkins and Webster 2009). MANOVA and PERMANOVA tests were performed as described above in order to compare the allometry-free variables.

To represent the major trends in shape differences (with and without the allometric effect), shape variables were transformed to a Euclidean distance dissimilarity matrix and the two corresponding non-metric multidimensional scaling ordination plots were obtained.

RESULTS

Carapace shape allometry

The multivariate regression of shape on carapace size was highly significant after 10000 permutations (P<0.001) and allometry accounted for 41.76% of the total variation in carapace shape. The changes in carapace shape during growth are represented in the scatterplot shown in Figure 2. Shape changes along the size axis are gradual, without any evident discontinuity. The rate of shape changes relative to size was higher in smaller specimens, decreases with size and there were no dramatic shape changes in specimens with Log centroid size values higher than 4.3 (carapace width greater than approximately 12 mm) (Fig. 2).

Shape differences between geographic locations

The differences between sampling locations were confirmed in both shape variables and allometry-free shape variables. The MANOVA and PERMANOVA tests performed on both datasets yielded significant differences (Table 2). The post hoc pairwise comparisons

TABLE 2. – Statistical analyses to test for shape differences between sampling locations.

	MANOVA			PERMANOVA	
	Wilk's λ	F	P	F	P
Shape variables (partial warps and uniforms scores)	0.2974	13.90	<0.001	29.57	<0.001
Allometry-free shape variables (*)	0.0549	54.48	<0.001	32.59	<0.001

(*) Allometric effects were removed by using the residuals of a polynomial regression of shape variables (partial warps and uniforms scores) on Log centroid size.

were significant between "Madryn" and both estuary samples, and not significant between samples within the estuary.

Despite the significant shape differences found between estuarine and gulf specimens, this is not clearly visualized in the non-metric multidimensional scaling ordination computed on both datasets (shape variables and allometry-free shape variables; Figure 3a and b, respectively).
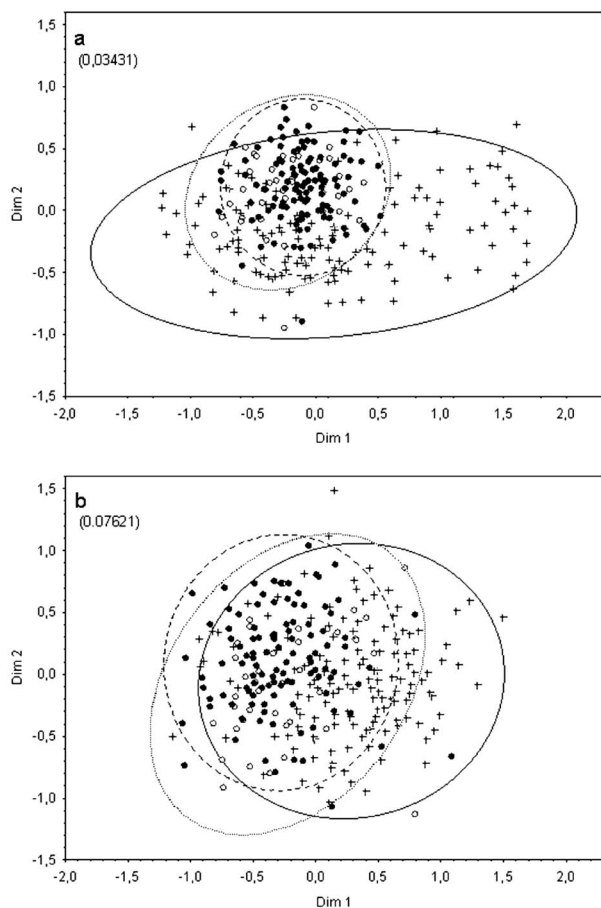

FIG. 3. – Non-metric multidimensional scaling (MDS) computed on shape variables (a) and allometry-free shape variables (b). Ellipses containing 95% of data are presented for each group. Stress values are between brackets and symbols are as in Figure 2.

DISCUSSION

Here we report a geometric morphometric study on the carapace shape of *Cyrtograpsus affinis* and *C. altimanus* that allows us to conclude that the former is a junior synonym of the latter. The study aimed to test for carapace shape differences between three different coastal regions, two of them within the Río de la Plata estuary and the third in the Nuevo Gulf (approximately 1150 km to the southwest). Previously we analyzed the regression between shape and size in order to test for shape allometry and to provide a graphical visualization of the relation between carapace shape of all the specimens included in the study and the graphical descriptions of the carapace used in previous morphological descriptions (Rathbun 1918, Spivak and Cuesta 2000).

The allometry in carapace shape was highly significant and the pattern of allometric shape variation precludes the existence of two morphologically dissimilar groups in the sample. The previous descriptions of the carapace shape of *C. affinis* and *C. altimanus* match different regions of a continuous allometric trajectory. The shape of the first instar of *C. affinis* previously described (Spivak and Cuesta 2000) matched with the smaller specimens (~2 mm carapace width [CW]) captured in the Nuevo Gulf. The first descriptions of *C. affinis* and *C. altimanus* by Rathbun (1918) match with the allometric trajectory in the mid-region (~10 mm CW) and in the extreme of the larger specimens (~20 mm CW), respectively (Fig. 2). The relative position of previously described carapace shapes in a continuous allometric trajectory could be considered evidence supporting Spivak and Schubart's hypothesis that "the two forms represent different life stages of the same species". Several studies based on ontogeny (Guinot 1984 and Otani and Takahashi 1996) and genetics (Mantelatto *et al.* 2007) allowed two crab species originally described on the basis of juvenile or adult specimens, respectively, to be synonymized. Our work goes further in providing formal geometric morphometric tests aimed at detecting, quantifying and removing allometric effects on a sample of carapaces previously considered as belonging to two different species.

In traditional crustacean taxonomy, carapace shape was largely used and almost all geographic forms that could be separated (e.g. the absence of "linking forms", Mallet 2005) were assigned to a species rank. According to our results, the carapace shape of *C. affinis* is clearly affected by ontogenetic allometry. Furthermore, this is the main source of morphological variation and is possibly the cause of Rathbun's original taxonomic misclassification. Several authors highlight the taxonomic implications of ontogenetic allometry (Rincón 2000, Klingenberg *et al.* 2001, Rosas and Bastir 2004)

and emphasize the need for a careful evaluation of the allometric effect when taxonomic assignation is focused on morphological variability. Shape allometry has also become important because of a trend towards using morphometrics also in the fields of conservation, biogeography, evolutionary biology (Santos Moreno and Hortelano 1997) and stock identification (Cadrin 2000, Chang and Hsu 2004).

Two different methods were used to compare carapace shape between geographic locations and yielded the same results: specimens from the Nuevo Gulf are different from those from the Río de la Plata estuary. Shape changes according to habitat variations have been recorded in other decapods species (Giri and Loy 2008, Barría *et al.* 2011). Organisms frequently develop specialized phenotypes adapted to local environmental conditions (Kingsolver *et al.* 2002) and the size-independent (allometry-free) morphological variation between locations may reflect local adaptation to contrasting habitats. The two locations sampled in this study are distinctive in the hydrographic conditions of the water masses. The sampling sites in the estuary are subtidal (10 and 13 m depth) and located 21 and 43 km from the nearest rocky intertidal, a muddy habitat with high turbidity, high temperature and low and variable salinity (Framiñan and Brown 1996, Guerrero *et al.* 1997); the sampling site in the Nuevo Gulf is also subtidal (15-20 m depth), but it is a near-shore (<1 km from the shoreline) muddy bottom with lower temperature and turbidity, and higher salinity (Rivas and Ripa 1989). Also, both the estuary and the gulf have been classified into different zoogeographic provinces (the Argentinian and the Magellanic provinces, respectively), in which different communities are expected to occur (Balech 1954, Boschi 2000).

Differences in maximum body size have been mentioned as a partial source of morphological divergence among crab carapaces (Hopkins and Thurman 2010). The maximum size is consistently lower in specimens previously classified as *C. affinis* than in *C. altimanus* (Table 1 and Spivak and Schubart 2003). Furthermore, the size at maturity of females is lower in the former (see Spivak and Schubart 2003, Fig. 4). Intraspecific changes in size range and size at maturity of decapod crustaceans has been mainly related to latitude (e.g. Garth 1957, Hines 1989, Orensanz *et al.* 2007) but also to water quality (i.e. estuarine vs freshwater populations; Mashiko 2000), predation pressure and food availability (Hines 1989). These changes were generally considered to be the result of phenotypic plasticity rather than genetic differences. Recently, the dramatic differences within the two forms of adult squat-lobsters has been attributed to heterochronies leading to phenotypic plasticity and associated with a response of the species to a varying environment (Haye *et al.* 2010). The two successive life stages studied in this paper could be an excellent model for testing the hypothesis of heterochronic development as an explanation for the observed carapace shape differences, also testing

whether one or several of the environmental features are associated with the observed phenotypic plasticity.

Morphometric research is more biologically meaningful if coupled with functional hypotheses regarding the adaptive significance of differences in body shape, a synthesis that is needed for the advancement of the field (Cadrin *et al.* 2005). The evidence of significant allometry of carapace shape found in this study highlights the need for a careful evaluation of the allometric effect when taxonomic assignation is focused on morphological variability. Furthermore, the geographic differences suggest an effect of the environmental variables on carapace shape, and a potential adaptive value of this trait. A fruitful line of research was developed in crab morphometrics when cheliped shape was related to habitat, especially in durophagous species (Silva *et al.* 2010 and references thereof). However, the link between the carapace shape and the environment conditions and epigenetic factors is poorly understood (Giri and Loy 2008, Barría *et al.* 2011). Studies manipulating habitat composition, predators and competitors will be required in order to test specific hypotheses and elucidate this association.

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