

Claw asymmetry in crabs: approaching an old issue from a new point of view

FEDERICA SPANI^{1*}, MASSIMILIANO SCALICI¹, KEITH A. CRANDALL^{2,3,◉} and PAOLO PIRAS^{4,5}

¹Department of Sciences, University of Roma Tre, Viale Guglielmo Marconi, 446, 00146 Rome, Italy

²Computational Biology Institute, George Washington University, Ashburn, VA 20132, USA

³Department of Invertebrate Zoology, US National Museum of Natural History, Smithsonian Institution, Washington, DC 20012, USA

⁴Department of Cardiovascular, Respiratory, Nephrological, Anesthesiological and Geriatric Sciences, “La Sapienza” University of Rome, Viale del Policlinico, 155, 00100 Rome, Italy

⁵Department of Structural and Geotechnical Engineering, “La Sapienza” University of Rome, Via Eudossiana 18, 00100 Rome, Italy

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Crabs are considered exceptional examples of antisymmetry resulting from the phenomenon of heterochely. Here we investigate morphometrically both the size and the shape of heterochely in 28 crab species, distributed unequally along a brachyuran phylogeny. We address the importance of investigating claw size and shape for interspecific comparisons by linking geometric morphometric outputs to phylogenetic data for 134 brachyuran species. New indices introduced as new sexual dimorphic characters of size and shape, namely heterometry (right chela size/left chela size) and heteromorphy (Procrustes distance between right and left chelae shape), revealed sexually dimorphic differences in diverse crab species. We demonstrate that both size and shape heterochely occur amongst the examined species, but there are no ecological correlations. Our study demonstrates that claw similarity between two or more species was due mainly to phylogenetic relatedness rather than ecological convergence, suggesting that claw morphological features could be useful morphological markers in phylogenetic studies. Although further investigation is needed, this study represents one of the first to thoroughly analyse the origin and evolution of heterochely within the Brachyura clade.

ADDITIONAL KEYWORDS: Brachyura – handedness – heterochely – heterometry – heteromorphy.

INTRODUCTION

Asymmetry in size or shape is a widespread pattern in evolution (Palmer, 1996). This condition may be interpreted as the deviation of an organism, or part of it, from perfect symmetry (Van Valen, 1962). This deviation from bilateral symmetry produces handedness (Graham *et al.*, 2010). According to the distribution of handedness, three main kinds of asymmetries have been recognized (see Klingenberg, 2015): fluctuating asymmetry, where the variance in the right–left (R–L) differences is distributed around a mean of value 0 (Gaussian distribution); directional asymmetry, where R–L differences are distributed

around a mean that is significantly different from 0 (Gaussian distribution); and antisymmetry, where the lack of symmetry in normally developing traits is distinguished by a departure from a Gaussian distribution of R–L differences (bimodal distribution).

Asymmetries are common among living organisms. Studies have shown that more than 450 species from 67 families in eight phyla of animals and plants exhibit antisymmetry (Palmer, 2005), and current studies are continuing to identify more asymmetries within Animalia (Klingenberg, 2015). Among these animals are some species of crabs (*Gecarcinus* spp., *Cardisoma* spp., *Uca* spp., *Acanthocyclus* spp., etc.) that have claws of very different sizes and shapes (Rathbun, 1918, 1930; Williams & Heng, 1981; Palmer, 2005; Graham *et al.*, 2010; Klingenberg, 2015). This phenomenon is known

*Corresponding author. E-mail: federica.spani@uniroma3.it

as ‘heterochely’: the two chelae are normally referred to as major and minor because of the size difference. Traditionally, major and minor claws were named crusher and cutter, respectively, depending on their functions during alimentary and predator activities. Indeed, a common shape difference is that the occlusal surfaces of the major chela bear rounded ‘molariform’ teeth while those of the minor chela bear numerous conical teeth (Abby-Kalio & Warner, 1989). Usually both sexes of heterochelous species display heterochely, although to different degree, so it is a secondary sexual characteristic in both marine (Castiglioni & Coelho, 2011; Alencar *et al.*, 2014) and freshwater (Trevisan *et al.*, 2012; Spani & Scalici, 2016) crabs.

Decapods are considered to represent interesting models for evaluating variations in claw size and shape through geometric morphometrics, due to their rigid exoskeleton with many spines and sutures that allow for accurate biometric measurements (Trevisan *et al.*, 2012; Alencar *et al.*, 2014). The phenomenon of claw asymmetry in the adaptive radiation of brachyurans has been thoroughly studied (see Hartnoll, 1978, 1982; Micheli *et al.*, 1990; Duarte *et al.*, 2008; Juanes *et al.*, 2008; Scalici *et al.*, 2013), but several questions remain (see Scalici & Gherardi, 2008, and references therein). Indeed, some studies have been carried out to better understand chelae use in predation, food manipulation, mate acquisition, defence of resources (such as food and territories), parental care (see Stein, 1976; Raubenheimer, 1986; Gherardi & Micheli, 1989; Liu & Li, 2000; Schenk & Wainwright, 2001) and the generation of different inter-sexual aggressive behaviours during antagonistic fights (see Gabbanini *et al.*, 1995, and references therein). In addition, allometric analyses of the chela have been investigated (e.g. Hartnoll, 1978; Daniels, 2001), but unfortunately most of these studies focused on the major chela (see Spani & Scalici, 2016, and references therein).

The well-known phenomenon of heterochely is no longer considered as a difference between claw size solely but rather differences in both size and shape. Here we propose for the first time, to our knowledge, the two new dimensionless indices ‘heterometry’ and ‘heteromorphy’ to quantify differences in chela size and shape of brachyurans by means of geometric morphometrics. Specifically, heterometry is the ratio between the ‘size index’ of the right and left claw, while heteromorphy is the numerical quantification of the shape differences between the right and left claw. By doing so, we overcome the problems of traditional analysis of heterochely, and recognize three different heterochelic patterns in each analysed species (size-heterochely, shape heterochely or both), and whether they could represent new sexually dimorphic characters.

In addition, we test for possible links between both heterometry and heteromorphy, and some ecological features (i.e. environment, habitat, feeding types). Finally, we assess possible relationships between claw form (=size + shape *sensu* Cardini & O’Higgins, 2005) and phylogenetic signal among brachyuran species by evaluating geometric morphometric results in a phylogenetic context.

MATERIAL AND METHODS

BIOLOGICAL MODEL

A total of 843 right-handed specimens belonging to 28 brachyuran species were included in the study (Table 1). All studied claws were checked to ensure that they were not in regeneration after autotomy. The specimens came from three sources. First, 198 freshwater and marine specimens (six brachyuran species) were sampled by F.S. from river water and Latium sea coasts: Rio Fiume (Rome, Italy) for freshwater crabs; and Santa Marinella (Rome, Italy) and Passoscuro (Rome, Italy) for several marine individuals captured by snorkelling. Second, the dataset was expanded with 465 specimens (13 brachyuran species) stored in the ‘La Specola’ Zoological Museum (University of Florence, Florence, Italy) and the National Museum of Natural History, Smithsonian Institution (Washington, DC, USA). Selected specimens were photographed by F.S. *in loco*. Finally, 180 individuals (nine brachyuran species) were recovered, before they had been discarded, from commercial fishermen of Latium (Passoscuro, Santa Marinella and Fiumicino, Rome, Italy) and Veneto (Chioggia, Venice, Italy). Among the 28 species analysed, 21 had five or more male individuals, 18 had five or more female individuals, and 18 had five or more individuals of both male and female individuals. Therefore, the numbers of species and specimens analysed are different amongst our statistical analyses depending on the type of test as well as the individuals involved in the analysis. Specifically, statistical analyses performed on males represented 21 species while analyses performed solely on females represented 18 species. Statistical analyses of sexual dimorphism, which require both males and females, involved those 18 species for which more than five male and female individuals were available.

GEOMETRIC MORPHOMETRIC EXPERIMENTAL DESIGN

Both the right and the left claws of each individual were photographed with a Tamron SP 90-mm F/2.8 Di VC USD 1:1 macro lens mounted on a Canon EOS 700D camera. The camera was set on a stand, and the crabs were positioned in sand to hold the claws

Table 1. List of investigated crab species, number of specimens separated by sex (M = male, F = female), and marks used for each species

	Species	M	F	Marks
1	<i>Atelecyclus rotundatus</i> (Olivi, 1792)	0	1	Ate_rot
2	<i>Acanthocyclus albatrossis</i> Rathbun, 1898	17	22	Ash_alb
3	<i>Ashtoret lunaris</i> (Forskål, 1775)	25	23	Ash_lun
4	<i>Bathynectes maravigna</i> (Prestandrea, 1839)	3	2	Bat_mar
5	<i>Calappa granulata</i> (Linnaeus, 1758)	11	20	Cal_gra
6	<i>Callinectes sapidus</i> (Rathbun, 1896)	11	10	Cln_sap
7	<i>Cancer borealis</i> Stimpson, 1859	25	24	Can_bor
8	<i>Carcinus aestuarii</i> (Nardo, 1847)	26	16	Car_aes
9	<i>Cardisoma guanhumi</i> Latreille, 1828	17	10	Crd_gua
10	<i>Carpilius maculatus</i> (Linnaeus, 1758)	9	14	Crp_mac
11	<i>Daira perlata</i> (Herbst, 1790)	15	25	Dai_per
12	<i>Derilambrus angulifrons</i> (Latreille, 1825)	1	0	Der_ang
13	<i>Ergasticus clouei</i> A. Milne-Edwards, 1882	1	0	Erg_clo
14	<i>Eriphia verrucosa</i> (Forskål, 1775)	0	3	Eri_ver
15	<i>Geryon longipes</i> A. Milne-Edwards, 1882	17	14	Ger_lon
16	<i>Goneplax rhomboides</i> (Linnaeus, 1758)	30	9	Gon_rho
17	<i>Herbstia condyliata</i> (Fabricius, 1787)	1	0	Her_con
18	<i>Liocarcinus depurator</i> (Linnaeus, 1758)	26	29	Lio_dep
19	<i>Macropipus tuberculatus</i> (Roux, 1830)	32	19	Mac_tub
20	<i>Medorippe lanata</i> (Linnaeus, 1767)	19	20	Med_lan
21	<i>Ocypode ryderi</i> Kingsley, 1880	24	4	Ocy_ryd
22	<i>Pachygrapsus marmoratus</i> (Fabricius, 1787)	2	2	Pac_mar
23	<i>Pilumnus townsendi</i> Rathbun, 1923	17	22	Pil_tow
24	<i>Portunus latipes</i> (Pennant, 1777)	30	30	Por_lat
25	<i>Potamon fluviatile</i> (Herbst, 1785)	30	28	Pot_flu
26	<i>Potamonautes obesus</i> A. Milne-Edwards, 1868	30	37	Ptm_obe
27	<i>Uca vocans</i> (Linnaeus, 1758)	22	0	Uca_voc
28	<i>Xantho pilipes</i> A. Milne-Edwards, 1867	14	4	Xan_pil
	Total specimens: 843			

parallel to both the camera lens and the stand plane. A total 1686 photographs were taken. To analyse the variation in claw shape, 11 landmarks were selected on homologous structures on the manus and pollex of the propodus (Fig. 1A; Table 2) using tpsDig 2.16 (Rohlf, 2010) according to previous studies (Rosenberg, 2002; Silva & Paula, 2008; Trevisan *et al.*, 2012; Alencar *et al.*, 2014). All landmarks were chosen for (1) their ease of identification and homology in all specimens, and (2) their suitability to capture the general shape of the chela according to Rosenberg (2002). In addition, 23 semi-landmarks were fixed at equal distances along the claw's external margin by using tpsDig 2.16 to capture shape differences where it was not possible to define homologous landmarks (Silva & Paula, 2008) (Fig. 1B). Semi-landmarks are points with a reduced degree of freedom depending upon landmarks (for details, see Perez *et al.*, 2006). No points were chosen on the dactyl because (1) only a homologous landmark could be identified on the tip, and (2) it represents a supple (hard but not stationary) structure.

STATISTICAL ANALYSIS OF HETEROCHELY

The size of each configuration was estimated using centroid size (CS), a dimensionless parameter computed as the square root of the sum of the squares of the distances of each landmark from the centroid (Bookstein, 1986). Once CS values for each right (major) and left (minor) chela of males and females of each species had been obtained, the ratio 'right chela CS/left chela CS' (heterometry) for each specimen was calculated to give chela size difference in a single parameter.

To evaluate possible sexually dimorphic size characteristics within each species, a series of one-way analysis of variance (ANOVA) was then performed on the CS values of both right and left chelae. The effects of variation in position, orientation and scale of the photographed claws may generate a non-shape variation (NSV) after digitization. In this case, NSV must be mathematically removed because the use of raw coordinates as shape variables in the subsequent statistical analyses would be inappropriate (Adams

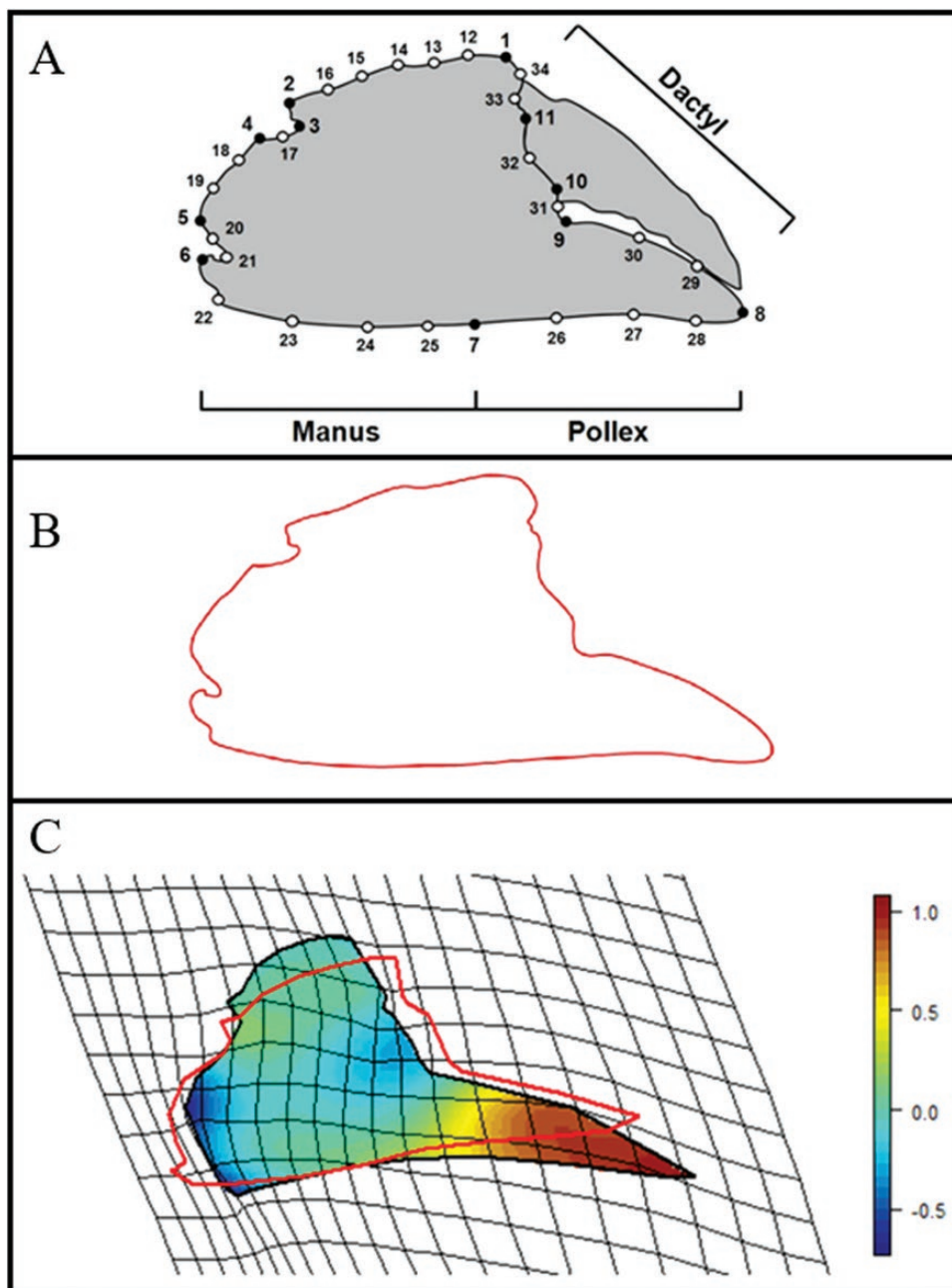


Figure 1. A, claw regions and landmark + semi-landmark (black and white circles, respectively) configurations. B, claw outline obtained by fixing both landmarks and semi-landmarks. C, example of visual output of the geometric morphometric analyses on deformation grids: red line, medium shape of claw; black line, claw shape at maximum deformation degree; the range of colours shows areas of morphological variation from highest (red) to lowest (blue) deformation degrees.

et al., 2004). To do so, for any series of configurations, we used Generalized Procrustes Analysis (GPA; Bookstein, 1991; Goodall, 1991), which align shapes by minimizing the 'Procrustes distance'. The Procrustes distance is the square root of the sum of squared differences between the positions of the landmarks in optimally (by least-squares) superimposed and

scaled configurations. GPA rotates, aligns and scales landmark configurations to unit CS so that shape differences between specimens are not due to rotation, position or size (Rohlf & Slice, 1990). The alignment of landmarks due to GPA generated new coordinates of landmarks and semi-landmarks in each configuration. These new coordinates were used as variables in

subsequent multivariate statistical analyses (Rohlf *et al.*, 1996; Rohlf, 1998).

A principal component analysis (PCA) was performed on the landmark configurations (see Rohlf & Slice, 1990; Bookstein, 1991; Dryden & Mardia, 1998; Polly, 2003) of both chelae for males and females separately. The principal component scores (PCs) were used in a multivariate analysis of variance (MANOVA) to (1) evaluate the occurrence of statistically significant sexual dimorphism in claw shape and (2) assess interspecific differences for each sex. Finally, Procrustes distances (PDs, vectorial distances) between right and left claws were calculated for each individual to obtain an individual heterochelic shape index (heteromorphy). To calculate heteromorphy, the left chela was reflected along the *y*-axis. An ANOVA was then performed on PDs to assess both sexually dimorphic and interspecific differences. All procedures described above, and associated statistics, were performed using the R package Morpho (Schlager, 2013).

THE OVERLAP BETWEEN FORM AND ECOLOGY

Information about habitat and feeding types of crabs are very fragmented in the scientific literature and for many species they are not available. In this study, data for environment (i.e. macroscopic conditions where species live: marine, brackish, estuarine and freshwaters), habitat (i.e. benthic conditions where crabs inhabit: rocks, sand and mud) and feeding types of the studied crabs were collected (Supporting Information, Table S1) from several published scientific papers and/or from the World Register of Marine Species (WoRMS; <http://www.marinespecies.org>). All previous ecological trends were correlated with shape and size variables to understand if heterometry and/or heterochely are associated with these ecological factors. In particular, environment, habitat and feeding types (Table S1) against heterometry and heteromorphy in both sexes were tested by a series of pair-wise ANOVAs to determine if those ecological features could explain heterometry and heteromorphy for interspecific variation and sexual dimorphism.

MAPPING FORM ON BRACHYURAN PHYLOGENY

To compute the phylogenetic signal, several gene regions (12S, 16S, 18S, 28S, Enolase, GADPH, H3, NAK, PEPCK; K.A.C., pers. data) of 134 different crab species (including species from which we collected morphological data) were used to reconstruct a time-calibrated phylogenetic tree aligning all genes using PASTA. PartitionFinder was used to find an optimal data partitioning scheme, Raxml to estimate a phylogeny with the concatenated dataset, and we

calibrated tree to time using penalized likelihood in the program 'treePL' (the c++ version of the program 'r8s').

We tested a number of associations of claw morphology with phylogeny: (1) for both the size and the shape of right and left claws in males and females; (2) for sexual dimorphism in right and left claw size and shape; and (3) for heterometry and heteromorphy in males and females. Specifically, branch lengths of each of our taxa were compared with previous morphometric variables (i.e. size, shape, sexual dimorphism in size and shape, heterometry and heteromorphy) by applying a K test (Adams, 2014). This was performed using the R function *phylosig* from the R package *phytools*, which computes phylogenetic signals using two different methods (Revell, 2012), namely 'K' or 'lambda'.

RESULTS

CENTROID SIZE AND HETEROMETRY

The CS of both right (R) and left (L) claws, in both sexes of all species, showed significant variation (Figs 2, 3). Statistically significant sexually dimorphic differences in CS were found in ten species in right and/or left claws, while the remaining eight did not show significant sexual dimorphism. Although the number of species was the same for both right and left claws, the taxa were different for each. Among species showing significant sexual dimorphism, nine showed sexually dimorphic differences in CS for both claws. *Calappa granulata* (Linnaeus, 1758) had significant sexual dimorphism only for the right claw, while *Potamon fluviatile* (Herbst, 1785) was only sexually dimorphic in the left claw (Table 3).

Interspecific variability was also observed for heterometry in both sexes (Fig. 4). Specifically, significant values of heterometry existed in eight of 21 and seven of 18 species for males and females, respectively (Table 3). Among 18 species with five or more individuals of both sexes, five species showed a significant heterometry while *Medorippe lanata* (Linnaeus, 1767) showed significant heterometry only in males. Both *Carpilius maculatus* (Linnaeus, 1758) and *Daira perlata* (Herbst, 1790) showed significant heterometry only in females.

Heterometry was a significant sexually dimorphic character for seven of 18 species (Table 3). Males had higher mean values than females in five of these species. *Geryon longipes* A. Milne-Edwards, 1882 and *Macropipus tuberculatus* (Roux, 1830) showed the opposite trend with females having higher mean values than males. The sexually dimorphic variation

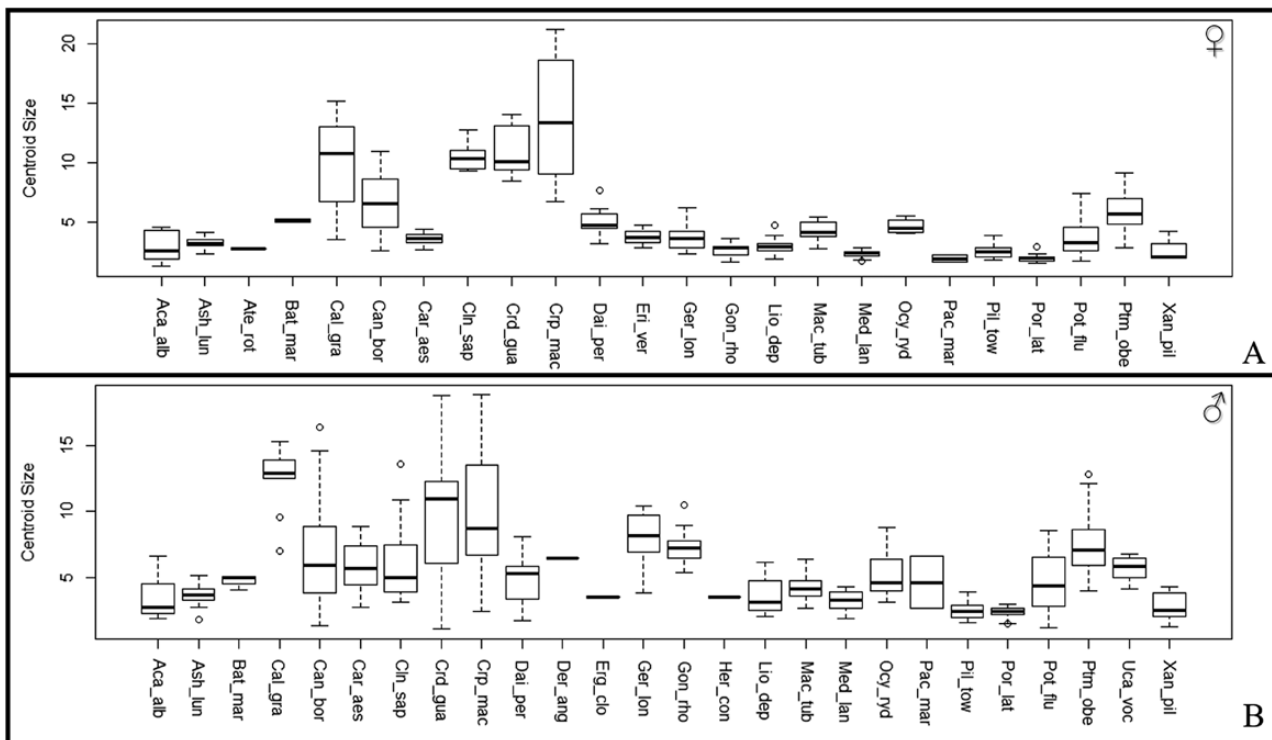


Figure 2. A, centroidsize values for right claw in female crabs. B, centroid size values for right claw in male crabs.

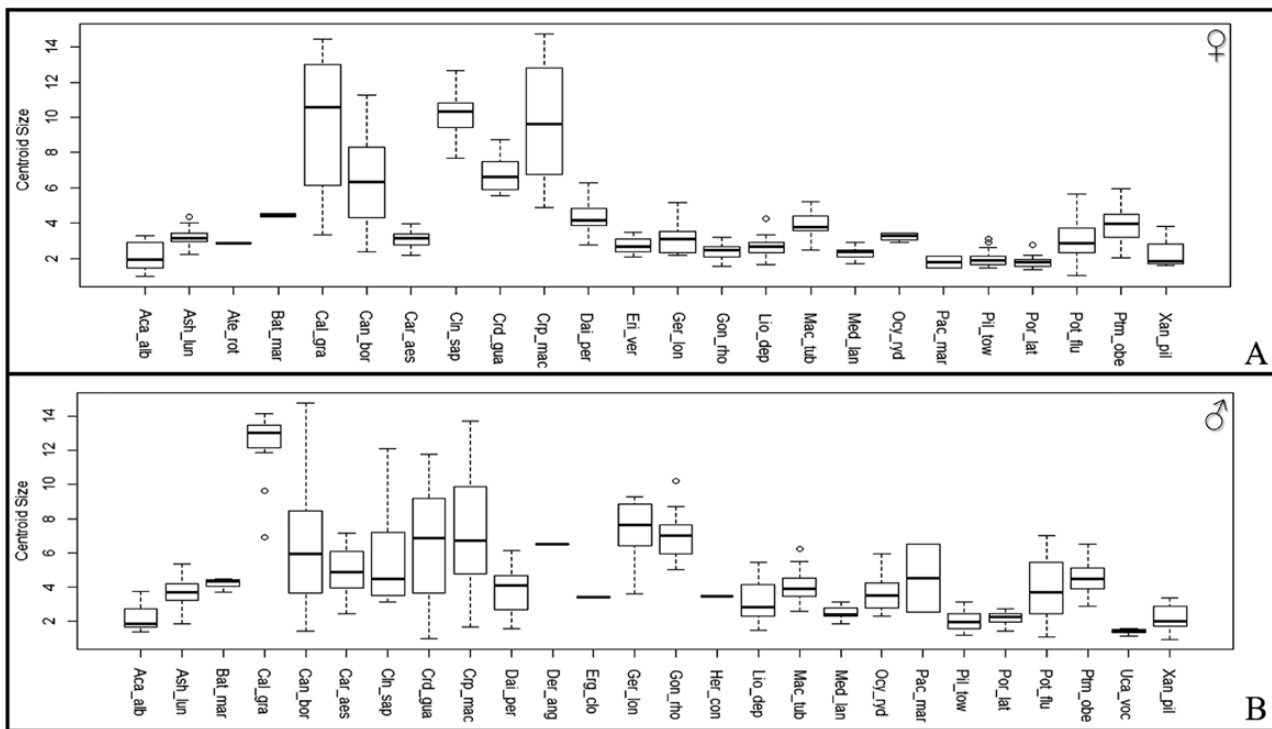


Figure 3. A, centroid size values for left claw in female crabs. B, centroid size values for left claw in male crabs.

Table 2. List of landmarks and their definition

Landmark list	
1	Tip of the manus near the dactyl
2	Tip of the manus upper tubercle
3	Base of the manus upper tubercle
4	Upper attachment points of the carpus with the manus, at the edge of the carpal cavity
5	Lower attachment points of the carpus with the manus, at the edge of the carpal cavity
6	Lower tip of the manus
7	Junction between the manus and the pollex on the ventral margin of the claw
8	Tip of the pollex
9	End of the internal margin of the manus
10	Lower point that marks the articulation of the dactyl with the manus
11	Upper point that marks the articulation of the dactyl with the manus

Table 3. Significance (setting $\alpha = 0.05$) obtained by applying ANOVA for (1) sexual dimorphism (M = male, F = female) in size of both right and left (R = right, L = left) claws, and (2) intra- (for M and F, separately) and intersexual (that is dimorphic) heterometry. Significance of MANOVA for (3) sexual dimorphism in shape of both R and L claws, and (4) intra- and intersexual heteromorphy

	Centroid size M vs. F		Heterometry			Shape M vs. F		Heteromorphy		
	R	L	M	F	M vs. F	R	L	M	F	M vs. F
Aca_alb	0.172	0.704	0.003	0.008	0.001	0.006	0.095	0.001	0.001	0.981
Ash_lun	0.010	0.005	0.9	0.979	0.338	0.001	0.001	0.035	0.137	0.641
Ate_rot	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bat_mar	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cal_gra	0.042	0.053	0.748	0.819	0.552	0.002	0.001	0.001	0.001	0.068
Can_bor	0.914	0.986	0.838	0.881	0.889	0.069	0.376	0.001	0.001	0.788
Car_aes	0.001	0.001	0.027	0.028	0.009	0.001	0.004	0.001	0.001	0.003
Cln_sap	0.003	0.003	0.779	0.675	0.248	0.109	0.006	0.002	0.125	0.328
Crd_gua	0.433	0.688	0.024	0.001	0.372	0.017	0.261	0.001	0.001	0.214
Crp_mac	0.137	0.160	0.22	0.012	0.515	0.099	0.042	0.001	0.001	0.337
Dai_per	0.984	0.339	0.077	0.015	0.001	0.004	0.349	0.001	0.001	0.014
Der_ang	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Erg_clo	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eri_ver	NA	NA	NA	NA	NA	NA	NA	0.001	NA	NA
Ger_lon	0.001	0.001	0.219	0.198	0.014	0.050	0.001	0.001	0.001	0.355
Gon_rho	0.001	0.001	0.376	0.413	0.082	0.001	0.001	0.001	0.002	0.442
Her_con	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lio_dep	0.012	0.016	0.267	0.1	0.201	0.003	0.038	0.001	0.001	0.453
Mac_tub	0.948	0.418	0.507	0.169	0.004	0.001	0.001	0.001	0.001	0.561
Med_lan	0.001	0.036	0.002	0.921	0.001	0.001	0.001	0.001	0.001	0.001
Ocy_ryd	NA	NA	0.001	NA	NA	NA	NA	0.001	NA	NA
Pac_mar	NA	NA	NA	NA	NA	NA	NA	0.067	NA	NA
Pil_tow	0.819	0.855	0.023	0.001	0.528	0.117	0.151	0.001	0.001	0.367
Por_lat	0.001	0.001	0.109	0.134	0.158	0.001	0.003	0.001	0.001	0.004
Pot_flu	0.058	0.036	0.181	0.097	0.251	0.012	0.005	0.001	0.001	0.633
Ptm_obe	0.001	0.004	0.001	0.001	0.002	0.001	0.008	0.001	0.001	0.106
Uca_voc	NA	NA	0.001	NA	NA	NA	NA	0.001	NA	NA
Xan_pil	NA	NA	0.128	NA	NA	NA	NA	0.001	NA	NA

Statistically significant values are in bold. NA = data not available due to number of specimens being < 5.

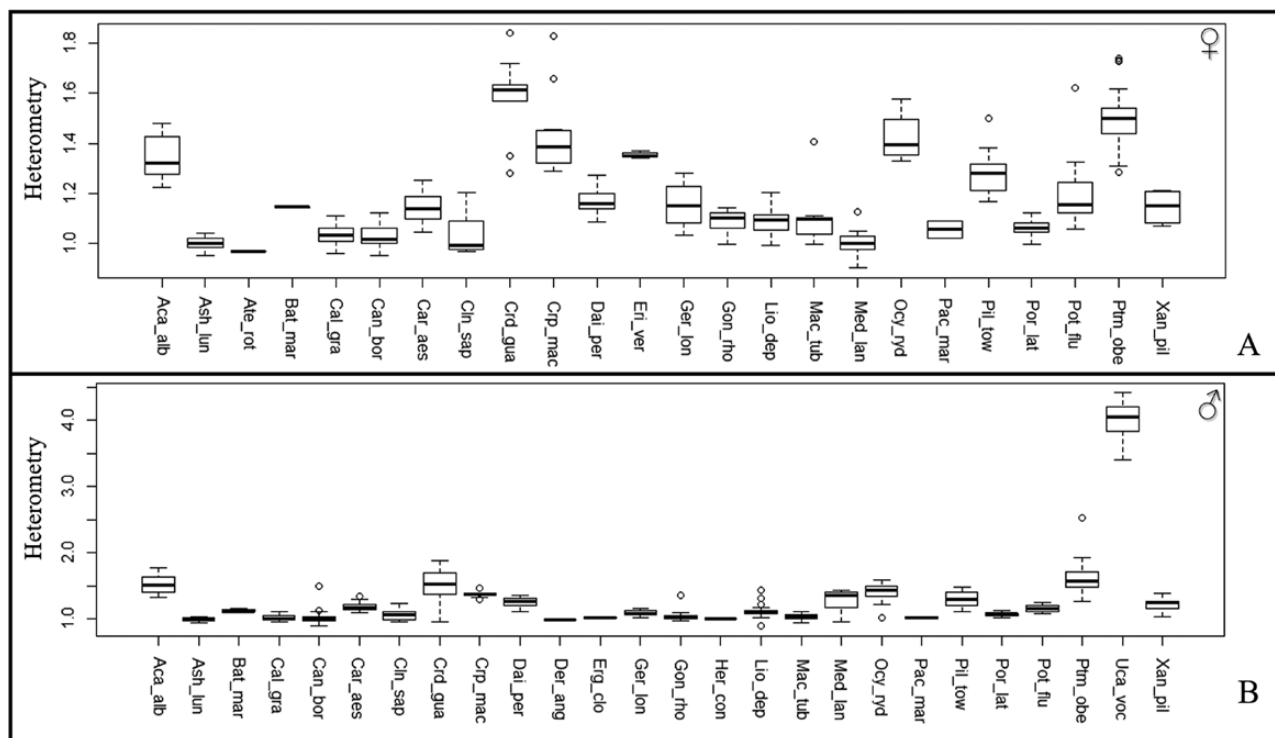


Figure 4. Observed values for heterometry in female (A) and male (B) crabs.

in heterometry is shown in [Figure 5A](#) for all studied species using the right claw's CS as the reference.

SHAPE ANALYSIS AND HETEROMORPHY

Deformation grids were constructed in association with PCA graphs and consisted of two red and black lines ([Fig. 1C](#)): the red line shows medium claw shape (the centroid has coordinates 0,0), while the black line shows the claw shape associated with the two extremes of the axes. [Figure 6](#) shows PCA graphs and relative deformation grids for both claws in males and females. PC1 explained 50.97% of the variation in the right claws of males and 54.65% of the variation in females. Moving from negative to positive PC1 values, shape variation is seen in the stretching of the pollex region and lowering of the manus region ([Fig. 6](#), top and bottom left) in the right claws of both sexes. For the left claws, the same shape variation is observed in both males and females, with PC1 explaining 48.04% and 55.53% of the variation, respectively ([Fig. 6](#), top and bottom right). PC2 explained 27.92% and 13.88% of the variation in the right chela of males and females, respectively, corresponding to stretching of the pollex and heightening of the manus ([Fig. 6](#), top and bottom right). Finally, PC2 explained 30.61% and 13.78% of the variation in the left chela of males and females, respectively, with the variation corresponding to a

shortening of the pollex and lowering of the manus ([Fig. 6](#), top and bottom left).

Statistically significant sexual dimorphism was observed in 13 of 18 species for the right claw. The same number of species (but not the same taxa) had statistically significant sexual dimorphism in the left claw. Ten out of 18 species showed significant dimorphism for both chelae ([Table 3](#)). The exceptions included *Acanthocyclus albatrossis* Rathbun, 1898, *Cardisoma guanhumii* Latreille, 1828 and *Daira perlata*, which only showed statistically significant dimorphism in the right claw. *Callinectes sapidus* (Rathbun, 1896), *Carpilius maculatus* and *Geryon longipes* showed statistically significant dimorphism only in the left claw ([Table 3](#)). The other species showed no significant sexual dimorphism in either claw.

PDs, or heteromorphy, revealed variation in shape among species for males and females ([Fig. 7](#)). Significant heteromorphy existed for both sexes. Males showed significant R-L shape differences in all 21 species, while females showed significant differences in 16 of 18 species. *Ashtoret lunaris* (Forskål, 1775) and *Callinectes sapidus* showed significant heteromorphy only in males ([Table 3](#)). Heteromorphy was found to be a sexually dimorphic characteristic in only four of the 18 species ([Table 3](#)), with males having greater values than females

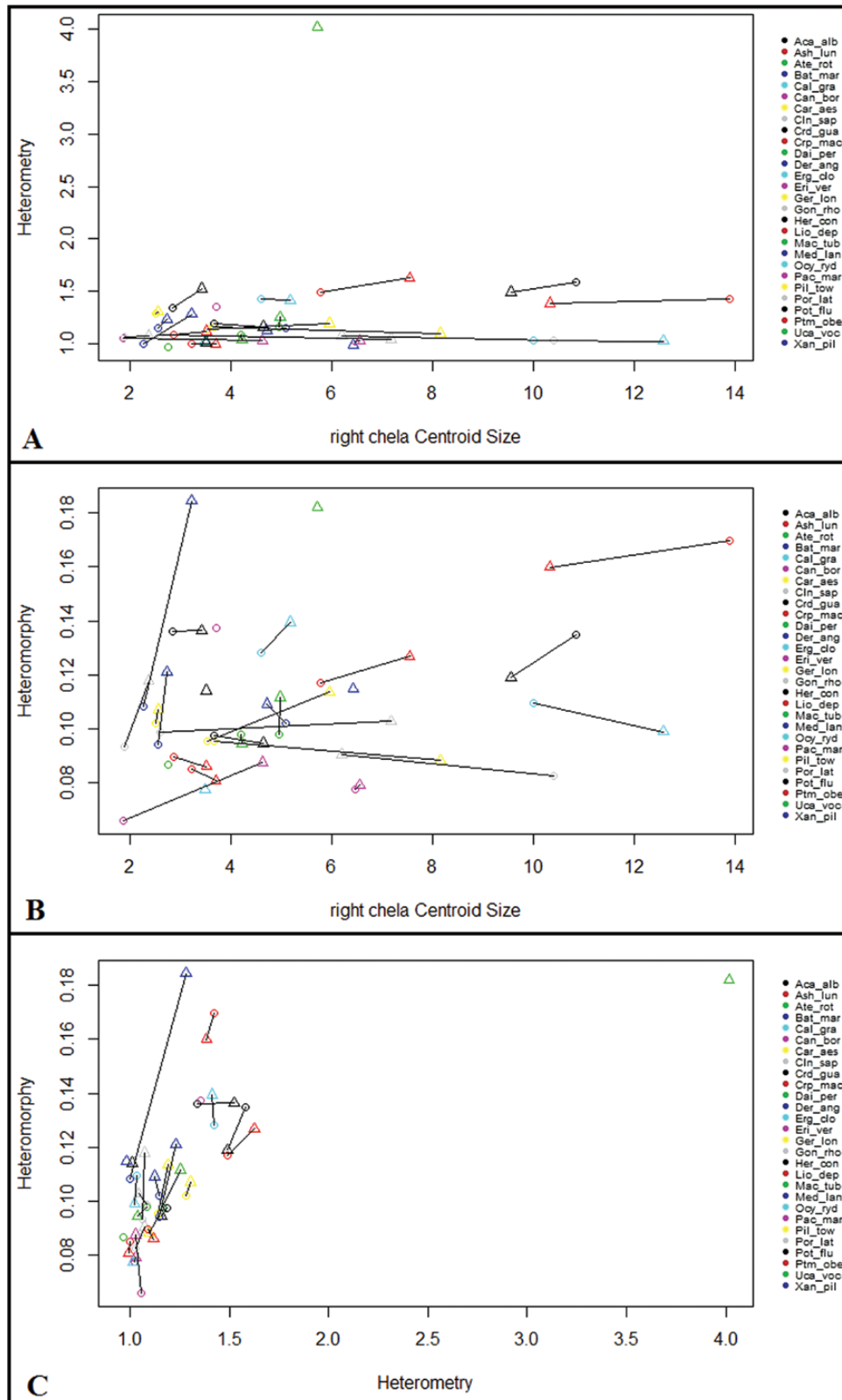


Figure 5. A, variation in heterometry in conspecific (linked) male (triangles) and female (circles) crabs. B, variation in heteromorphy in conspecific (linked) male (triangles) and female (circles) crabs. C, variation in heterometry and heteromorphy in conspecific (linked) male (triangles) and female (circles) crabs. For marks see [Table 1](#).

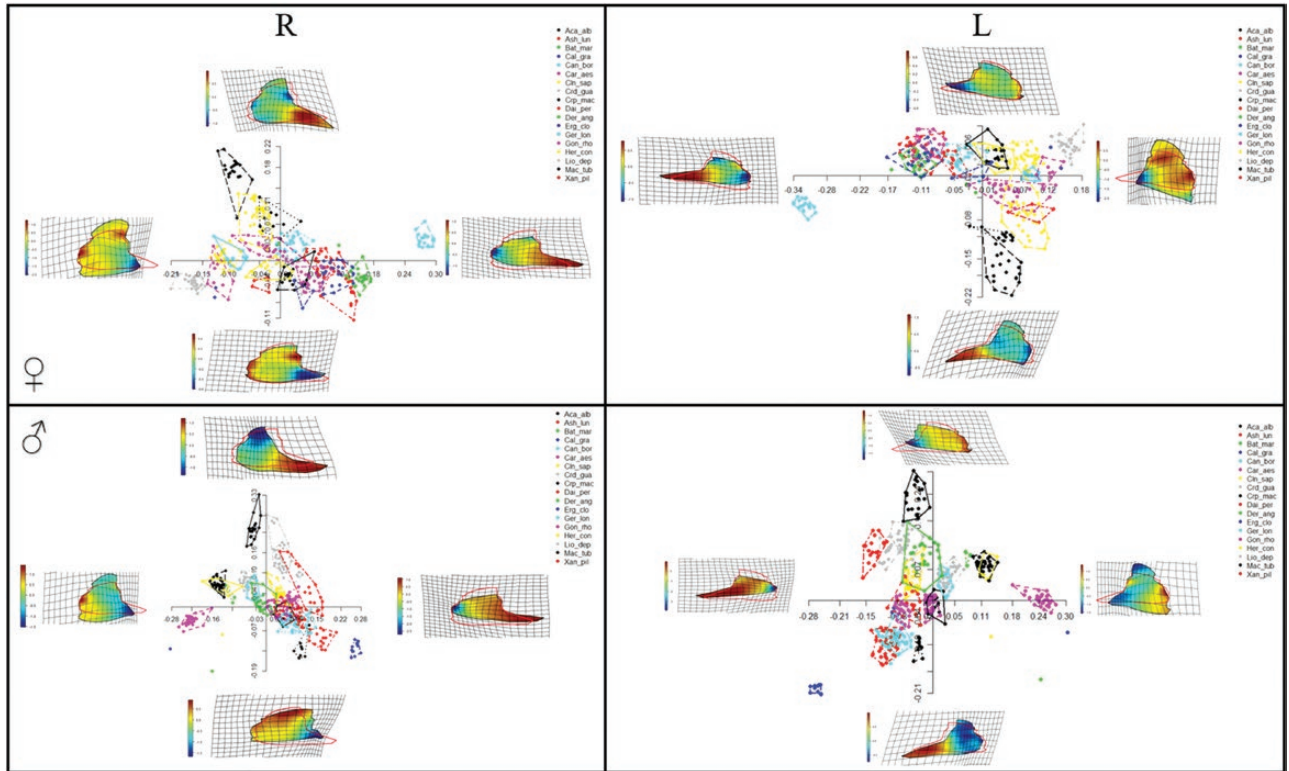


Figure 6. Graphs of principal component analyses and relative deformation grids for both claws (R = right; L = left) in female (top) and male (bottom) crabs. See text for the percentage variance explained by each principal component.

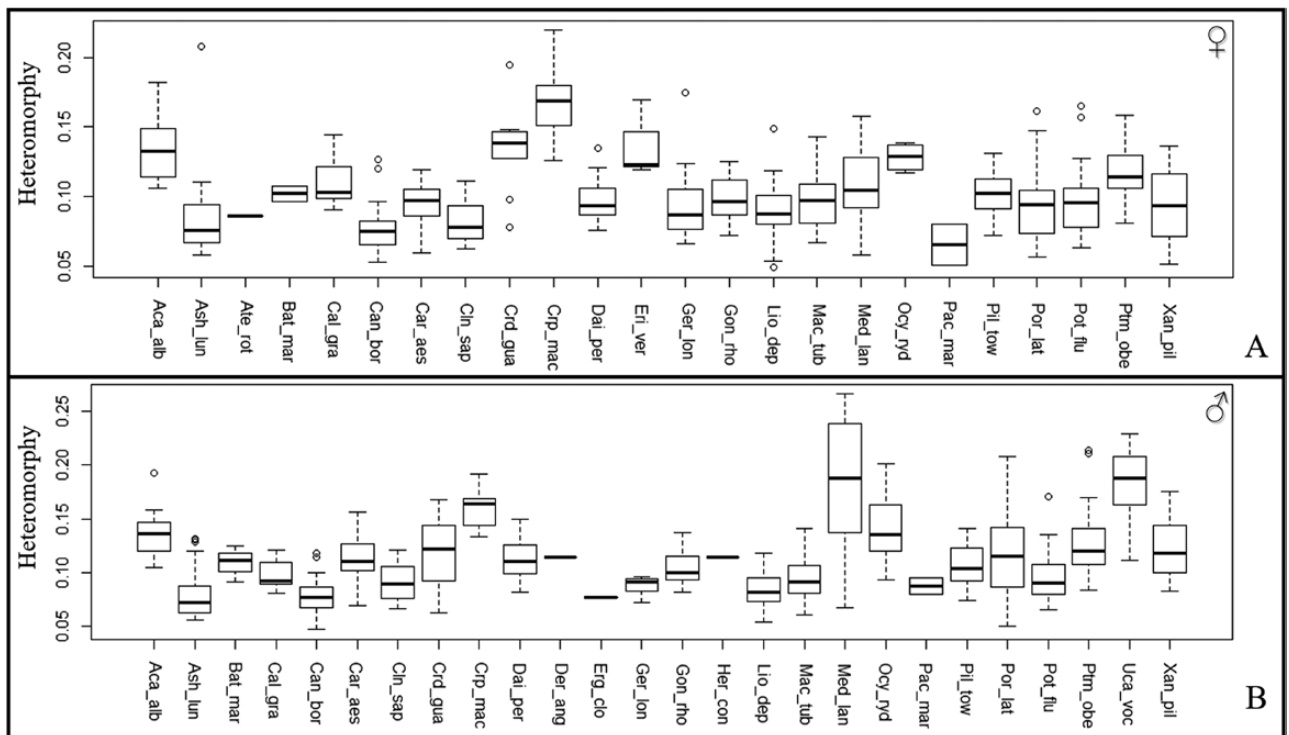


Figure 7. Values of heteromorphy (i.e. Procrustes distances) for female (A) and male (B) crabs.

Table 4. Significance (setting $\alpha = 0.05$) obtained by applying the K test for phylogenetic signals calculated for: (1) right (R) and left (L) claw (ch) size and shape, in males (M) and females (F); (2) sexual dimorphism (Sex. dim.) in right and left claw size and shape; and (3) heterometry within and between males and females, and heteromorphy within and between males and females

	M	F	Sex. dim.
R_ch_size	0.483	0.249	0.703
L_ch_size	0.084	0.408	0.695
R_ch_shape	0.003	0.006	0.113
L_ch_shape	0.002	0.001	0.454
Heterometry	0.521	0.13	0.819
Heteromorphy	0.181	0.476	0.143

in all species. The sexually dimorphic variation in heteromorphy is shown in [Figure 5B](#) for all the studied species, using the right claw CS as the reference measurement.

HETEROMETRY VS. HETEROMORPHY

Combining information for heterometry and heteromorphy in the same plot ([Fig. 5C](#)) allowed us to understand how size and shape varied in males and females, in both intraspecific and interspecific comparisons. The results showed interspecifically large variation in heterometry and small variation in heteromorphy. Despite differences in scale of the x - and y -axes, [Figure 5C](#) clearly shows that total variation of heteromorphy occurs within one-tenth

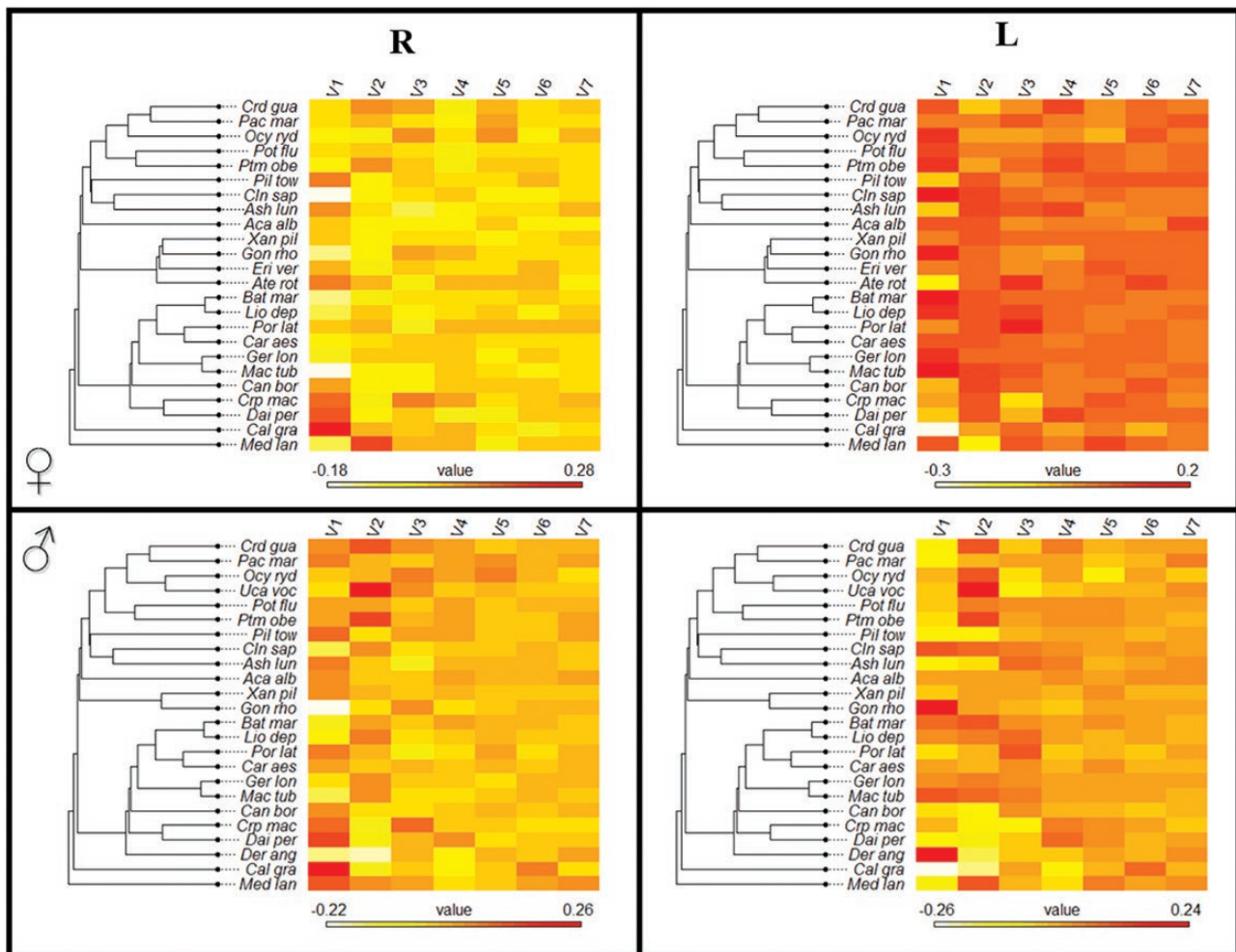


Figure 8. Significant (setting $\alpha = 0.05$) phylogenetic signal obtained by applying the K test for both right (R) and left (L) claw shape in female (top) and male (bottom) crabs. V is a vector of phenotypic trait values for each investigated species calculated in R using the shape matrix in phytools. For statistical significance values, see [Table 4](#).

of the heteromorphy index (y-axis in Fig. 5C), while total variation of heterometry occurs within two units, excluding *Uca vocans* (Linnaeus, 1758), which has a total variation of heterometry occurring within four units.

ECOLOGICAL ANALYSIS

The series of pairwise ANOVAs performed between heterometry and heteromorphy in both sexes, and other ecological features (i.e. environment, habitat, feeding types), revealed no significant differences (all $P > 0.05$).

PHYLOGENETIC SIGNAL

Statistically significant phylogenetic signal ($P < 0.05$) was obtained for the right and left claw shape (i.e. PCs) in both males and females (Table 4; Fig. 8). However, other morphological characters previously considered did not show any statistically significant phylogenetic signal ($P = n.s.$), namely (1) size of right and left claws in males; (2) size of right and left claws in females; (3) sexual dimorphism in right and left claw size; (4) sexual dimorphism in right and left claw shape; (5) heterometry within and between sexes; and (6) heteromorphy within and between sexes.

DISCUSSION

Heterochely in brachyurans is a well-known but not well-understood phenomenon. Studies published in the last 40 years have generally focused on ontogenetic patterns of claw asymmetry, mainly investigating the functional use of the larger chela through traditional morphometric methods (Hartnoll, 1974; Govind & Blundon, 1985; Abby-Kalio & Warner, 1989; Scalici & Gherardi, 2008; Silva *et al.*, 2014). Many other studies have evaluated the distribution of right and left handedness in one or more crab species (Barnwell, 1982; Ng & Tan, 1985; Ladle & Todd, 2006), and only a few studies have analysed shape variation of claws using landmarks (e.g. Rosenberg, 2002; Silva & Paula, 2008; Alencar *et al.*, 2014).

Here, we have thoroughly investigated the heterochely phenomenon (heterometry + heteromorphy) within the brachyuran tree by using the widest sample available of crab species with an adequate number of specimens per species (as compared with similar studies). The description of claw form was well supported by the largest number of landmarks (11) + semi-landmarks (23) used in landmark-based studies

of brachyurans. Our study focused on variation in the size and shape of both major and minor chela. Furthermore, statistical analysis of size and shape were carried out through geometric morphometric modelling to determine if: (1) heterometry and heteromorphy represent new sexually dimorphic characteristics for the investigated species; and (2) interspecific variation in claw size and shape has a phylogenetic signal.

The main findings of this study might contribute to debates regarding heterochely in crabs by providing innovative tools (heterometry index and heteromorphy index) for considering and describing shape and size variation of claws independently, in and between crab species. By using these two indices we were able to recognize three different kinds of heterochely affecting each analysed species: (1) size-heterochely, when the right and left claws are significantly different only in size and not in shape (not observed in our sample); (2) shape-heterochely, when the right and left claws are significantly different only in shape and not in size; and (3) size- and shape-heterochely, when the right and left claws are significantly different in both size and shape. In fact, heterometry and heteromorphy were widely observed in the examined species. Considering our sample, some taxa showed both size- and shape-heterochely, such as *Carcinus aestuarii* (Nardo, 1847) and *Cardisoma guanhumi*. Others, such as *Calappa granulata*, showed only heterochely in shape but not in size. Just one species, *Pachygrapsus marmoratus* (Fabricius, 1787), was homochelic as previously described by Silva & Paula (2008).

Regarding interspecific comparisons between males and females, we offer a new point of view considering heterochely as a sexually dimorphic parameter. In fact, some of our species showed heterometry and/or heteromorphy only for one sex while others showed them for both, thus resulting in the large amount of interspecific variation attributed to sexual dimorphism.

We also tested for ecological associations of the claw variation in size and shape by overlapping them with ecological descriptors for each species found in the literature (i.e. environment, habitat, feeding types). We did not find any pattern of association between heterochely and ecological descriptors due to a lack of detail in the published data. In fact, the scattered literature available regarding the ecology and feeding habit of crab species considered in the present work forced us to recognize macro-categories of environment, habitat and feeding types, which may have flattened all interspecific ecological differences. This was probably the main reason we found no significant ecological associations with size/shape.

Claw shape in both sexes showed significant phylogenetic signal, meaning that closely related species have similar claw shape and that in

each species displaying heterochely it evolved independently for different uses (feeding, mating, fighting). These functions could be therefore considered morphological attributes with sex-dependent differential expression (Hartnoll, 1974; Rosemberg, 1997; Mariappan *et al.*, 2000; Tsuchida *et al.*, 2000; Barria *et al.*, 2014). Thus, the shape of the right and left claws in male and female brachyurans could be interpreted as a fixed genetic character that needs to be decoded throughout targeted molecular studies (Lewis, 1969), as demonstrated for *Carcinus maenas* (Linnaeus, 1758) by Ladle & Todd (2006). On the other hand, Smith & Palmer (1994) observed that *Cancer productus* Randall, 1840 may show diverse heterochely depending on the administered food in an indoor experiment. Additionally, they demonstrated that when one claw was immobilized, the chelae became asymmetrical. They advanced the hypothesis that short-term adaptive responses to environmental stress, if heritable, could produce long-term evolutionary changes in claw size and could also promote the evolution of claw dimorphism. Proposing a solution to the origin, evolution and adaptive meaning of heterochely within Brachyura therefore remains a challenge and goes beyond the aims of our work.

In conclusion, determining the evolutionary and molecular processes involved in the development of heterochely in crabs is complex given all the functions in which claws are involved (e.g. feeding, burrowing, intraspecific antagonism, courtship). Such a variety of functions are evolutionary forces acting simultaneously on these characters, resulting in current claw size and shape, and their pattern of asymmetry.

Many previous studies have focused on heterochely in one or a few crab species, but there are no previous comprehensive analyses of the morphological variability of this trait in crabs. In the above context, this study provides advanced numerical indices of heterochely that capture shape and size variation in greater detail, allowing a comparison of chelae polymorphism among species, possibly identifying relationships among phylogenetic history, ecological traits and patterns of asymmetry. Our results show great potential to generate novel and useful information regarding the evolutionary and molecular processes involved in the development of heterochely in crabs. However, more interdisciplinary studies are needed, to link claw morphology, specifically heterochely, and molecular biology. In light of our findings, it is clear that future studies need to investigate the widespread phenomenon of heterochely not only in crabs, but also in all animal groups showing asymmetrical claws, possibly by applying quantitative methods, such as the indices of heterometry and heteromorphy presented here.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Table S1. Data about environment, habitat, feeding types and depth (min = minimum depth; max = maximum depth; mean = mean depth) of the studied crabs (species column), collected either from the World Register of Marine Species (WoRMS, <http://www.marinespecies.org>, indicated by *), or from different published scientific papers (see references column). ND = no data available.