

SPECIES STATUS IN QUESTION: A MORPHOMETRIC AND MOLECULAR
COMPARISON OF *CYRTOGRAPSUS AFFINIS* AND *C. ALTIMANUS*
(DECAPODA, BRACHYURA, VARUNIDAE)

Eduardo D. Spivak and Christoph D. Schubart

(EDS, correspondence) Departamento de Biología, Facultad de Ciencias Exactas y Naturales,
Universidad Nacional de Mar del Plata, Casilla de Correos 1216, 7600 Mar del Plata,
Argentina (e-mail: espivak@mdp.edu.ar);

(CDS) Biologie I (Zoologie), Universität Regensburg; 93040 Regensburg; Germany
(e-mail: christoph.schubart@biologie.uni-regensburg.de)

A B S T R A C T

Three species of the varunid crab genus *Cyrtograpsus* (Brachyura: Grapsoidea) occur in apparent sympatry along temperate coastlines of the southwestern Atlantic. Two of them, *C. angulatus* Dana, 1851, and *C. altimanus* Rathbun, 1914, are common in intertidal habitats and are easily separable by their morphology. In contrast, there is very limited knowledge on the ecology and distribution of the third species, *C. affinis* (Dana, 1851). In the present study, several specimens of *C. affinis* were collected in the Río de la Plata Estuary between Argentina and Uruguay. The study of these specimens showed that *C. affinis* tends to be smaller than the other two species of the genus and seems to be restricted to subtidal habitats. However, morphometric comparisons suggest the existence of a continuous gradient rather than discrete differences between specimens of *C. affinis* and *C. altimanus*. Morphometric differences were as common among subtidal samples as between subtidal and intertidal samples. Likewise, comparison of mitochondrial DNA sequences (16S rRNA) did not reveal diagnostic differences between the two putative species, suggesting a very close relationship between *C. altimanus* and *C. affinis*. Possibly the two forms represent a single species with a high ecological and phenotypic plasticity.

Marine benthic invertebrates with wide geographical ranges and highly mobile planktonic larvae are generally believed to show little genetic divergence throughout their range. In recent years, however, molecular studies have revealed that there can be a remarkable degree of local endemism and genetic structure within and between coastlines (Knowlton, 1993; Hellberg, 1998; Cuesta and Schubart, 1998; Klautau *et al.*, 1999; Fratini *et al.*, 2002). In many of these cases, the involved populations/species are morphologically indistinguishable.

In traditional invertebrate taxonomy, almost all geographic forms that could be morphologically separated were assigned species rank. Later, the biological species concept made it necessary to also infer reproductive isolation of the respective populations. In consequence, there was a tendency toward synonymizing of species names. Forms that originally were described as monotypic species often became polytypic species and were used as evidence of intermediate stages in the speciation process (Mayr and Ashlock, 1991).

The crab family Varunidae (*sensu* Schubart *et al.*, 2000a; Schubart *et al.*, 2002) includes

marine, brackish, and freshwater species (Anger, 1995). The genus *Cyrtograpsus* comprises three species that inhabit temperate marine and brackish waters of the southwestern Atlantic: *C. angulatus* Dana, 1851; *C. altimanus* Rathbun, 1914; and *C. affinis* (Dana, 1851). The three species differ in size and habitat. *Cyrtograpsus angulatus* adults (maximum carapace width: 58.8mm) are three and five times larger than maximum size specimens of *C. altimanus* (19 mm) and *C. affinis* (12.1 mm), respectively (Spivak, 1999; present study). Adults of *C. angulatus* and *C. altimanus* coexist in rocky intertidal habitats (Scelzo and Lichtschein de Bastida, 1979) and show different abilities for invading estuarine habitats (Spivak, 1999). These species are very different in their morphology (Rathbun, 1918; Boschi, 1964). The third species of the genus, *C. affinis*, is morphologically similar to *C. altimanus* and only distinguished by a more squarish carapace. However, these two species apparently differ in habitat requirements, with *C. affinis* being mostly reported from subtidal habitats. To date, there is very little published information on the latter species (Boschi, 1964).

The discovery of a dense population of *C. affinis* in a subtidal muddy estuarine habitat in the Río de la Plata, near the coasts of Montevideo, Uruguay (Spivak, unpublished), allowed us to quantify and compare morphometric traits of adult *C. affinis* and *C. altimanus* and to demonstrate genetic identity of both species along 554 basepairs of the 16S mtDNA gene. In the present paper, we discuss whether the two putative species could be conspecifics. In that case, observed morphometric and allometric differences would be due to ontogenetic or phenotypic plasticity and probably reflect differences in age or habitat. Until further evidence is provided, we suggest to retain species status for *Cyrtograpsus affinis* and *C. altimanus*. However, according to the results of this study, these forms must be considered closely related sister taxa. The observed niche differentiation may have occurred after or during a recent speciation event.

MATERIALS AND METHODS

A total of 567 crabs were examined. New specimens of *Cyrtograpsus affinis* were collected with a bottom trawl (1 cm mesh size) in the Río de la Plata Estuary during fishing cruises of the "BIP Dr. Eduardo Holmberg" and "BIP Capitán Cánepa" (Instituto Nacional de Investigación y Desarrollo Pesquero), Argentina between 1997 and 1998. In addition, we studied material of the same species collected by the Bureau of Fisheries Steamer "Albatross" during its 1887–88 voyage to South America. *Cyrtograpsus altimanus* was collected by hand during low tides in Mar Chiquita and Santa Clara del Mar (Buenos Aires Province, Argentina) between 1995 and 1998 and in the mouth of San Antonio Bay (Gulf of San Matías, Río Negro Province, Argentina) in November 1997 and October 2000. Part of this material was deposited at the Senckenberg Museum und Forschungsinstitut (SMF) Frankfurt am Main, Germany. We also studied the type series of *C. altimanus*, collected in "San Matías Bay, Patagonia" in 1872. Additional intertidal material included in this analysis, but originally identified as *C. affinis*, was from Piriapolis and Puerto La Paloma (Uruguay), and from Rio Grande do Sul (Brazil). This and other material was loaned from the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, U.S.A.; and U.S. National Museum of Natural History (USNM), Smithsonian Institution, Washington D.C., U.S.A.

Crabs were sexed and females were assigned to three different categories (immature, mature, and ovigerous) on the basis of pleon width and the presence of eggs under the pleon. Female crabs were considered immature if the pleon did not touch the coxae of the walking legs. The following measurements were taken with a stereo-microscope furnished with a micrometer eyepiece (10 \times): carapace width between the first, second, third, and fourth lateral teeth, carapace length, length and height of the chelal propodus, width of the fourth abdominal somite, total length (ventral) and merus length (dorsal) of the third pereopod (longest appendage), length and width of the carpus and propodus of

the fifth pereopod. Overall, the following material was examined and measured (abbreviations: M, males; F, females; F_o, ovigerous females; F_m, mature, nonovigerous females; and F_i, immature females):

Cyrtograpsus affinis (Dana, 1851)

Material Examined.—2 M (SMF 24545, DNA vouchers), 18 M, 22 F_o and 1 F_i, (collection Spivak 001), Uruguay: Río de la Plata (35°07'S, 56°02'W, 10 m depth), 19 November 1997, leg. "BIP Dr. Eduardo Holmberg"; 2 M, 1 F_m, (collection Spivak 002), Uruguay: Río de la Plata (35°05'S, 56°10'W), 19 November 1997, leg. "BIP Dr. Eduardo Holmberg"; 1 M, (collection Spivak 003), Uruguay: Río de la Plata (35°16'S, 56°10'W, 10 m depth), 19 November 1997, leg. "BIP Dr. Eduardo Holmberg"; 3 M, 6 F_o, 3 F_m, (collection Spivak 004), Uruguay: Río de la Plata (35°01'S, 55°51'W, 8.5 m depth), 19 November 1997, leg. "BIP Dr. Eduardo Holmberg"; 59 M, 44 F_o, 5 F_m, 27 F_i, (collection Spivak 005), Uruguay: Río de la Plata, (36°05'S, 56°37'W, 13 m depth), 9 March 1998, leg. "BIP Capitán Cánepa"; 25 M, 2 F_o, 29 F_i, (USNM 22104), "off Río de la Plata" (36°42'S, 56°23'W, 11 f. depth), 12 January 1888, leg. "Albatross"; 45 M, 6 F_o, 28 F_i, (USNM 22105), "off Río de la Plata" (36°43'S, 56°23'W, 10 f. depth), 12 January 1888, leg. "Albatross"; 1 F_i, (USNM 22107), "off Gulf of San Matías" (43°03'S, 58°56'W, 52 f. depth), 13 January 1888, leg. "Albatross"; 1 M (USNM 23345), Brazil, "off Cape St. Roque" (36°59'S, 34°47'W, 20 f. depth), 16 December 1887, leg. "Albatross"; 2 M (USNM 71203, DNA voucher) Uruguay: Montevideo (from dogfish stomach), 12 November 1925, leg. W. L. Schmitt.

Cyrtograpsus altimanus Rathbun, 1914

Material Examined.—6 M, 4 F (SMF 25973, DNA voucher), Argentina: Mar Chiquita, (37°45'S, 57°19'W, intertidal), December 1997, leg. E. D. Spivak; 1 M, 1 F (SMF 24544, DNA voucher), Argentina: Santa Clara del Mar, 17 km N of Mar del Plata (37°56'S, 58°11'W, intertidal), 14 January 1998, leg. E. D. Spivak; 105 M, 40 F_i, 4 F_m, 55 F_o (collection Spivak 006), Argentina: mouth of San Antonio Bay, Gulf of San Matías, (40°46'S, 64°50'W, intertidal), 23 November 1997, leg. T. A. Luppi; 2 M (SMF 28102 DNA voucher), Argentina: mouth of San Antonio Bay, Gulf of San Matías, (40°46'S, 64°50'W, intertidal), 24 October 2000, leg. T. A. Luppi; 3 M, (MCZ 6126, holotype; USNM 22835, paratypes), Argentina: "San Matías Bay, Patagonia", (intertidal), February 1872, leg. Hassler Expedition; 1 M, (MCZ 6127), Brazil: Rio Grande do Sul, (32°00'S, 50°15'W, intertidal), 1859, leg. Capt. G. Harrington; 4 M (USNM 56710) Uruguay: Piriapolis, (34°55'S, 54°15'W, intertidal), 3 December 1922, leg. H. M. Smith (originally identified as *C. affinis* by M. J. Rathbun); 4 M, 1 F_o, 1 F_m (USNM 70925 and 70926), Uruguay: Puerto La Paloma, (34°39'S, 54°08'W, intertidal), 6 December 1925, leg. W. L. Schmitt (originally identified as *C. affinis* by M. J. Rathbun).

The carapace maximum width (between fourth lateral teeth) of subtidal specimens collected in the Río de la Plata by the "Albatross" in January 1888 (samples pooled, here referred to as "Albatross samples"), by the BIP "Eduardo Holmberg" in November 1997 (samples pooled, here referred to as "Holmberg samples"), and by the BIP "Capitán Cánepa" in March 1998 (here referred to as "Cánepa samples") were compared between samples and

Table 1. *Cyrtograpsus affinis* (Dana, 1851) and *C. altimanus* Rathbun, 1914. Specimens used for DNA comparisons, with locality of collection, museum catalog number, sequence length in basepairs, and genetic database (EMBL) accession number. SMF: Senckenberg Museum, Frankfurt a.M.; USNM: Smithsonian Institution, National Museum of Natural History, Washington, D.C.

Species	Locality and date of collection	Catalogue No.	Sequence length	EMBL No.
<i>C. affinis</i>	Argentina/Uruguay: Rio de la Plata (19.11.1997, "Holmberg")	SMF 24545	554 bp	AJ130801
<i>C. affinis</i>	Argentina/Uruguay: Rio de la Plata (19.11.1997, "Holmberg")	SMF 24545	554 bp	AJ130801
<i>C. affinis</i>	Uruguay: Montevideo: English Bank "from dog fish stomach" (17.11.1925, id. M. J. Rathbun)	USNM 71203	282 bp	AJ487318
<i>C. altimanus</i>	Argentina: Sta. Clara del Mar (14.1.1998)	SMF 24544	554 bp	AJ130801
<i>C. angulatus</i>	Argentina: Mar Chiquita (12.1997)	SMF 25973	554 bp	AJ130801
<i>C. altimanus</i>	Argentina: San Antonio Oeste (24.10.2000)	SMF 28102	554 bp	AJ487319
<i>C. altimanus</i>	Argentina: Mar Chiquita (11.1996)	SMF 24546	550 bp	AJ278836

sexes with the intertidal sample from San Antonio Bay (November 1997). Because these data were not normally distributed and/or variances were not equal ($P < 0.0001$), the median values were compared among samples by means of a Mann-Whitney rank sum test (two samples) or a Kruskal-Wallis one-way analysis of variance on ranks (more than two samples), followed by an all pairwise multiple comparison procedure (Dunn's Method).

Quantitative relationships between morphometric variables were described with least-square regressions (after G -tests of homogeneity for normal distribution). The maximum carapace width, i.e., width between fourth lateral teeth, was chosen as reference dimension for size in most relationships. Log-log regression slope values between 0.9 and 1.1 were considered indicative of isometry ("conservative definition"; Clayton, 1990: 285). Slopes and intercepts of different regression lines were compared with an analysis of covariance (ANCOVA). Differences were considered statistically significant when $P < 0.05$.

Genomic DNA was extracted from the muscle tissue of walking legs or claws from several specimens of *C. altimanus* and *C. affinis* using a phenol-chloroform or a Puregene protocol. Species and localities included are presented in Table 1. An approximately 595-basepair region (including the primers) of the mitochondrial large ribosomal subunit rRNA gene (16S rRNA) was amplified by polymerase-chain-reaction (PCR) (38–40 cycles with 1 min at 94°, 1 min at 48–55°, 2 min at 72° denaturing, annealing, and extension temperatures, respectively) with the primers 16sar, 16sbr, 1472, and 16H16 (see Schubart *et al.*, 2000b). The internal primer 16H16 was necessary to successfully amplify a shorter fragment of a museum specimen dating from 1925 (USNM 71203) and originally obtained from a dogfish stomach. The PCR products were purified and sequenced by dideoxy chain termination with S35 radioactive labeling (Pennsylvania State University) or with the ABI Prism 310 Genetic Analyzer using the ABI BigDye terminator mix (University of Louisiana at Lafayette, Universität Regensburg). All sequences were aligned manually using the multisequence editing program ESEE (Cabot and Beckenbach, 1989). The DNA-sequences have been submitted to the EMBL genetic database and can be retrieved under the accession numbers AJ130801 (*C. affinis* and *C. altimanus* common haplotype), AJ487318 (*C. affinis*

USNM 71203), AJ487319 (*C. altimanus* San Antonio), and AJ278836 (*C. angulatus*) (Table 1).

RESULTS

Most crabs identified as *Cyrtograpsus affinis* had been obtained from the subtidal, mainly from estuarine waters of Río de la Plata; those identified as *C. altimanus* always originated from the intertidal. Type specimens of *C. altimanus* were from the intertidal in "San Matías Bay, Patagonia" (Rathbun, 1914); new material was obtained for this study in 1997 and 2000 from the intertidal of San Antonio Bay, a small bay within the Golfo de San Matías (present geographic term of San Matías Bay). Only the crabs collected in the intertidal of Piriapolis and Puerto La Paloma, Uruguay, did not seem to match the above pattern of species-habitat relationship, and they were originally identified as *C. affinis* by M. J. Rathbun in 1925. However, the present comparisons suggested that they morphologically correspond to *C. altimanus*.

There were significant differences in size of both sexes between three subtidal and one intertidal sample (Kruskal Wallis ANOVA on ranks, $H_{\text{males}} = 124.5$; $H_{\text{females}} = 115.2$; $P < 0.0001$; Fig. 1). However, differences were not consistently related to habitat (i.e., species). Specimens of the "Albatross" sample were significantly smaller from those belonging to the other two subtidal and the intertidal samples (Dunn's Method, $P < 0.05$). Males reached significantly larger sizes than females in the "Holmberg" sample and in the intertidal sample (Mann-Whitney rank sum test, $t = 845.5$,

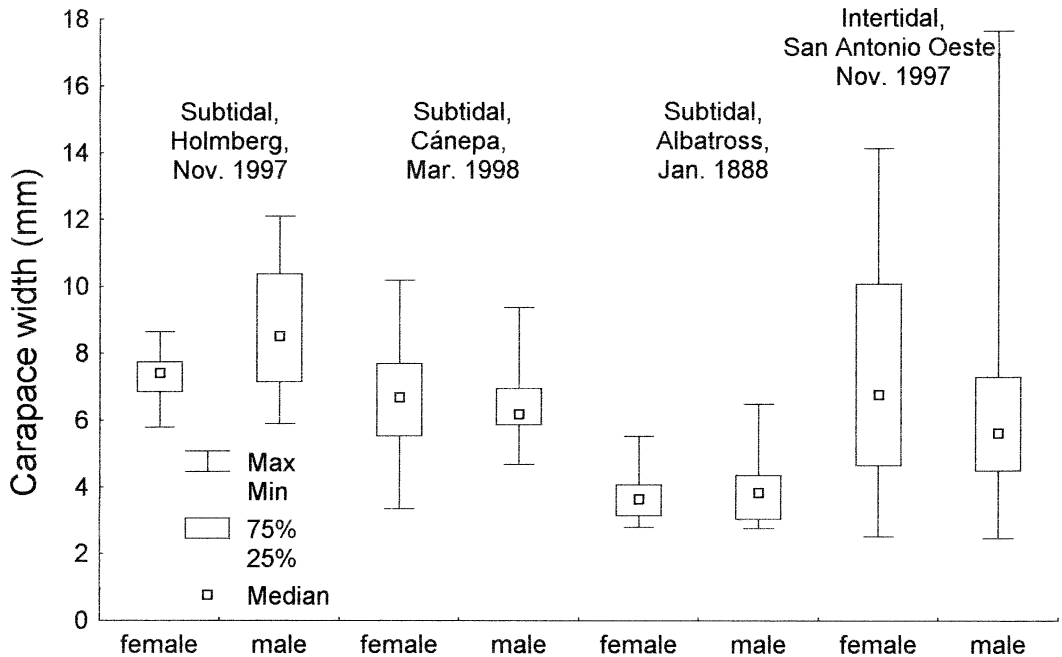


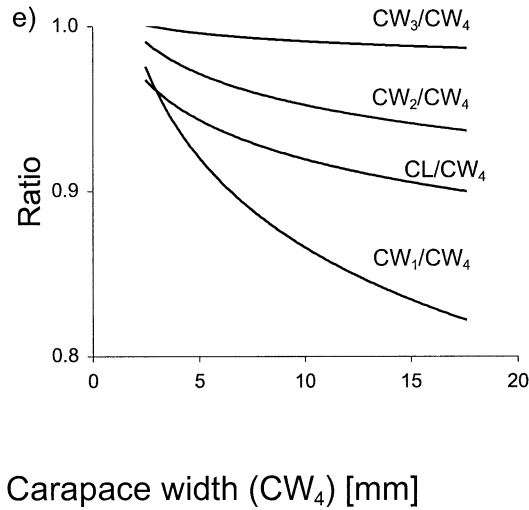
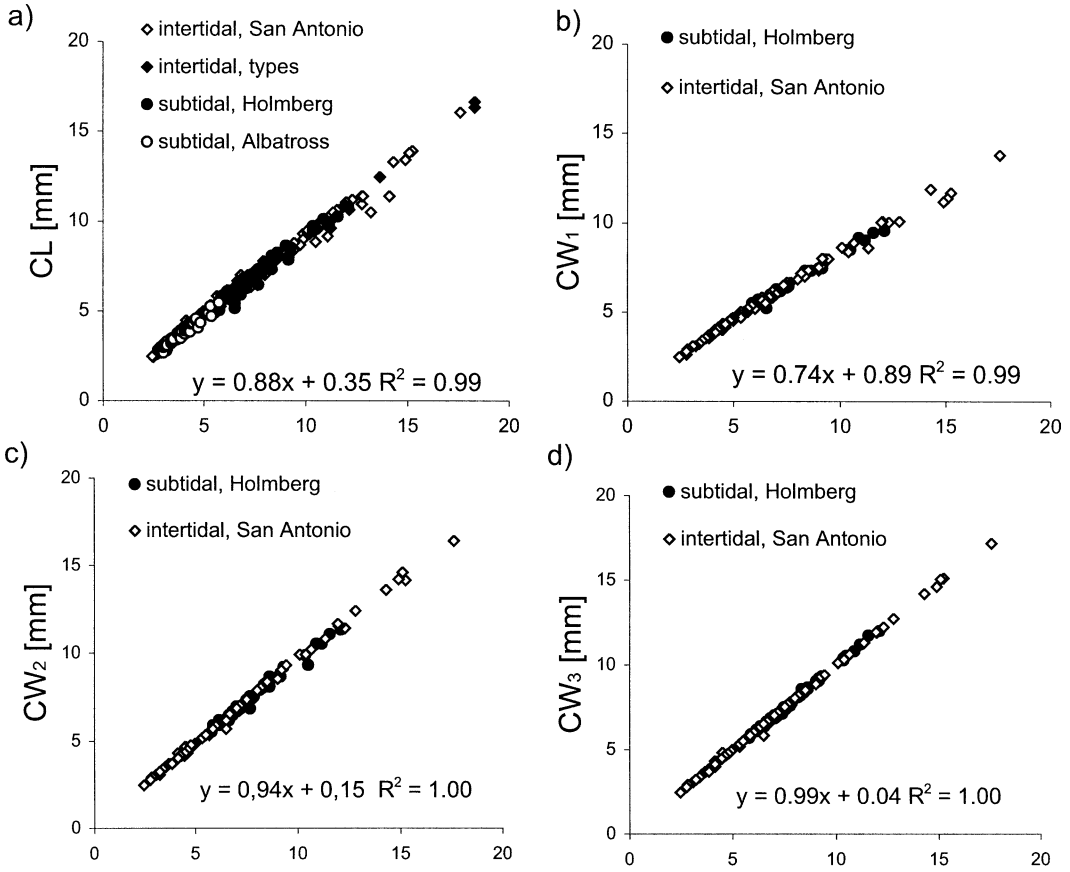
Fig. 1. *Cyrtograpsus affinis* (Dana) and *C. altimanus* Rathbun. Carapace maximum width (between fourth lateral teeth) of specimens from different subtidal collections from Río de la Plata (see Materials and Methods) and of intertidal specimens from San Antonio Bay. The maximum, minimum, median and 25–75 percentile values of both sexes are represented.

$P = 0.016$, $t = 11.067$, $P = 0.0206$, respectively; Fig. 1). There were no significant differences of any type when comparing intertidal crabs with subtidal ones from the “Holmberg” sample. The largest subtidal males measured 6.5, 10.2, and 12.1 mm (“Albatross,” “Cánepa,” and “Holmberg” samples, respectively), and the largest intertidal male from San Antonio Bay measured 17.6 mm. The type specimen of *Cyrtograpsus altimanus* is a male, 18.3 mm in carapace width. The largest male of *C. altimanus* from Uruguay, identified as *C. affinis* by M. J. Rathbun, measured 16.6 mm in carapace width (USNM 56710).

Despite the reported differences in carapace shape (see Introduction), linear relationships between maximum carapace width (CW_4 between fourth lateral teeth) versus carapace length (CL) and different carapace widths (CW_1 , CW_2 , CW_3 , between first, second and third lateral teeth) revealed no significant differences between subtidal and intertidal crabs ($P > 0.05$). The linear regressions of pooled data (subtidal + intertidal) were the following: $CL = 0.88CW_4 + 0.35$ (Fig. 2a); $CW_1 = 0.74CW_4 + 0.89$ (Fig. 2b); $CW_2 = 0.94CW_4 + 0.17$ (Fig. 2c); and $CW_3 = 0.99CW_4 + 0.04$ (Fig. 2d). The ratios between several carapace

dimensions (Fig. 2e) suggested that the overall carapace shape changes through growth. In both species, small specimens seem to have squarish carapaces, but large specimens seem to have hexagonal carapaces that are always wider than long.

The chelae were clearly more elongate in the subtidal males from the “Albatross” and “Holmberg” samples as compared to the “Cánepa” subtidal and intertidal males with similar carapace width (Fig. 3). The slopes of the regressions of chelar propodus length versus carapace width indicated positive allometry in males and significant variation among samples ($P < 0.0001$, Appendix 1). Significant differences in the slopes of these regressions were found for the males of intertidal ($b = 1.34$) and the “Albatross” ($b = 1.53$) and “Holmberg” ($b = 1.53$) subtidal samples (Fig. 3, $P < 0.0001$, $P = 0.014$, respectively, Appendix 1) and between the “Albatross” and “Cánepa” ($b = 1.35$) subtidal samples (Fig. 3, $P = 0.044$, Appendix 1). However, the slope of the “Cánepa” subtidal crabs was not significantly different from the “Holmberg” subtidal crabs and the intertidal ones (Appendix 1). In females, the propodus length grew isometrically relative to carapace width, and slopes did not vary among samples



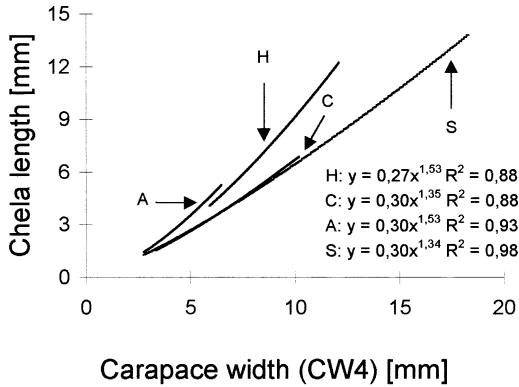


Fig. 3. *Cyrtograpsus affinis* (Dana) and *C. altimanus* Rathbun. Chelar propodus length plotted against carapace maximum width (CW_4) of males. S: intertidal from San Antonio Bay; A: subtidal, "Albatross" sample, January 1888; H: subtidal, "Holmberg" sample, November 1997; and C, subtidal, "Cánepa" sample, March 1998.

($P = 0.936$, Appendix 1). In the regressions of propodus height versus carapace width, the slopes did not vary significantly among samples (males, $P = 0.242$; females, $P = 0.147$; data not shown).

The slopes of the regressions of pleon width versus carapace width varied significantly among samples in males, immature females, and mature females ($P < 0.0001$, Fig. 4, see Appendix 1 for details). The relative growth of the pleon was negative allometric in "Holmberg" ($b = 0.77$) and San Antonio ($b = 0.86$) males, but isometric in "Cánepa" ($b = 1.03$) and "Albatross" ($b = 0.98$) males. It was positive allometric in all immature and in mature "Albatross" females ($b = 1.63$) and isometric in the rest of mature females. Mature females from the "Albatross" sample (CW range: 2.5–5.0 mm) were smaller than those from other subtidal samples (range: 4.0–7.7 mm) and intertidal females (range: 4.0–11.0 mm).

The length of the third and fifth pereiopods is linearly related to carapace width. For a given carapace width, the third merus, the total length of the third pereiopod, and the fifth carpus + propodus did not differ significantly among subtidal samples ($P = 0.821$, $P = 0.697$, $P = 0.607$). However, the carpus + propodus of the

fifth pereiopod was significantly wider in intertidal crabs ($P = 0.004$, Fig. 5, Appendix 1).

Five of the six crabs from which mtDNA was obtained shared the exact same sequence over the studied 554 (228 in the museum specimen USNM 71203) nucleotide region of the 16S rRNA gene. The sixth sequence, corresponding to the most geographically distant crab population (*C. altimanus* from San Antonio), differed by one transition from the remaining sequences. In contrast, the sequence of *C. angulatus* differed in 39 positions (19 transitions, 6 transversions, and 4 indels) from all sequences of *C. affinis* and *C. altimanus*.

DISCUSSION

In her description of *Cyrtograpsus altimanus*, Rathbun (1914) only compares the new species with *C. angulatus*. Even though *Cyrtograpsus altimanus* and *C. affinis* were both included in Rathbun's (1918) monograph of American grapsoid crabs, the latter was listed under the genus *Hemigrapsus*. Rathbun's (1918) descriptions indicate many morphological similarities between these genera, but a clear difference regarding the shape of the carapace, which narrows anteriorly in *Cyrtograpsus* and is quadrate in *Hemigrapsus*. All specimens of *C. affinis* examined by Rathbun (1918: 266) came from the "Albatross" 1887–88 cruise to South America. They are relatively small (Fig. 1) and thus have a quadratic carapace shape according to our size-shape relationships (Fig. 2). Whereas *C. affinis* has three lateral carapace teeth, all other species of *Hemigrapsus* included in Rathbun (1918) (*H. crenulatus*, *H. nudus*, and *H. oregonensis*) have two. That is probably why Rathbun (unpublished) subsequently modified her taxonomic classification and placed *C. affinis* in *Cyrtograpsus*. However, she indicated this change only to Fenner A. Chace, Jr., as a personal communication (cited by Boschi, 1964: 58). In consequence, *C. affinis* became a "quadrate *Cyrtograpsus*" rather than a "*Hemigrapsus* with three teeth".

In this study, we observed that carapace shape gradually changes from quadrate to

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Fig. 2. *Cyrtograpsus affinis* (Dana) and *C. altimanus* Rathbun. Carapace length (CL) and widths (CW_1 , CW_2 , CW_3 , between first, second and third lateral teeth respectively) plotted against carapace maximum width (CW_4 , between fourth lateral teeth) of Río de la Plata (subtidal) and San Antonio Bay (intertidal) crabs. In the carapace length/width graph, data from the material collected by the "Albatross" in 1888 and *C. altimanus* types were included. Regression equations correspond to the pooled data. a) CL versus CW_4 ; b) CW_1 versus CW_4 ; c) CW_2 versus CW_4 ; d) CW_3 versus CW_4 ; e) pooled ratios of different carapace dimensions versus CW_4 .

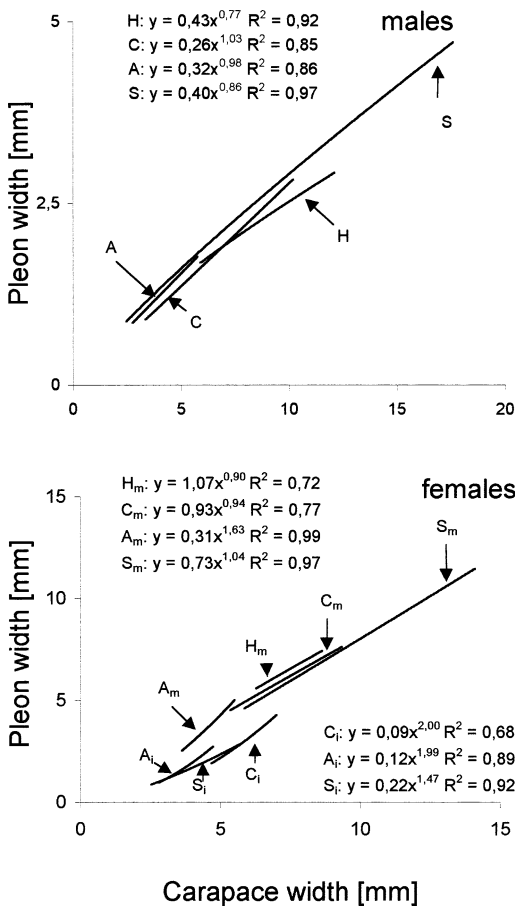


Fig. 4. *Cyrtograpsus affinis* (Dana) and *C. altimanus* Rathbun. Pleon width plotted against carapace maximum width (CW_4) of males and females (i: immature; m: mature). S: intertidal from San Antonio Bay; A: subtidal, "Albatross" sample, January 1888; H: subtidal, "Holmberg" sample, November 1997; and C, subtidal, "Cánepa" sample, March 1998.

roughly hexagonal when larger subtidal *Cyrtograpsus affinis*, as well as small intertidal *C. altimanus*, are included in the analysis (Fig. 2). We suspect that this was also noticed by the late Mary J. Rathbun and that she might have decided the two species were conspecifics. It is otherwise hard to explain why she identified a sample of intertidal crabs from Piriapolis and Puerto La Paloma, Uruguay (USNM 56710, 70925, 70926 collected by H. M. Smith in 1922 and W. L. Schmitt in 1925), as *C. affinis*, despite the fact that they perfectly match the morphology of her species *C. altimanus*.

Recent larval morphological studies do not render any additional separating characters. Zoeae and megalopae of *C. altimanus*, *C.*

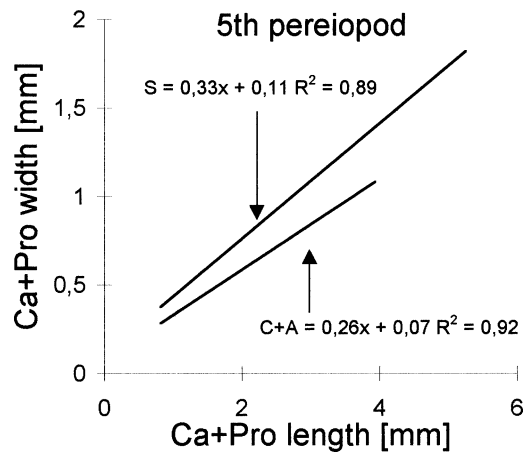


Fig. 5. *Cyrtograpsus affinis* (Dana) and *C. altimanus* Rathbun. Carpus and propodus length versus width of the fifth pereopod of males. S: intertidal from San Antonio Bay; C+A: subtidal, "Albatross" sample, January 1888, and "Cánepa" sample, March 1998.

angulatus, and *C. affinis* have been described from laboratory-reared individuals (Scelzo and Lichtschein de Bastida, 1979; Rieger and Vieira, 1997; Spivak and Cuesta, 2000). The minor differences found in zoeal morphology and size are listed by Spivak and Cuesta (2000). The megalopae of *C. altimanus*, *C. angulatus*, and *C. affinis* have similar morphologies and practically the same setation in antennae, mouthparts, pleopods, and uropods. The most important differences observed among the megalopae are the morphology of antennules and mandibles (Spivak and Cuesta, 2000) and size; *C. altimanus* and *C. affinis* megalopae are similar in size and larger than those of *C. angulatus* (Spivak, unpublished).

In several cases, detailed studies of ontogenetic development have allowed synonymizing of two species that were previously described on the basis of juvenile or adult specimens (e.g., Guinot, 1984, for Majidae and Otani and Takahashi, 1996, for Pinnotheridae). In addition, there are several known cases of intraspecific variation in morphometry and reproductive strategies (i.e., size at maturity) among local populations as well as over a broad geographic range in intertidal crabs. Both size range and size at maturity can change with latitude in several grapsoid and other crabs (Garth, 1957; Jones and Simons, 1983; Hines, 1989; Flores and Negreiros-Fransozo, 1999; Schubart *et al.*, 1999). Variations in size structure and reproductive tactics can be related

to differences in habitat (estuarine vs. marine), food supply, or shelter as in the case of the crabs *Macrophthalmus hirtipes* (see Simons and Jones, 1981), *Sesarma intermedium* (as *Sesarma intermedia*, see Kyomo, 1986, 2000), and *Pilumnus sayi* (see Kuhlmann and Walker, 1999), respectively, as well as in a palaemonid shrimp (Alon and Stancyk, 1982). In this context, the observed differences in size range and morphometry between *Cyrtograpsus affinis* and *C. altimanus* could be considered interpopulational differences related to life in different habitats.

Our preliminary molecular results also support synonymy of *C. affinis* and *C. altimanus*. Three *C. affinis* sequences (from Río de la Plata) were identical to two sequences from *C. altimanus* from Mar Chiquita and Santa Clara del Mar. On the other hand, *C. altimanus* from San Antonio Oeste (Gulf of San Matías), which is roughly 1,000 km farther south, differed by one transition from the previous sequences. Additional sequencing of many more specimens of *C. affinis* and *C. altimanus* will be necessary to determine whether there is genetic structure along the geographic range of these species.

Up to now, two different morphs (presently considered species) were always associated with different habitats, i.e., subtidal versus intertidal (except identifications by M. J. Rathbun of intertidal specimens of *C. affinis* from Uruguay). However, morphometric extrapolations of carapace and leg measures and molecular comparisons do not provide consistent significant differences between these forms (except in one leg length/width relationship, Fig. 5). On the other hand, differences were recorded in secondary sexual characters not only between intertidal and subtidal populations, but also between different subtidal samples (chela length in males, Fig. 3, and pleon width in both sexes, Fig. 4). One possible factor accounting for these differences may be that sampling of different localities was carried out at different seasons and with different methods. Nevertheless, the variability and gradients of morphometric ratios encountered, plus the lack of good diagnostic characters separating *C. angulatus* and *C. altimanus*, suggest that the two species represent different ecophenotypes or life stages of the same species. However, more specimens and more variable molecular markers will have to be analyzed before the morphological variability can definitely be attributed to phenotypic

plasticity. A similar case has recently been discovered in the Mediterranean genus *Brachynotus*. Also in this case, two closely related species, *B. sexdentatus* and *B. gemmellari*, differ only in habitat preferences, whereas they have overlapping morphometries and are identical in their 16S mtDNA (see Cuesta *et al.*, 2000; Schubart *et al.*, 2001).

One possibility to test for synonymy between the two species of *Cyrtograpsus* would be to keep subtidal *C. affinis* alive in captivity. If squarish specimens become more hexagonal after several molts, this would support the hypothesis that the two species are synonymous (either ecophenotypes or successive life stages).

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LITERATURE CITED

- Alon, N. C., and S. E. Stancyk. 1982. Variation in life-history patterns of the grass shrimp *Palaemonetes pugio* in two South Carolina estuarine systems.—*Marine Biology* 68: 265–276.
- Anger, K. 1995. The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics.—*Journal of Experimental Marine Biology and Ecology* 193: 119–145.
- Boschi, E. E. 1964. Los crustáceos decápodos Brachyura del litoral bonaerense (R. Argentina).—*Boletín del Instituto de Biología Marina, Mar del Plata* 6: 1–99.
- Cabot, E. L., and A. T. Beckenbach. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE.—*Computer Applications in the Biosciences* 5: 233–234.
- Clayton, D. A. 1990. Crustacean allometric growth: a case of caution.—*Crustaceana* 58: 27–290.
- Cuesta, J. A., and C. D. Schubart. 1998. Morphological and molecular differentiation between three allopatric populations of the littoral crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae).—*Journal of Natural History* 32: 1499–1508.
- , ———, and A. Rodríguez. 2000. Larval development of *Brachynotus sexdentatus* (Risso, 1827)

- (Decapoda, Brachyura) reared under laboratory conditions, with notes on larval characters of the Varunidae.—*Invertebrate Reproduction and Development* 383: 207–223.
- Flores, A., and M. L. Negreiros-Fransozo. 1999. Allometry of the secondary sexual characters of the shore crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura: Grapsidae).—*Crustaceana* 72: 1051–1066.
- Fratini, S., and M. Vannini. 2002. Genetic differentiation in the swimming crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean.—*Journal of Experimental Marine Biology and Ecology* 272: 103–116.
- Garth, J. S. 1957. The Crustacea Decapoda Brachyura of Chile.—Reports of the Lund University Chile Expedition 1948–49, n°29.—*Lunds Universitets Arsskrift N.I. Avd. 2*, 53(7): 1–127.
- Guinot, D. 1984. Le genre *Leurocyclus* Rathbun (Crustacea, Decapoda, Brachyura).—*Bulletin du Museum National d'Histoire Naturelle, Paris 4*, ser. 9, sec A, 1: 211–247.
- Hellberg, M. E. 1998. Sympatric sea shells along the sea's shore: the geography of speciation in the marine gastropod *Tegula*.—*Evolution* 52: 1311–1324.
- Hines, A. H. 1989. Geographic variation in size at maturity in brachyuran crabs.—*Bulletin of Marine Science* 45: 356–368.
- Jones, M. B., and M. J. Simons. 1983. Latitudinal variation in reproductive characteristics of a mud crab, *Helice crassa*.—*Bulletin of Marine Science* 33: 656–670.
- Klautau, M., C. Russo, C. Lazoski, N. Boury-Esnault, J. P. Thorpe, and A. Solé-Cava. 1999. Does cosmopolitanism in morphologically simple species result from overconservative systematics? A case study using the marine sponge *Chondrilla nucula*.—*Evolution* 53: 1414–1422.
- Knowlton, N. 1993. Sibling species in the sea.—*Annual Review of Ecology and Systematics* 24: 189–216.
- Kuhlmann, M., L. and R. E. Walker. 1999. Geographic variation in size structure and size at maturity in the crab *Pilumnus sayi* (Crustacea; Decapoda: Xanthidae) in the Northern Gulf of Mexico.—*Bulletin of Marine Science* 64: 535–541.
- Kyomo, J. 1986. Reproductive activities in the sesarmid crab *Sesarma intermedia* in the coastal and estuarine habitats of Hakata, Japan.—*Marine Biology* 91: 319–329.
- . 2000. Intraspecific variation of reproductive strategies of the crab *Sesarma intermedia*: a consequence of habitat variations.—*Bulletin of Marine Science* 66: 157–171.
- Mayr, E., and P. D. Ashlock. 1991. Principles of Systematic Zoology, 2nd edition. McGraw Hill Book Co., Singapore. 475 pp.
- Otani, T., and T. Takahashi. 1996. Cheliped transformation in the swimming pea crab *Tritodynamia horvathi* Nobili.—*Crustacean Research* 25: 137–141.
- Rathbun, M. J. 1914. New genera and species of American brachyrhynchous crabs.—*Proceedings of the United States National Museum* 47: 117–129.
- . 1918. The grapsoid crabs of America.—*Bulletin of the United States National Museum* 87: 1–461.
- Rieger, P. J., and R. R. R. Vieira. 1997. Desenvolvimento larval de *Cyrtograpsus angulatus* Dana (Crustacea, Decapoda, Grapsidae) em laboratório.—*Revista brasileira de Zoologia* 14: 601–623.
- Scelzo, M. A., and V. Lichtschein de Bastida. 1979. Desarrollo larval y metamorfosis del cangrejo *Cyrtograpsus altimanus* Rathbun 1914 (Brachyura, Grapsidae) en laboratorio, con observaciones de la ecología de la especie.—*Physis, Buenos Aires* 38A: 103–126.
- Schubart, C. D., J. A. Cuesta, R. Diesel, and D. L. Felder. 2000a. Molecular phylogeny, taxonomy, and evolution of non-marine lineages within the American Grapsoidea (Crustacea: Brachyura).—*Molecular Phylogenetics and Evolution* 15: 179–190.
- , ———, and D. L. Felder. 2002. Glyptograpsidae, a new brachyuran family from Central America: larval and adult morphology, and a molecular phylogeny of the Grapsoidea.—*Journal of Crustacean Biology* 22: 28–44.
- , ———, and A. Rodríguez. 2001. Molecular phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA gene.—*Hydrobiologia* 449: 41–46.
- , D. Horst, and R. Diesel. 1999. First record of *Sesarma rectum* Randall (Brachyura, Grapsidae, Sesarminae) from the Lesser Antilles.—*Crustaceana* 72: 537–538.
- , J. E. Neigel, and D. L. Felder. 2000b. The use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea.—*Crustacean Issues* 12: 817–830.
- Simons, M. J., and M. B. Jones. 1981. Population and reproductive biology of the mud crab *Macrophthalmus hirtipes* (Ocypodidae) from marine and estuarine habitats.—*Journal of Natural History* 15: 981–994.
- Spivak, E. 1999. Effects of reduced salinity on juvenile growth of two co-occurring congeneric grapsid crabs.—*Marine Biology* 134: 249–257.
- , and J. A. Cuesta. 2000. Larval development of *Cyrtograpsus affinis* (Dana) (Decapoda, Brachyura, Varunidae) from Río de la Plata estuary, reared in the laboratory.—*Scientia Marina* 64: 29–47.

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Appendix 1. *Cyrtograpsus affinis* (Dana, 1851) and *C. altimanus* Rathbun, 1914. Statistical comparison (ANCOVA) of allometric and morphometric relationships between subtidal specimens collected in the Río de la Plata by the "Albatross" in January 1888 (samples pooled, "A"), by the BIP "Eduardo Holmberg" in November 1997 (samples pooled, "H"), and by the BIP "Capitán Cánepa" in March 1998 ("C"), and intertidal specimens from San Antonio Bay, collected in November 1997 ("S"). (Figs. 3, 4, 5) (*, **, *** regression coefficient significantly different from 0 at $P < 0.05$, $P < 0.01$, and $P < 0.001$ respectively; NS not statistically significant, i.e., $P > 0.05$).

Trait, sex, sample, and source of variation	Slope		Intercept	
	F-ratio	P	F-ratio	P
Chela length, males, all samples ($n = 217$), Fig. 3				
Size	1,598.481	***	3,739.193	***
Habitat	1.549	NS	74.154	***
Interaction	6.616	***		
Chela length, males, H \times C ($n = 72$), Fig. 3				
Size	372.014	***	492.175	***
Habitat	0.145	NS	49.682	***
Interaction	1.538	NS		
Chela length, males, H \times A ($n = 77$), Fig. 3				
Size	663.186	***	844.812	***
Habitat	0.253	NS	5.170	**
Interaction	0.000	NS		
Chela length, males, H \times S ($n = 106$), Fig. 3				
Size	667.028	***	2,988.095	***
Habitat	1.427	NS	122.021	***
Interaction	6.275	*		
Chela length, males, C \times A ($n = 111$), Fig. 3				
Size	1,010.906	***	982.631	***
Habitat	0.000	NS	75.094	***
Interaction	4.140	*		
Chela length, males, C \times S ($n = 140$), Fig. 3				
Size	1,603.748	***	3,100.928	***
Habitat	1.639	NS	0.020	NS
Interaction	1.735	NS		
Chela length, males, A \times S ($n = 145$), Fig. 3				
Size	2,022.402	***	3,806.079	***
Habitat	3.351	NS	11.407	***
Interaction	18.904	***		
Chela length, females, all samples ($n = 218$), Not shown				
Size	647.436	***	2,309.682	***
Habitat	0.224	NS	1.193	NS
Interaction	0.140	NS		
Pleon, males, all samples ($n = 240$), Fig. 4				
Size	1,233.161	***	2,981.874	***
Habitat	10.637	***	37.189	***
Interaction	6.344	***		
Pleon, males, H \times C ($n = 76$), Fig. 4				
Size	286.631	***	409.741	***
Habitat	5.229	*	1.572	NS
Interaction	6.063	*		
Pleon, males, H \times A ($n = 85$), Fig. 4				
Size	407.936	***	497.780	***
Habitat	3.459	NS	5.882	*
Interaction	6.210	*		
Pleon, males, H \times S ($n = 125$), Fig. 4				
Size	699.899	***	3,725.406	***
Habitat	0.243	NS	67.834	***
Interaction	2.112	NS		
Pleon, males, C \times A ($n = 115$), Fig. 4				
Size	626.688	***	645.524	***
Habitat	2.325	NS	19.593	***
Interaction	0.316	NS		

Appendix 1. Continued.

Trait, sex, sample, and source of variation	Slope		Intercept	
	<i>F</i> -ratio	<i>P</i>	<i>F</i> -ratio	<i>P</i>
Pleon, males, C × S (<i>n</i> = 155), Fig. 4				
Size	1,460.733	***	2,389.877	***
Habitat	23.147	***	77.505	***
Interaction	12.161	***		
Pleon, males, A × S (<i>n</i> = 164), Fig. 4				
Size	1,455.613	***	3,076.024	***
Habitat	13.844	***	32.006	***
Interaction	6.914	**		
Pleon, immature females, C × A × S (<i>n</i> = 126), Fig. 4				
Size	1,002.490	***	2,093.047	***
Habitat	74.026	***	7.907	***
Interaction	61.411	***		
Pleon, immature females, C × A (<i>n</i> = 85), Fig. 4				
Size	284.392	***	453.848	***
Habitat	0.595	NS	40.086	***
Interaction	0.000	NS		
Pleon, immature females, C × S (<i>n</i> = 66), Fig. 4				
Size	205.486	***	344.585	***
Habitat	4.943	*	0.551	NS
Interaction	4.668	*		
Pleon, immature females, A × S (<i>n</i> = 99), Fig. 4				
Size	874.505	***	712.230	***
Habitat	13.931	***	18.889	***
Interaction	20.158	***		
Pleon, mature females, all samples (<i>n</i> = 139), Fig. 4				
Size	531.617	***	1,401.406	***
Habitat	8.006	***	17.102	***
Interaction	7.252	***		
Pleon, mature females, H × C (<i>n</i> = 72), Fig. 4				
Size	142.087	***	213.120	***
Habitat	0.217	NS	11.550	***
Interaction	0.080	NS		
Pleon, mature females, H × A (<i>n</i> = 31), Fig. 4				
Size	210.652	***	122.274	***
Habitat	17.466	***	0.011	NS
Interaction	17.784	***		
Pleon, mature females, H × S (<i>n</i> = 82), Fig. 4				
Size	326.624	***	1,587.253	***
Habitat	3.094	NS	56.360	***
Interaction	1.734	NS		
Pleon, mature females, C × A (<i>n</i> = 57), Fig. 4				
Size	212.890	***	203.927	***
Habitat	16.152	***	0.779	NS
Interaction	15.367	***		
Pleon, mature females, C × S (<i>n</i> = 108), Fig. 4				
Size	791.129	***	1,378.605	***
Habitat	2.909	NS	12.113	***
Interaction	1.915	NS		
Pleon, mature females, A × S (<i>n</i> = 67), Fig. 4				
Size	554.182	***	1,493.578	***
Habitat	25.455	***	0.781	NS
Interaction	27.412	***		
Fifth carpus + propodus width vs. length, males, C+A × S (<i>n</i> = 168), Fig. 5				
Size	260.459	***	915.888	***
Habitat	0.336	NS	53.284	***
Interaction	4.548	**		