

Gastric mill morphology in the genus *Cyrtograpsus* (Crustacea: Decapoda: Grapsoidea: Varunidae)

A.V. HUESPE¹, E. GÓMEZ-SIMES² AND C.T. PASTOR-DE-WARD¹

¹Centro Nacional Patagónico, CONICET, Bvrd Brown 2825 – (U9120ACV) Puerto Madryn, Chubut, Argentina, ²Universidad Nacional de la Patagonia ‘San Juan Bosco’, Facultad de Ciencias Naturales, Sede Puerto Madryn, Bvrd Brown 3700—(U9120ACX) Puerto Madryn, Chubut, Argentina

The gastric mill on the genus Cyrtograpsus (Varunidae) has not been described previously. A morphological comparative study of the gastric mill ossicles within its species (C. angulatus, C. altimanus and C. affinis) was carried out. This research attempts to give new evidence to validate the existence of these three species and to serve as a guide to identify them. Urocardiac (dorsal median tooth shape), zygocardiac (shape and number of ridges and molar processes—cusps and plates—and their arrangement in the lateral teeth) and pectineal (number and shape of accessory teeth) ossicles, and cardiopyloric valve descriptions and comparisons are based on microscopical, stereomicroscopical, scanning electron microscope observations, and digital images. Morphological differences found in zygocardiac (lateral teeth) and urocardiac (dorsal-median tooth) ossicles allow to identify species. Considering these ossicles morphology in C. altimanus and C. affinis it can be concluded that the studied specimens belong to a single species as stated in morphometric and molecular comparative studies of the above species. These new data validate the existence of only two species (C. affinis and C. angulatus). This information will also be useful to recognize the species in the stomach content of their predators throughout the ossicles morphology.

Keywords: taxonomy, stomach, ossicles, comparative, scanning electron microscopy (SEM)

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INTRODUCTION

In Argentina, the superfamily Grapsoidea comprises marine, brackish and freshwater crabs; there are five species grouped in three genera and two families (Martin & Davis, 2001). The family Varunidae is represented by *Cyrtograpsus angulatus* (Dana, 1851), *Cyrtograpsus altimanus* (Rathbun, 1914), *Cyrtograpsus affinis* (Dana, 1851) and *Neohelice granulata* (Dana, 1851) (= *Chasmagnathus granulata*). The last one included in the subfamily Cyclograpsinae. *Metasesarma rubripes* (Rathbun, 1897) belongs to the family Sesarmidae (Boschi, 1964).

This study is focused on *Cyrtograpsus*, an endemic South Atlantic and Pacific Ocean genus (Rathbun, 1918). Other previous studies on this genus were carried out on larval development (Scelzo & Litchstein de Bastida, 1979; Rieger & Vieira, 1997; Spivak & Cuesta, 2000), ecological and population structure parameters (Rognone, 1984; Rascio, 1986; Castiglioni & Santos, 2000), moult and growth (Spivak, 1988), use of habitat, mating systems and sexual selection (Gavio & Spivak, 1994; Gavio, 2003), feeding (Capítoli & Ortega, 1996), fecundity (Luppi *et al.*, 1997), reproduction (López Greco & Rodríguez, 2004) and morphometric and molecular comparisons (Spivak & Schubart, 2003).

Cyrtograpsus species present a wide distribution: *C. angulatus* is distributed along the south-western Atlantic Ocean coast from Rio de Janeiro, Brazil to Puerto Deseado, Argentina and in the south-eastern Pacific Ocean from San Lorenzo Island, Perú to Talcahuano Bay, Chile; *C. altimanus* is distributed from Rio Grande do Sul, Brazil to Puerto Deseado, Argentina; and *C. affinis* is distributed from San Roque Cape, Brazil to San Matías Gulf, Argentina (Boschi, 1964).

Spivak (1999) and Spivak & Schubart (2003) report differences in body size and habitat for these species; maximum carapace width in adult specimens of *C. angulatus* is three to five times larger than that of *C. altimanus* and *C. affinis*, respectively. *Cyrtograpsus angulatus* and *C. altimanus* coexist in rocky intertidal habitats (Rathbun, 1918; Boschi, 1964; Scelzo & Lichtschein de Bastida, 1979) and display diverse abilities to invade estuarine habitats (Spivak, 1999). These species are very different in their external morphology; whereas *C. altimanus* is morphologically similar to *C. affinis*. The latter species can be distinguished by a more squarish carapace and because it inhabits subtidal habitats. At present, *C. altimanus* and *C. affinis* are considered putative species (Spivak & Schubart, 2003), but there is a great controversy about their taxonomic status.

Due to the fact that no other study regarding gastric mill was found in the literature on the genus *Cyrtograpsus*, it was considered relevant to undertake a descriptive and comparative morphological study of the gastric mill ossicles within

Corresponding author:
A.V. Huespe
Email: anahuespe@yahoo.com.ar

the species that this genus comprises. The present study increases the knowledge on *Cyrtograpsus* species' morphological characteristics that allow them to be identified. The chitinous gastric mill ossicles are resistant to the digestive process because of their hardness. Thus, they are useful to recognize the species in predator stomach contents when there are no other external morphological features available.

Stomach and gastric mill studies have been carried out on many species of decapod crustaceans; those of Nauck (1880), Patwardhan (1934, 1935), Meiss & Norman (1977), Dall & Mortiary (1983), Felgenhauer & Abele (1989), Brösing (2002), Brösing *et al.* (2002) and Castro & Bond-Buckup (2003) include a critical review of the current terminology in Reptantia (Macrura: Anomura: Brachyura).

Several authors (Boschi & Angelescu, 1962; Dall & Mortiary, 1983; Felgenhauer & Abele, 1989; Huang *et al.*, 1998; Brösing *et al.*, 2001; Brösing, 2002; Sakai *et al.*, 2006) have used the gastric mill structure as an additional characteristic to define genera, families and species. These authors have discussed the gastric mill structural complexity, stating that this structure reflects the evolutionary trends and relationships within decapods.

The gastric mill is a triturative device with several strongly chitinized ossicles of which the dorsal ones are important for the shape and stabilization of the anterior stomach chamber (Brösing, 2002). The anterior, posterior, ventral and dorsal side refers to the position of the gut within the body. In this study only the most conspicuous ossicles, easy to identify and considered of taxonomic value, were included (Brösing *et al.*, 2001; Brösing, 2002), those supporting the stomach chamber being excluded.

Mesocardiac (I), pterocardiac (II), post-pterocardiac (IIb), zygocardiac (V), urocardiac (VII), propyloric (VI), exopyloric (IV) and pyloric (III) ossicles are placed dorsally in the cardiac chamber; the central unpaired ossicles (mesocardiac and urocardiac) can be fused according to the species. The unpaired mesocardiac ossicle is laterally hinged to the paired post-pterocardiac ossicle and this articulates with the paired post-pterocardiac which acts as a pivot for the zygocardiac (paired) and prepectineal (IX) (paired) ossicles, the latter is hinged anteriorly to pectineal ossicle (VIII) (paired). In some species prepectineal and pectineal ossicles are fused; both are placed in the lateral walls of cardiac stomach. Urocardiac, zygocardiac and pectineal ossicles have a dorsal median tooth, lateral and accessory teeth, respectively. All these teeth are responsible for mastication and grinding of the food that enters through the oesophagus. The accessory teeth assist in pushing the material towards the central region of the cardiac chamber.

The cardiopyloric valve (CPV) placed ventrally between the cardiac and pyloric stomach, lies under the urocardiac median dorsal tooth. This valve is conformed by the unpaired anterior (XVI) and posterior (XVII) ossicles, and the lateral ossicle (XVIII) (paired). This valve directs and regulates food material passage from the cardiac to pyloric stomach. Meiss & Norman (1977), Brösing (2002) and Brösing *et al.* (2002) compare the cardiopyloric valve ossicles in different decapod crustacean species.

The objectives of this research were to: (i) describe *C. angulatus*, *C. altimanus* and *C. affinis* gastric mill; and (ii) differentiate each species by comparing the gastric mill structures. Morphology of the dorsal median tooth in urocardiac ossicle; number of ridges and molar processes (cusps and plates) in zygocardiac ossicle; number of accessory teeth in

pectineal ossicle and in cardiopyloric valve development and shape of median tooth on the posterior margin, and length, density and distribution of setae on its lateral and posterior margins were considered.

MATERIALS AND METHODS

Sampling and study area

Cyrtograpsus altimanus (N = 193) and *Cyrtograpsus angulatus* (N = 97) specimens were manually collected during low tide in Punta Cuevas (42°46' 50.8" S 65°0' 06.0" W), April 2003 and Punta Este (42°47' 06.6" S 64°57' 0.9" W), December 2002, February and March 2004. These sampling sites are located in Golfo Nuevo (Chubut, Argentina). *Cyrtograpsus affinis* (N = 8), were supplied by E. Spivak, and collected at 13 m depth, in Río de la Plata estuary (36°05' S 56°37' W) during the field work of the fishing research ship 'Capitán Cánepa' (Instituto Nacional de Investigación y Desarrollo Pesquero, INIDEP).

Methodology

Crabs were transported alive to the laboratory, once there some specimens were: (i) fixed in 10% formaldehyde for a week and then they were kept in 70% ethanol; or (ii) frozen at -20°C. Frozen animals facilitate stomach dissection and muscles removal; in these specimens appendages lost is less frequent.

In all specimens carapace length (CL) and carapace width (CW) were measured with a Vernier caliper and then the stomach was extracted by lifting up the carapace, since it is adhered to it. Ossicles were measured in a stereomicroscope and microscope with an ocular micrometer.

Stomachs were cleaned and observed under a stereomicroscope (Zeiss, Stemi SV6). Afterwards, they were opened in an antero-posterior direction through the ventral mid-line from the oesophagus up to the pleuropyloric valve and the stomach content was discarded so the gastric mill ossicles (urocardiac, zygocardiac and pectineal) and cardiopyloric valve could be observed, described and compared in order to establish interspecific and intraspecific differences in the three studied species.

Those stomachs to be observed under scanning electron microscopy (SEM) (ALUAR S.A.I.C.) were prepared as follows: (i) organic debris and tissues adhered to the stomach structures were removed with 10% sodium hydroxide, time varied from 5 to 20 minutes according to stomach size and ossicles hardness (intermoult or premoult crabs); (ii) alkali was neutralized with 5% acetic acid and then rinsed in distilled water; and (iii) each stomach was extended and mounted with a double-sided adhesive tape on a labelled slide.

Under a JEOL JSM-6460 LV microscope dry SEM samples without gold coating were viewed using the back-scattered electron (BSE) technique, at low vacuum (15–20 Pa). Digital images of gastric mills' general views and ossicles details were taken, as well as each photograph condition (magnification, plate inclination and rotation, scale, resolution, etc.).

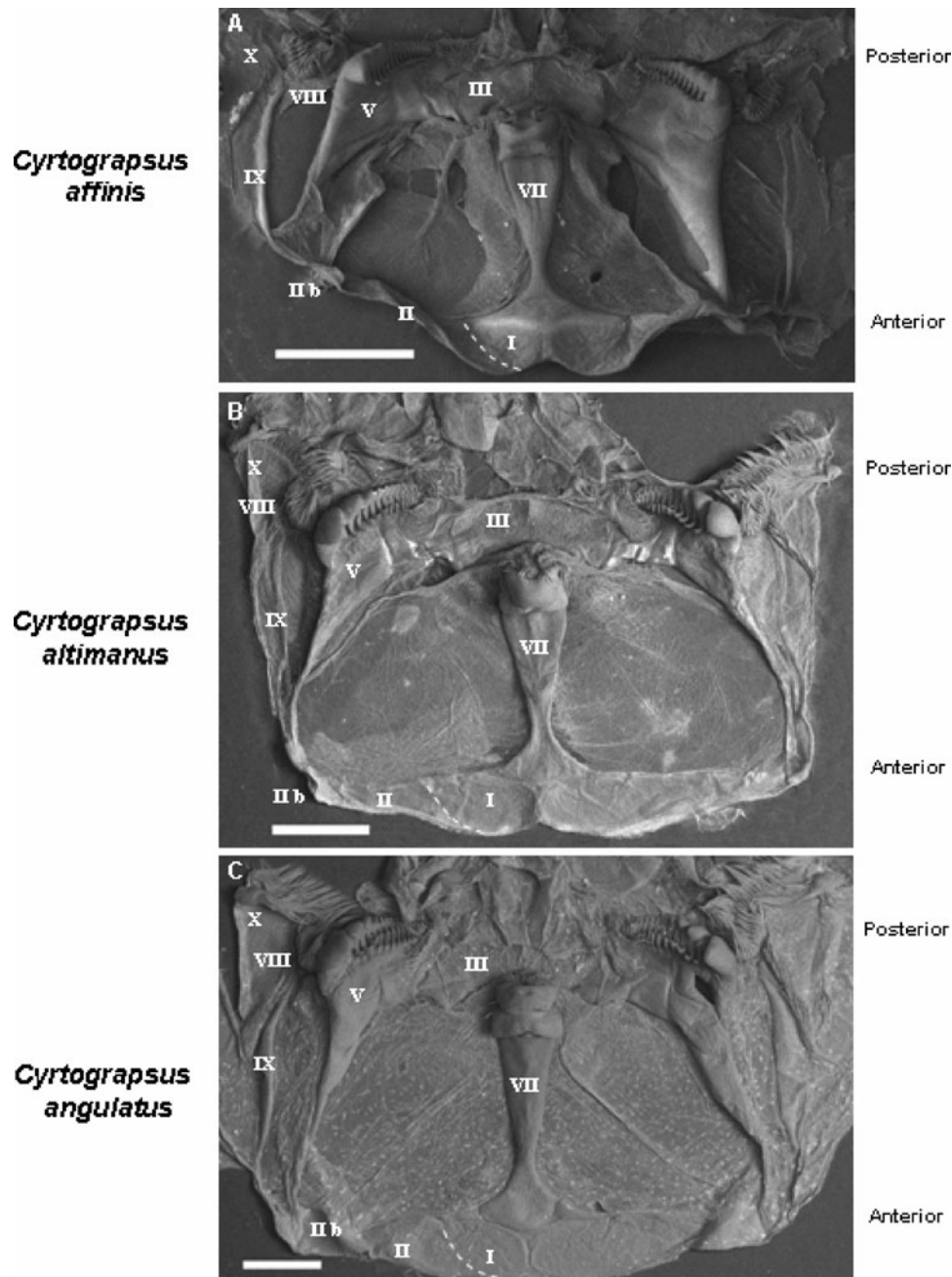


Fig. 1. Gastric mill, internal dorsal view: (A) *Cyrtograpsus affinis*; (B) *Cyrtograpsus altimanus*; and (C) *Cyrtograpsus angulatus*. Ossicles: I, mesocardiac; II, pterocardiac; IIb, postpterocardiac; III, pyloric; V, zygocardiac; VII, urocardiac; VIII, pectineal; IX, prepectineal; X, post-pectineal. Scale bars: 1 mm.

RESULTS

Cyrtograpsus gastric mill ossicles shape and their relative position follow the same general pattern in the studied species (Figure 1 A–C).

The unpaired urocardiac ossicle (VII) is an elongated centred T-shaped ossicle that narrows to its anterior region (Figure 3 A–C). There is a small protuberance at the end of this narrowing. The urocardiac ossicle articulates anteriorly with the mesocardiac ossicle and laterally is separated from the pterocardiac ossicle by an incision (Figure 2 B). The posterior end is hinged with the vertical unpaired subtriangular propyloric ossicle (VI) constituting a dorsal median tooth (Figures 2C & 3H).

The unpaired pyloric ossicle (III) looks like a paired one because it is thinner in the median line (Figure 3H). This ossicle is hinged anteriorly to the propyloric ossicle and laterally contacts the subtriangular exopyloric ossicle (IV) (Figure 3H).

The mesocardiac ossicle (I) (unpaired) is an anteriorly bilobed triangular plate which is thinner in the median line looking like a paired ossicle (Figures 1 & 2B), is laterally fused to the pterocardiac (II) ossicle and is posteriorly hinged to the urocardiac one. Mesocardiac (I) and pterocardiac (II) ossicles fusion is incomplete and is pointed out by an incision (Figures 1A–C, dotted line on the left side, and 2B).

The pterocardiac ossicle (II) (paired) is subrectangular, tapering to its distal lateral end (Figures 1 & 2B).

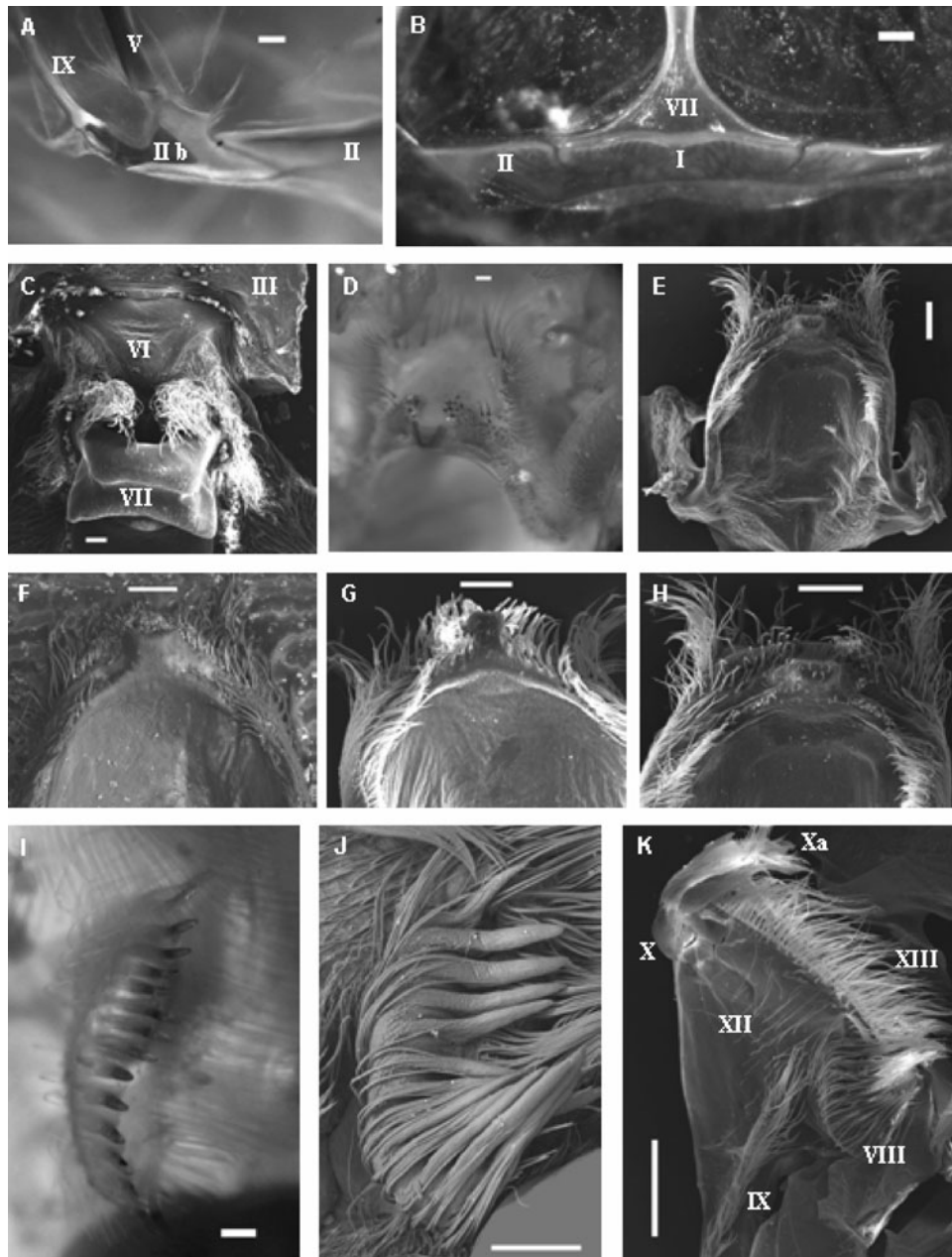


Fig. 2. (A) *Cyrtograpsus altimanus* ossicles: postptero-cardiac (Iib), pterocardiac (II), zygo-cardiac (V), prepectineal (IX); (B) *Cyrtograpsus affinis* ossicles: mesocardiac (I), pterocardiac (II), urocardiac (VII); (C) *Cyrtograpsus angulatus* ossicles: propyloric (VI), urocardiac (VII), pyloric (III), cardiopyloric valve general view; (D) *C. angulatus*, ventral posterior view; (E) *C. angulatus*, dorsal view. Detail: (F) *C. affinis*; (G) *C. altimanus*; (H) *C. angulatus*; (I) and (J) *C. angulatus* pectineal ossicle, accessory teeth; and (K) *C. altimanus* ossicles: pectineal (VIII), prepectineal (IX), postpectineal (X) and 'Quill' (Xa), inferior lateral cardiac (XIII) and posterior lateral cardiac plate (XII). (A), (B), (D) and (I) stereo-microscope photographs. Scale bars: 200 μm .

The postptero-cardiac ossicle (Iib) (paired) is a Y-shape ossicle, sometimes very difficult to observe due to its small size and location. It is hinged anteriorly to the pterocardiac ossicle and posteriorly to the paired zygo-cardiac and prepectineal ossicles (Figure 2A).

The zygo-cardiac ossicle (V) (paired) is a large triangular plate bearing the gastric mill lateral teeth. These heavily sclerotized teeth have dorsal ridges with upwardly curved endings and ventral molar processes with large cuspidate processes (cusps) shaping the anterior part followed by flattened ones (plates) (Figure 4A–F). This ossicle posteriorly articulates with the exopyloric (IV) one.

In the three *Cyrtograpsus* species, the number of molar processes (cusps and plates) is similar to that of the ridges in the zygo-cardiac ossicle (Table 1). Cusps are smooth and constitute half of the lateral teeth length; but the plate's surface differs within the species.

The long and narrow paired prepectineal ossicle (IX) (Figure 1A–C) articulates anteriorly with the postero-cardiac ossicle and posteriorly with the pectineal ossicle.

The pectineal ossicle (VIII) (paired) is a mobile oblong plate bearing the lateral accessory teeth, directed forward and posteriorly hinged to the postpectineal ossicle (X). The lateral accessory teeth (12–16) (Figures 1A–C & 2 I–J) are

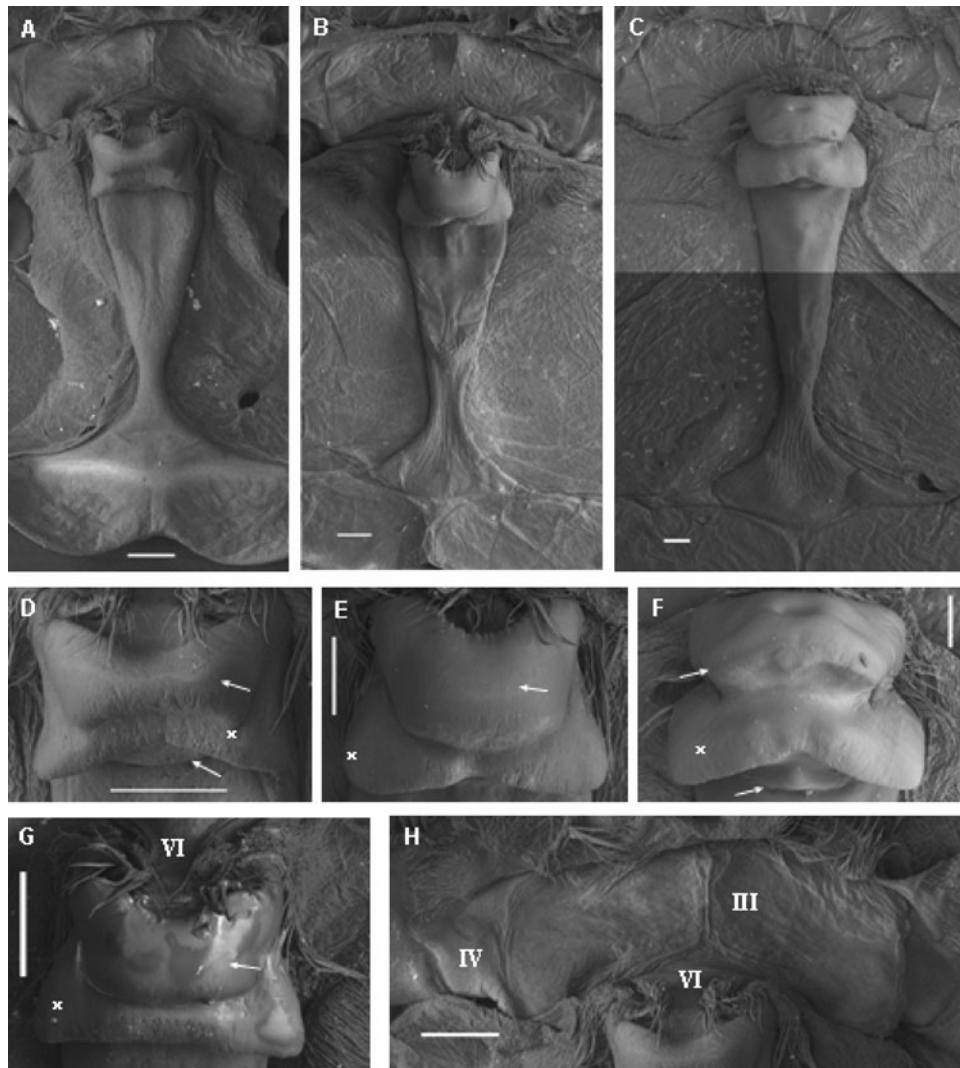


Fig. 3. Urocardiac ossicle. General view: (A) *Cyrtograpsus affinis*; (B) *Cyrtograpsus altimanus*; (C) *Cyrtograpsus angulatus*. Dorso-median tooth, detail: (D) *C. affinis*; (E) *C. altimanus*; (F) *C. angulatus* and (G) *C. affinis*; and (H) *C. affinis*: pyloric (III), exopyloric (IV) and propyloric (VI) ossicles. (Arrows show central teeth and crosslateral teeth in urocardiac dorsal median tooth.) Scale bars: 200 μm .

long, narrow and flexible setae arranged bordering the pectineal ossicle. These teeth externally surrounded with long setae, slightly project towards the centre where there is a group of long thin setae. Teeth number variation is less in small size specimens.

The postpectineal ossicles (X), 'quill' (Xa) of the postpectineal ossicles, posterior lateral cardiac plates (XII) and inferior lateral cardiac ossicles (XIII) are similar in the three *Cyrtograpsus* species (Figure 2K). Anterior lateral cardiac plates (XI) were not observed due to the way the stomach was opened.

Table 1. Number of teeth, molar processes (cusps and plates) and ridges in cardiac stomach ossicles.

| Ossicles | | | <i>Cyrtograpsus affinis</i> | <i>Cyrtograpsus altimanus</i> | <i>Cyrtograpsus angulatus</i> | |
|--------------------------------------|------|-------------------------------------|------------------------------------|-------------------------------|-------------------------------|------------------------|
| Gastric mill Dorsal region | V | Zygocardiac | Cusps and Plates | 5–6 6–9, smooth | 3–4 7–8, smooth | 4 6–10, mammillated |
| | | Ridges and inter-ridges distance | 13–14 20–42 μm | 11–13 20–42 μm | 11–14 62–83 μm | |
| | VII | Urocardiac | Dorsal median tooth projections | 1 or 2 median 2 lateral | 1 median 2 lateral | 2 median 2 lateral |
| Cardiac Stomach Lateral region | VIII | Pectineal | Accessory teeth | 12–14 | 12–14 | 12–15 |

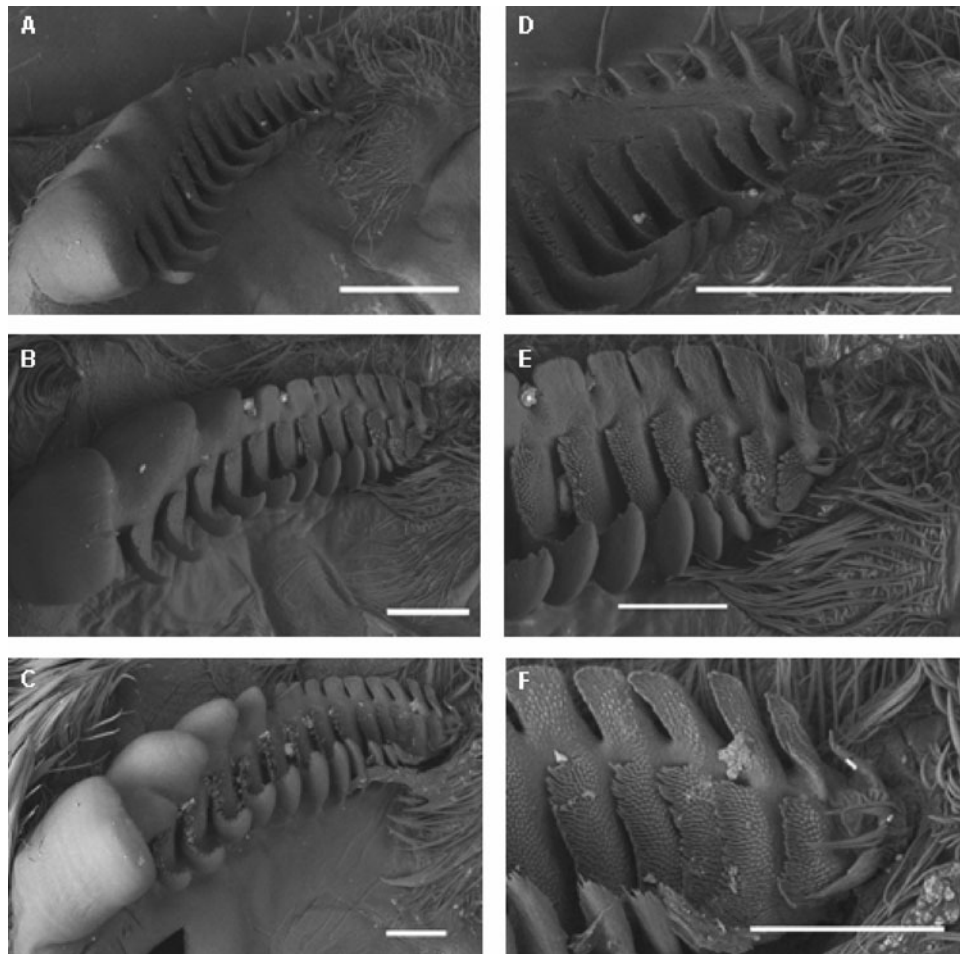


Fig. 4. Zygocardiac ossicle. General view: (A) *Cyrtograpsus affinis*; (B) *Cyrtograpsus altimanus*; (C) *Cyrtograpsus angulatus*. Lateral tooth, posterior end detail: (D) *C. affinis*; (E) *C. altimanus*; (F) *C. angulatus*. Scale bars: 200 μm .

The cardiopyloric valve (Figure 2D–H) is ventral and placed posteriorly to the urocardiac dorso-median tooth. This valve is conformed by three ossicles: (i) anterior (XVI), unpaired, forms a small middle ridge; (ii) posterior (XVII), unpaired, slightly curved with a well-developed tooth shaped median projection. Lateral to this median tooth there are eight spines usually strongly worn out (rounded and shorter or broken) (Figure 2F–H). The postero-dorsal surface of the median tooth has a smooth triangular area whose borders bear densely arranged setae (Figure 2D); and (iii) lateral (XVIII), paired, with setae of different length on the postero-median edge. These lateral ossicles have big ear-like projections on their antero-lateral border (Figure 2E). Posterior and lateral ossicles form a unit.

Table 1 shows the main structures in cardiac stomach ossicles that can be used to identify *Cyrtograpsus* species.

The interspecific and intraspecific variation observed in the zygocardiac (number of ridges) and pectineal (number or teeth) ossicles (Table 1) is based in data obtained from all the *Cyrtograpsus altimanus* (N = 193) and *Cyrtograpsus angulatus* (N = 97) specimens studied. Their size-range was 8.5–25.3 mm for *C. altimanus* and 16–47.4 mm for *C. angulatus*. In *Cyrtograpsus affinis* (N = 8) intraspecific variation could not be established because of the scanty specimens.

The differences observed in cardiac stomach ossicles in *Cyrtograpsus* species are the following ones:

UROCARDIAC OSSICLE (VII) (FIGURE 3A–G)

The well-developed quadrangular dorsal median tooth has two lobed projections on its anterior margin in *C. angulatus* (Figure 3C & F, cross) and *C. affinis* (Figure 3A, D & G, cross); there is another smaller tooth (Figure 3D & F, inferior arrow) anterior to this bilobate edge. The anterior small protuberance in the urocardiac ossicle is very conspicuous in *C. angulatus* (Figure 3F), less evident in *C. affinis* (Figure 3D & G) and absent in *C. altimanus* (Figure 3E).

ZYGOCARDIAC OSSICLE (V) (FIGURE 4A–F)

In *C. angulatus* (Figure 4C & F), this ossicle has four cusps and six to ten mammillated plates whose rough aspect is given by small rounded elevations (mammillas/mammelons). The dorsal ridges (11–14), spaced 62–100 μm , curve upward on their distal end. Stout setae form these ridges, in a compact arrangement; they appear on the surface as small cusps whose height increases gradually towards the ridge's anterior distal end and surpassing its edges. In the apex of the zygocardiac ossicle, there is a dense group of short and thin setae (Figure 4F).

In *C. altimanus* (Figure 4B & E) there are three to four cusps followed by six to eight smooth plates. Zygo-cardiac ridges (11–13) are similar to those of *C. angulatus*, except for the inter-ridges distance (20–60 μm) and the setae that barely surpass the ridge's edges. As in *C. angulatus* a dense group of setae is present at the apex of the zygo-cardiac ossicle (Figure 4E).

Cyrtograpsus affinis (Figure 4A & D) has five to six cusps, six to nine smooth plates and a row of 13–14 ridges spaced by 20–42 μm ; the ridges are very similar to those of *C. altimanus*. At the apex of the zygo-cardiac ossicle, there is a less dense group of thin setae and stout ones (Figure 4D).

DISCUSSION

Sakai *et al.* (2006) found that the differences in the form of the gastric mill in the genera *Helice/Chasmagnathus* complex support the generic groupings within families and can be used in brachyuran systematics. The authors stated that 'The gastric mill teeth differ significantly between genera but are relatively homogenous within a genus,' and also that 'The species can be sometimes distinguished by the number of the lateral comb-shaped teeth and by details of the median tooth plate. Such differences have been referred to in the species descriptions, but there is a great deal of overlap, and the structure of the teeth is not useful in distinguishing between closely related species (p.5).'

Within the family Varunidae, the ossicle structures in *Cyrtograpsus* differ from those shown by Sakai *et al.* (2006) and Covelo de Zolessi & Spiritoso (1985) for the genera *Helice/Chasmagnathus* complex and *Neohelice granulata* (= *Chasmagnathus granulata*), respectively.

In *Cyrtograpsus* the gastric mill has the same general pattern in the three studied species and there are no differences related to specimens' size or sex.

Zygo-cardiac and uro-cardiac ossicles' structures are useful to distinguish species. They can be observed under SEM, but can also be recognized using a microscope or a stereomicroscope, allowing their measurement with an ocular micrometer.

The incision that laterally separates the uro-cardiac ossicle from the pterocardiac ossicle is similar to that shown by Brösing (2002) and Nauck (1880) in *Pseudosesarma moeschi* (Sesarmidae) and *Cardisoma carnifex* (Gecarcinidae), respectively. In the latter species, the pterocardiac and postpterocardiac ossicles are like those found in *Cyrtograpsus*. Yang's (1986) drawings of mesocardiac and pterocardiac ossicles in *Uca* (Ocypodidae) and *Metaplax* (Grapsidae) are quite similar to *Cyrtograpsus* (Varunidae) ones.

The uro-cardiac ossicle dorso-median tooth and the zygo-cardiac ossicle lateral teeth (ridges, cusps and plates) are useful to identify *Cyrtograpsus* species. Since they show interspecific differences, these structures are helpful when found intact in the stomach contents of their predators.

The uro-cardiac dorso-median tooth is similar in *C. affinis* and *C. altimanus*, but *C. angulatus* differs only by a small well-developed central tooth in front of the lateral projections.

The zygo-cardiac ossicle differs in the type, surface and number of molar processes (cusps and plates) in the lateral tooth. There are 5–6 cusps and 6–9 smooth plates in *C. affinis*, 3–4 cusps and 6–8 smooth plates in *C. altimanus* and 4 cusps and 6–10 mammilated plates in *C. angulatus*. The

number of ridges (14) is similar in *Cyrtograpsus* species and the variability observed in *C. angulatus* and *C. altimanus* (11 to 14) is considered to be intraspecific variation. The *Cyrtograpsus altimanus* inter-ridge distance size-range (20–60 μm) overlaps that for *C. affinis* (20–42 μm); but, this distance size-range is quite different in *C. angulatus* (62–100 μm).

Shape and number of molar processes as well as inter-ridge distance differences in the zygo-cardiac ossicle could be related to the species feeding type associated to their habitat.

In *Cyrtograpsus* the pectineal (VIII) and prepectineal (IX) ossicles are slightly chitinized compared with uro-cardiac and zygo-cardiac ones. Therefore, it is less probable to find them complete in predators' stomach contents. In the three species studied, the pectineal ossicle is similar. The number of accessory teeth is quite constant (12–16) and the differences observed are considered as due to specimen size.

The morphology of the cardiopyloric valve is similar in all *Cyrtograpsus* species. According to Felgenhauer & Abele (1989), the morphology of the cardiopyloric valve provides an idea of the species feeding type, as this valve limits the size of the food that enters the pyloric chamber. Therefore, a valve with a prominent median tooth (posterior ossicle) indicates that together with zygo-cardiac lateral teeth it contributes to food grinding. In *Cyrtograpsus* species, cardiopyloric valve similarities suggest filtering of same size food particles. The well-developed median tooth in the posterior valve's ossicles (XVII) implies large food intake (macrophagy).

Gastric mill comparisons in *Cyrtograpsus* species have shown very few differences. Gastric mill morphology in *C. altimanus* and *C. affinis* are similar but they differ from *C. angulatus* in zygo-cardiac and uro-cardiac ossicles. Huang *et al.* (1998) have also found that these ossicles are different in two very closely related Ocypodidae species (*Ocypode sinensis* and *O. cordimanus*).

The few internal morphological differences found in the three *Cyrtograpsus* species are consistent with larval morphology studies (Spivak & Cuesta, 2000). The differences stated in zoeal and megalopae morphology descriptions were based on laboratory-reared larvae (Scelzo & Lichtschein de Bastida, 1979; Rieger & Vieira, 1997; Spivak & Cuesta, 2000), finding minimum differences in morphology and zoeae (I–IV) size. Megalopae sizes are similar in *C. altimanus* and *C. affinis*, but they are larger in *C. angulatus*. The morphology of this larval stage is similar in the three species and the main differences among the megalopae are in the morphology of antennule and the mandible (Spivak & Cuesta, 2000).

Spivak & Schubart's (2003) comparisons of morphometrics and 16S mtDNA did not find diagnostic differences between *C. affinis* and *C. altimanus* and these putative species are considered closely related species. These authors stated that *C. affinis* and *C. altimanus* differences found in molecular 16S mtDNA comparisons, external morphology and size-range could be considered interpopulational differences related to life in different habitats. *Cyrtograpsus altimanus* is distributed in subtidal and intertidal habitats but *C. affinis* is restricted to subtidal habitats.

Spivak & Schubart (2003) did not find diagnostic differences between these two putative species considering that both represent different morphs of a single species. These results are confirmed by Lezcano *et al.* (2006) in a geometric morphometric study carried out in Puerto Madryn (Chubut, Argentina). The authors' findings showed the same continuous

gradient in subtidal habitats and a strong allometric effect. They also consider that *C. affinis* and *C. altimanus* represent morphs of a single species. Spivak & Schubart (2003) attribute morphological variability to a high phenotypic and ecological plasticity. A similar case of closely related species is that of *Brachynotus sexdentatus* and *B. gemmellari* (Varunidae). Their 16S mtDNA are identical and have overlapping morphometrics, differing only in habitat preferences (Cuesta *et al.*, 2000; Schubart *et al.*, 2001). A 16S mtDNA molecular study should be performed in *C. angulatus* to establish if there are molecular differences with *C. affinis* and *C. altimanus*.

Our findings demonstrate enough evidence to conclude that *C. altimanus* and *C. affinis* should belong to a single species as mentioned by Spivak & Schubart (2003) and Lezcano *et al.* (2006). These new data validate the existence of only two species, *C. affinis* and *C. angulatus*, and allow us to recognize these species in the stomach content of their predators.

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Correspondence should be addressed to:

A.V. Huespe
Centro Nacional Patagónico
CONICET
Bvrd Brown 2825—(U9120ACV) Puerto Madryn
Chubut
Argentina
email: anahuespe@yahoo.com.ar