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ORIGINAL ARTICLE

Comparative morphology of the stomach ossicles of four freshwater crabs species of Dilocarcinini (Crustacea, Decapoda, Trichodactylidae)

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ABSTRACT

The morphology of the foregut ossicles of representatives of four genera of Dilocarcinini were described to assess the usefulness of the ossicles as possible morphological characters in studies on the intrafamilial relationships of Trichodactylidae. Stomachs of specimens of *Dilocarcinus pagei* Stimpson, 1861, *D. septemdentatus* (Herbst, 1783), *Moreirocarcinus emarginatus* (H. Milne Edwards, 1853) and *M. laevifrons* (Moreira, 1901) were heated for 60 minutes in 10% KOH solution and then were stained with Alizarin Red 1% added into the KOH solution. The foregut ossicles of Dilocarcinini consists of 11 ossicles of the gastric mill, 10 of the lateral supporting cardiac region, 4 of the cardio-pyloric valve, 6 supporting the dorsal pyloric stomach, 9 supporting the ventral pylorus and bulb, 3 supporting the supra-ampullary region, 5 supporting the lateral pylorus region, totaling 48 ossicles and the following 34 are similar in shape, size and degree of calcification. The morphological differences between the majority of the ossicles were not very clear among the species, showing inconsistency intrageneric differences, however, species of different genera showed small similarities between them. There is a clear need for including more taxa in a comparative analysis,

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combined with external morphology and perhaps even molecular data to properly evaluate the phylogenetic relationships within Trichodactylidae and their systematic position within Brachyura.

Key words

Gastric mill, anatomy, stomach, Amazon region, Neotropical region.

Introduction

The Neotropical freshwater crab family Trichodactylidae comprises of 49 species distributed in 15 genera and two subfamilies (Ng *et al.*, 2008). The suprageneric classification of the family was delineated by Rodríguez (1992) and reassessed by Magalhães and Türkay (1996), who used characters of the abdomen, the first male pleopod (gonopod) and the endofragmal skeleton as the basis of their classification system. These authors proposed a classification in which Trichodactylidae is divided into two subfamilies and two tribes [Trichodactylinae and Dilocarcininae (Dilocarcinini and Valdiviini)]. The information currently available on the gastric system of trichodactylid crabs is restricted to descriptions of the foregut ossicles of *Valdivia serrata* White, 1847, *Dilocarcinus septemdentatus* (Herbst, 1783), and *Sylviocarcinus pictus* (H. Milne-Edwards, 1853) by Alves *et al.* (2010), and to the study of Lima-Gomes *et al.* (2017), which added some previously unnoticed ossicles to the description of the foregut ossicles of *V. serrata* and *S. pictus*.

The ossicles of crustacean stomachs, in addition to offering a possible indication of how the digestive processes work in each group, might provide valuable information on phylogenetic relationships between the groups (Patwardhan, 1935; Brösing *et al.*, 2002; 2007). In this study, we analyzed the stomachs of four Dilocarcinini species, *Dilocarcinus pagei* Stimpson, 1861, *D. septemdentatus*, *Moreirocarcinus emarginatus* (H. Milne Edwards, 1853), and *M. laevifrons* (Moreira, 1901) for comparing the morphology of their foregut ossicless and to assess the usefulness of the ossicles as possible morphological characters in studies on the intrafamilial relationships of Trichodactylidae.

Material and Methods

Five uncatalogued specimens of each species of *Dilocarcinus pagei*, *D. septemdentatus, Moreirocarcinus emarginatus*, and two specimens of the *M. laevifrons*, adult and juvenile males and females, from the Crustacean Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus (Tab. 1), were used for the dissection of the stomachs. The specimens were separated according to sex and measured by carapace width (measured across the carapace at its widest point) and carapace length (measured along the midline from the frontal to the posterior margin). Their stomachs were obtained after removing the carapace and fixed in 10% formalin for 24 hours. For tissue maceration, stomachs were heated for 60 minutes in 10% Potassium Hydroxide (KOH) solution and further to 100°C (Mocquard, 1883; Brösing *et al.*, 2002). Then the skeleton was stained with Alizarin Red 1% added into the KOH solution to facilitate visualization of the internal structures such as setae and ossicles (Brösing *et al.*, 2002). Illustrations of the foregut ossicles were made in ventral, dorsal and lateral views, with drawings prepared with the aid of a camera lucida mounted on the stereoscopic microscope.

The nomenclature and abbreviations used in the morphological descriptions of the foregut ossicles follows Lima (2010), and a complete list was constructed with these names and abbreviations of all the described ossicles (Tab. 2).

Results

In the species studied, the esophagus is connected with the antero-ventral portion of the cardiac chamber. This chamber is responsible for grinding and maceration of food particles (Mocquard, 1883; Patwardhan, 1935;

Table 1. Species of Trichodactylidae, examined specimen (sex: AF, adult female; JF, juvenile female; AM, adult male; JM, juvenile male), measurements (CW, carapace width; CL, carapace length), and origin of the specimens (INPA, crustacean collection of the Instituto Nacional de Pesquisas da Amazônia).

Species	Specimen	Measurements (mm)	Origin of specimens
1. Dilocarcinus pagei	AF	CW: 44.45; CL: 39.52	INPA, uncatalogued
2. Dilocarcinus pagei	AM	CW: 47.19; CL: 39.29	INPA, uncatalogued
3. Dilocarcinus pagei	AM	CW: 37.21; CL: 31.05	INPA, uncatalogued
4. Dilocarcinus pagei	AM	CW: 34.49; CL: 28.32	INPA, uncatalogued
5. Dilocarcinus pagei	JM	CW: 20.01; CL: 16.15	INPA, uncatalogued
1. Dilocarcinus septemdentatus	AF	CW: 32.78; CL: 16.15	INPA 800
2. Dilocarcinus septemdentatus	JF	CW: 28.60; CL: 24.53	INPA 800
3. Dilocarcinus septemdentatus	JF	CW: 28.53; CL: 23.49	INPA 800
4. Dilocarcinus septemdentatus	AM	CW: 45.34; CL: 34.05	INPA 800
5. Dilocarcinus septemdentatus	JM	CW: 36.44; CL: 29.74	INPA 800
1.Moreirocarcinus emarginatus	AF	CW: 26.89; CL: 21.28	INPA 500
2. Moreirocarcinus emarginatus	AM	CW: 29.13; CL: 23.35	INPA 500
1. Moreirocarcinus laevifrons	AM	CW: 32.75; CL: 25.23	INPA, uncatalogued
2. Moreirocarcinus laevifrons	AF	CW: 39.37; CL: 32.09	INPA, uncatalogued
3. Moreirocarcinus laevifrons	AF	CW: 33.30; CL: 25.59	INPA, uncatalogued
4. Moreirocarcinus laevifrons	AF	CW: 37.10; CL: 30.43	INPA, uncatalogued
5. Moreirocarcinus laevifrons	JF	CW: 22.26; CL: 19.27	INPA, uncatalogued

Meiss and Norman, 1977), its shape is wider than long, and consisting of the cardiac sac in the anterior portion and the ossicles of the gastric mill in the posterior portion. This cardiac sac is composed of a fairly thin chitin membrane. Ventrally there is the pyloric chamber, which comprises the posterior portion of the stomach, and is generally longer than wide and much smaller than the cardiac chamber. The pyloric chamber is responsible for the digestion, absorption and elimination of food particles (Mocquard, 1883; Patwardhan, 1935; Meiss and Norman, 1977; Brösing *et al.*, 2002; Brösing, 2010).

Table 2. Nomenclature and abbreviations used in the morphological descriptions ossicles of the stomachs of four species of Dilocarcinini.

Name of the ossicles	Number assigned to the ossicles
Ossicles of the gastric mill:	
Mesocardiac ossicle	(I)
Pterocardiac ossicle	(II)
Post-pterocardiac ossicle	(IIb)
Pyloric ossicle	(III)
Exopyloric ossicle	(IV)
Zygocardiac ossicle	(V)
Pro-pyloric ossicle	(VI)
Urocardiac ossicle	(VII)
Pectineal ossicle	(VIII)
Dorsomedian cardiac plate	(VIIa)
Dorsolateral cardiac plate	(VIIb)
Lateral supporting cardiac ossicles:	
Suprapectineal lateral ossicle	(VIIIa)
Pre-pectineal ossicle	(IX)
Post-pectineal ossicle	(X)
Quill of the postpectineal ossicle	(Xa)
Anterior lateral cardiac plate	(XI)
Posterior lateral cardiac plate	(XII)
Inferior cardiac valve ossicle	(XIIa)
Inferior lateral cardiac ossicle	(XIII)
Subdentate ossicle	(XIV)
Lateral cardiac-pyloric ossicle	(XV)
Ossicles of the cardio-pyloric valve:	
Anterior ossicle of the cardio-pyloric valve	(XVI)
Posterior ossicles of the cardio-pyloric valve	(XVII)
Lateral ossicle of the cardio-pyloric valve	(XVIII)
Supporting ossicles of the dorsal pyloric chamber:	
Anterior mesopyloric ossicle	(XIX)
Lateral mesopyloric ossicle	(XIXa)
Posterior mesopyloric ossicle	(XX)
Uropyloric ossicle	(XXI)
Infra-uropyloric fragment	(XXIa)
Posterior uropyloric ossicle	(XXIb)
Supporting ossicles of the ventral pylorus and ampullae:	
Preampullary ossicle	(XXII)
Anterior inferior pyloric ossicle	(XXIII)
Inferior ampullary ossicle	(XXIV)
Ampullary roof ossicle, lower portion	(XXV)
Ampullary roof-medium portion ossicle	(XXVa)
Ampullary roof ossicle, upper portion	(XXVI)
Process of the ampullary roof-upper portion	(XXVIa)
Posterior inferior pyloric ossicle	(XXVII)
Lateral inferior post-ampullary plate	(XXVIIa)
Supporting ossicles of the supra-ampullary:	
Anterior supra-ampullary ossicle	(XXVIII)
Median supra-ampullary ossicle	(XXIX)
Posterior supra-ampullary ossicle	(XXX)
Supporting ossicles of the lateral pylorus:	
Anterior pleuro-pyloric ossicle	(XXXI)
Pleuro-pyloric valve's ossicle	(XXXIa)
Median pleuro-pyloric ossicle	(XXXII)
Lateral pleuro-pyloric plate	(XXXIIa)
Posterior pleuro-pyloric ossicle	(XXXIII)
Cardiac-pyloric valve	(v.c.p.)

The foregut ossicles consists of 11 ossicles of the gastric mill, 10 ossicles of the lateral supporting cardiac region, 4 ossicles of the cardio-pyloric valve, 6 ossicles supporting the dorsal pyloric chamber, 9 supporting ossicles of the ventral pylorus and ampullae, 3 supporting ossicles of the supra-ampullary, and 5 ossicles supporting the lateral pylorus region and cardiac-pyloric valve, totaling 48 ossicles for these species.

Of these 48 recognized ossicles in the four species studied (Figs. 1–4), the following 34 are similar in shape, size and degree of calcification: dorsomedian cardiac plate (VIIa); dorsolateral cardiac plate (VIIb); suprapectineal lateral ossicle (VIIIa); post-pectineal ossicle (X); quill of the postpectineal ossicle (Xa); anterior lateral cardiac plate (XI); posterior lateral cardiac plate (XII); inferior cardiac valve (XIIa); inferior lateral cardiac ossicle (XIII); lateral cardio-pyloric ossicle (XV); cardio-pyloric valve (v.c.p.); posterior ossicle of the cardio-pyloric valve (XVIII); lateral ossicle of the cardio-pyloric valve (XVIII); lateral mesopyloric ossicle (XIXa); uropyloric ossicle (XXI); infra-uropyloric fragment (XXIa); posterior uropyloric ossicle (XXIb); preampullary ossicle (XXII); anterior inferior pyloric ossicle (XXIII); inferior ampullary ossicle (XXIV); ampullary roof ossicle, lower portion (XXV); ampullary roof-mediun

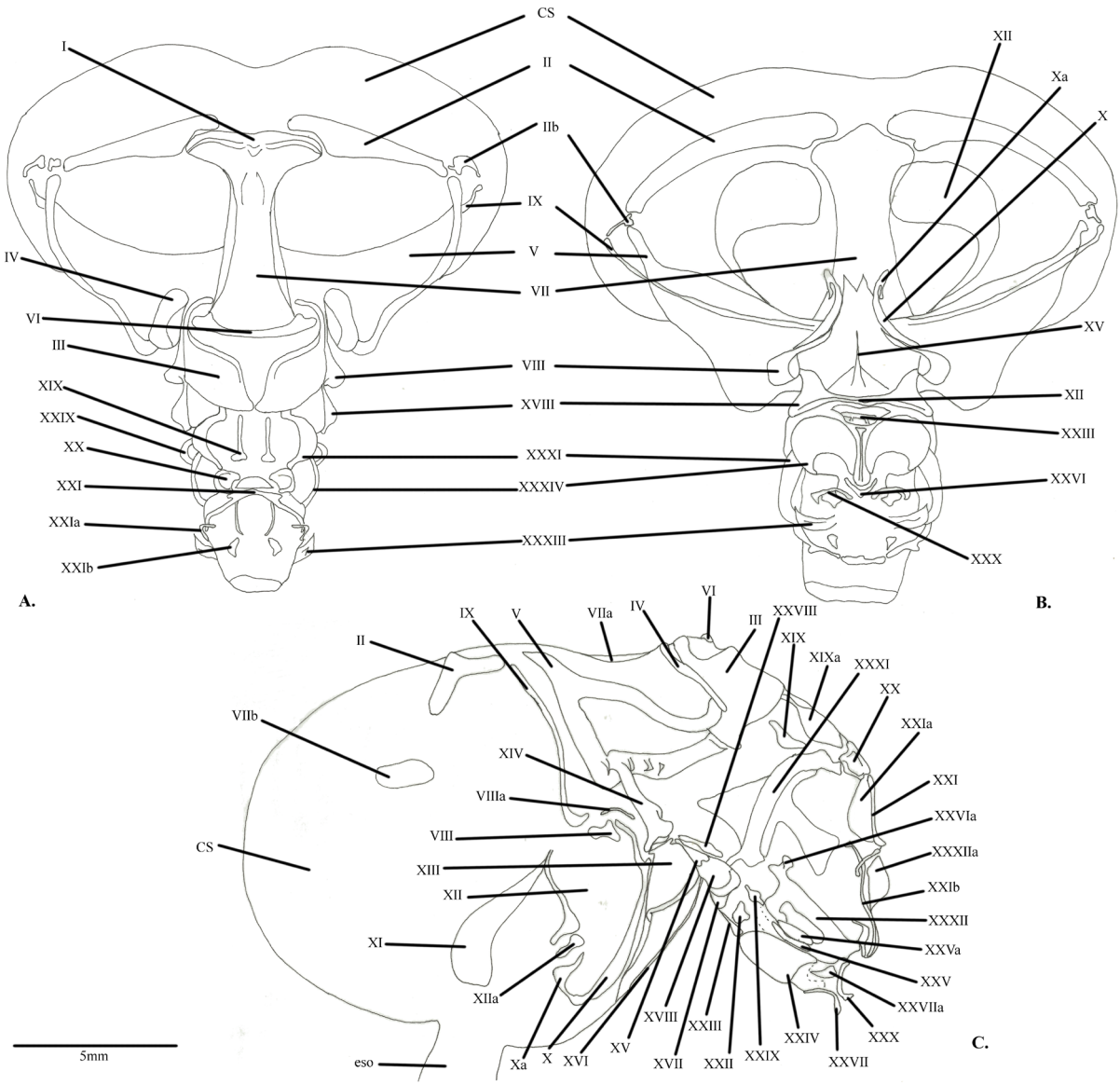

Figure 1. *Dilocarcinus pagei* Stimpson, 1861, cardiac and pyloric chambers of the foregut. A, Dorsal view; B, ventral view; C, lateral view. Abbreviations: CS, cardiac sac; eso, esophagus; see Tab. 2 for the nomenclature of the ossicles.

portion ossicle (XXVa); ampullary roof ossicle, upper portion (XXVI); process of the ampullary roof-upper portion (XXVIa); posterior inferior pyloric ossicle (XXVII); lateral inferior post-ampullary plate (XXVIIa); anterior supra-ampullary ossicle (XXVIII); median supra-ampullary ossicle (XXIX); posterior supra-ampullay ossicle (XXX); anterior pleuro-pyloric ossicle (XXXI); pleuro-pyloric valve's ossicle (XXXIa); median pleuro-pyloric ossicle (XXXII); lateral pleuro-pyloric plate (XXXIIa) and posterior pleuro-pyloric ossicle (XXXIII). The 14 remaining ossicles (mesocardiac ossicle (I); pterocardiac ossicle (II); post-pterocardiac ossicle (IIb); pyloric ossicle (III); exopyloric ossicle (IV); zygocardiac ossicle (V); pro-pyloric ossicle (VI); urocardiac ossicle (VII); pectineal ossicle (VIII); pre-pectineal ossicle (IX); subdentate ossicle (XIV); anterior ossicle of cardio-pyloric valve (XVI); anterior mesopyloric ossicle (XIX); posterior mesopyloric ossicle (XX)) exhibit morphological differences among the studied species.

The description of all ossicles follows, and the differences among the studied species are highlighted.

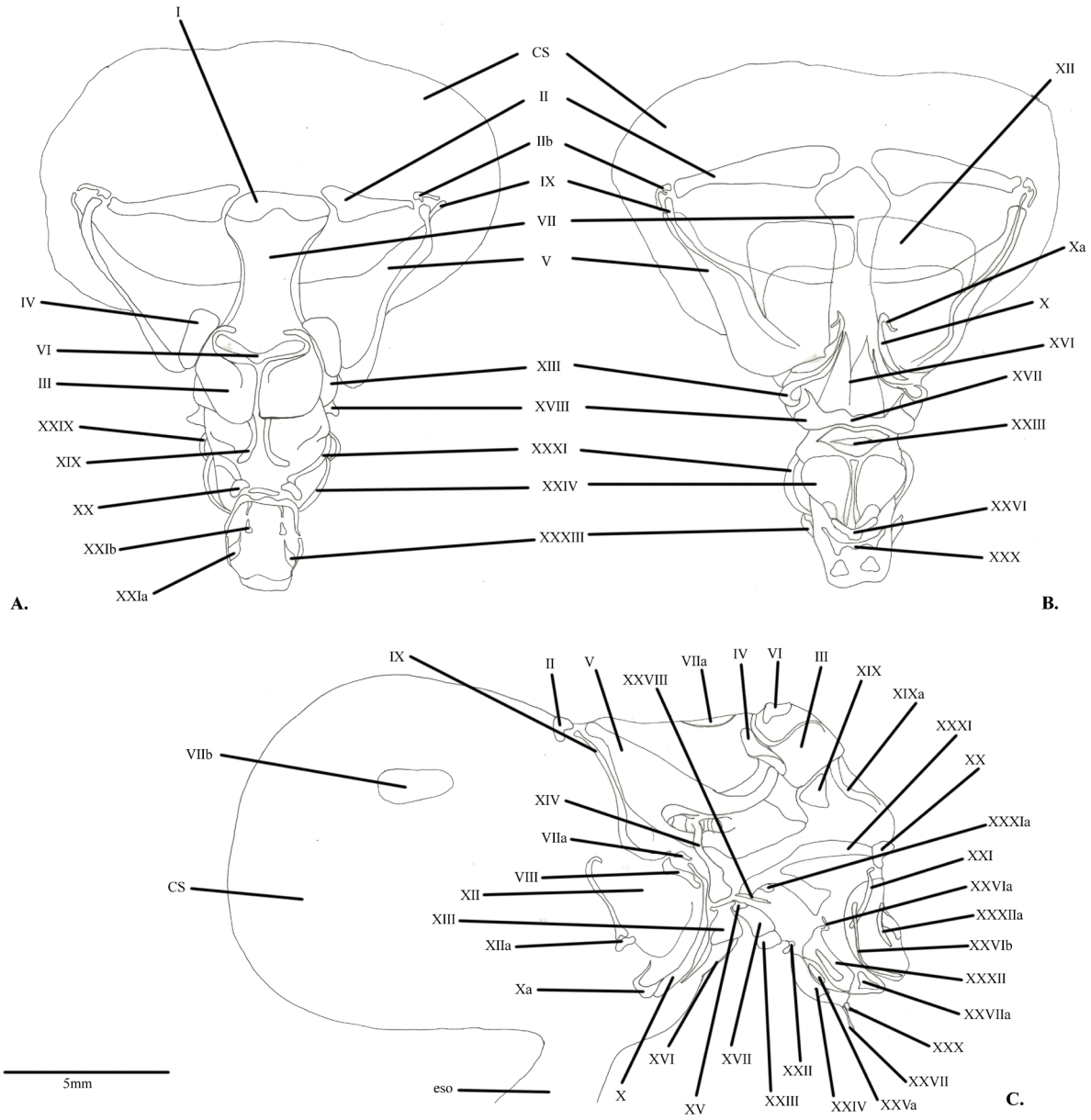

Figure 2. *Dilocarcinus septemdentatus* (Herbst, 1783), cardiac and pyloric chambers of the foregut. A, Dorsal view; B, ventral view; C, lateral view. Abbreviations: CS, cardiac sac; eso, esophagus; see Tab. 2 for the nomenclature of the ossicles.

Ossicles of the gastric mill
(Figs. 1A, C, 2A, C, 3A, C, 4A, C)

Mesocardiac ossicle (I): unpaired, compressed, heavily calcified, positioned in front of the cardiac stomach, between pterocardiac (II) and urocardiac ossicles (VII); upper border completely fused to basal portion of urocardiac ossicle (VII) forming distinct bar, heavily calcified. The anterior-lower margin is slightly calcified, projected ventrally and concave in *D. pagei* and *M. laevifrons*, but convex in *D. septemdentatus* and *M. emarginatus*. It is partially fused (incomplete suture line) from the pterocardiac ossicles (II) in *D. pagei* and *M. laevifrons*, and completely separated in *D. septemdentatus* and *M. emarginatus*.

Pterocardiac ossicle (II): paired, compressed, heavily calcified, located between mesocardiac (I) and post-pterocardiac (IIb). The lateral margin of the pterocardiac ossicle (II) is truncated in *D. septemdentatus* and *M. emarginatus*, and not truncated in *D. pagei* and *M. laevifrons*. The anterior surface is devoid of the carina in all four species; its margin is

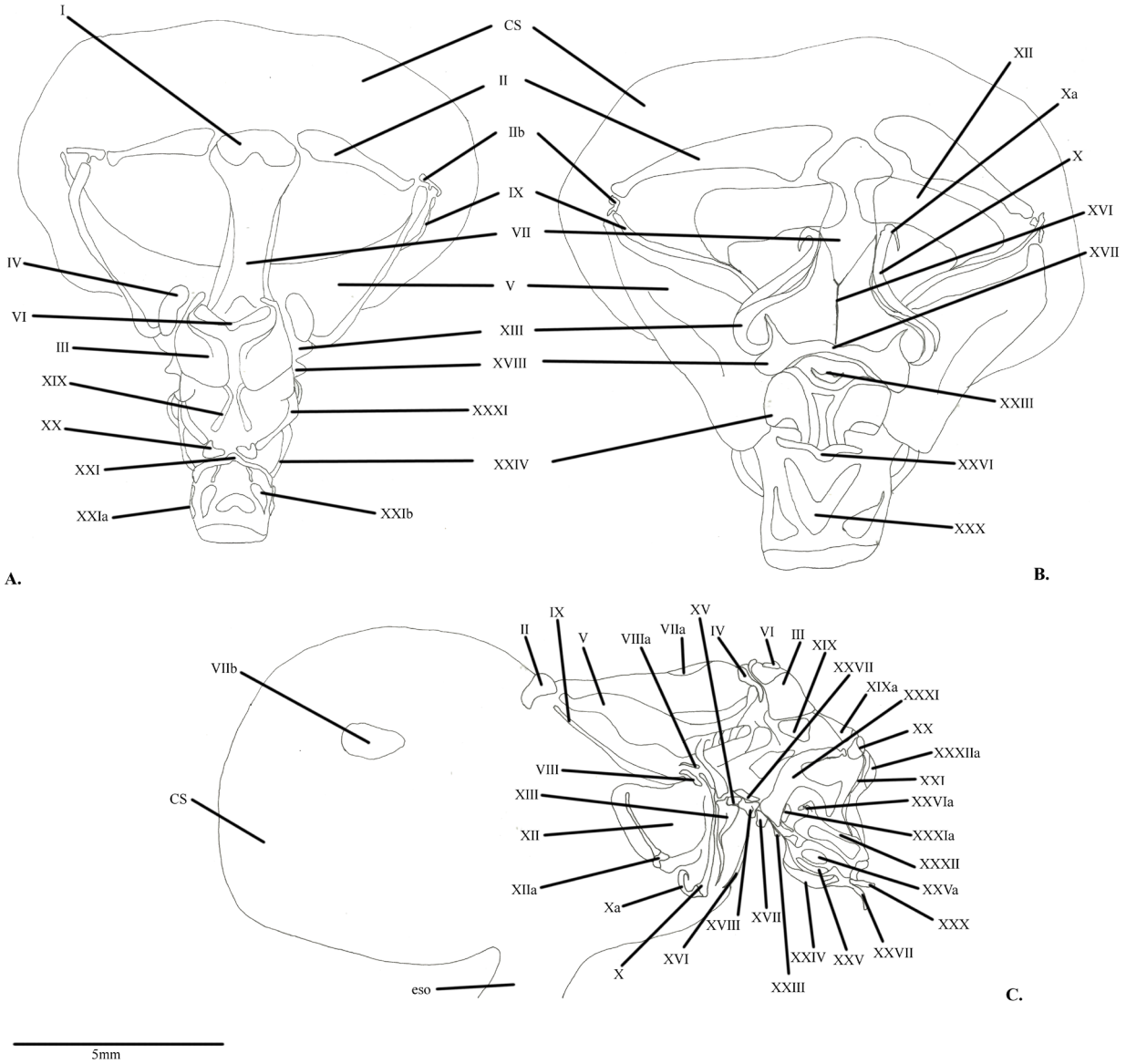

Figure 3. *Moreirocarcinus emarginatus* (H. Milne Edwards, 1853), cardiac and pyloric chambers of the foregut. A, Dorsal view; B, ventral view; C, lateral view. Abbreviations: CS, cardiac sac; eso, esophagus; see Tab. 2 for the nomenclature of the ossicles.

subtriangular in *D. pagei, D. septemdentatus* and *M. laevifrons*, but is elongated and slightly sinuous in dorsal view in *M. emarginatus*.

Post-pterocardiac ossicle (IIb): paired, compressed, located anterolaterally, forming a firm connection between pterocardiac (II), zygocardiac (V) and pre-pectineal ossicles (IX). It is lightly calcified in *D. pagei* and *M. emarginatus* but moderately so in *D. septemdentatus* and *M. laevifrons*. In all four species, the ossicle is rectangular conected by projections with the pre-pectineal and zygocardiac ossicles (V).

Pyloric ossicle (III): unpaired, compressed, convex, moderately calcified, located in the dorsal part of the stomach, connecting the pro-pyloric (VI), exopiloric (IV) and urocardiac ossicles (VII); superior edge slightly bend; anterolateral angle with long process. The apex of the anterolateral angle process is conspicuous in *D. pagei* and *M. laevifrons,* but inconspicuous in *D. septemdentatus* and *M. emarginatus*. Subterminal process curved mesially. Deep median notch along coth shells.

Exopyloric ossicle (IV): paired, heavily calcified, dorsolateral face compressed, forming ellipsoid plate in

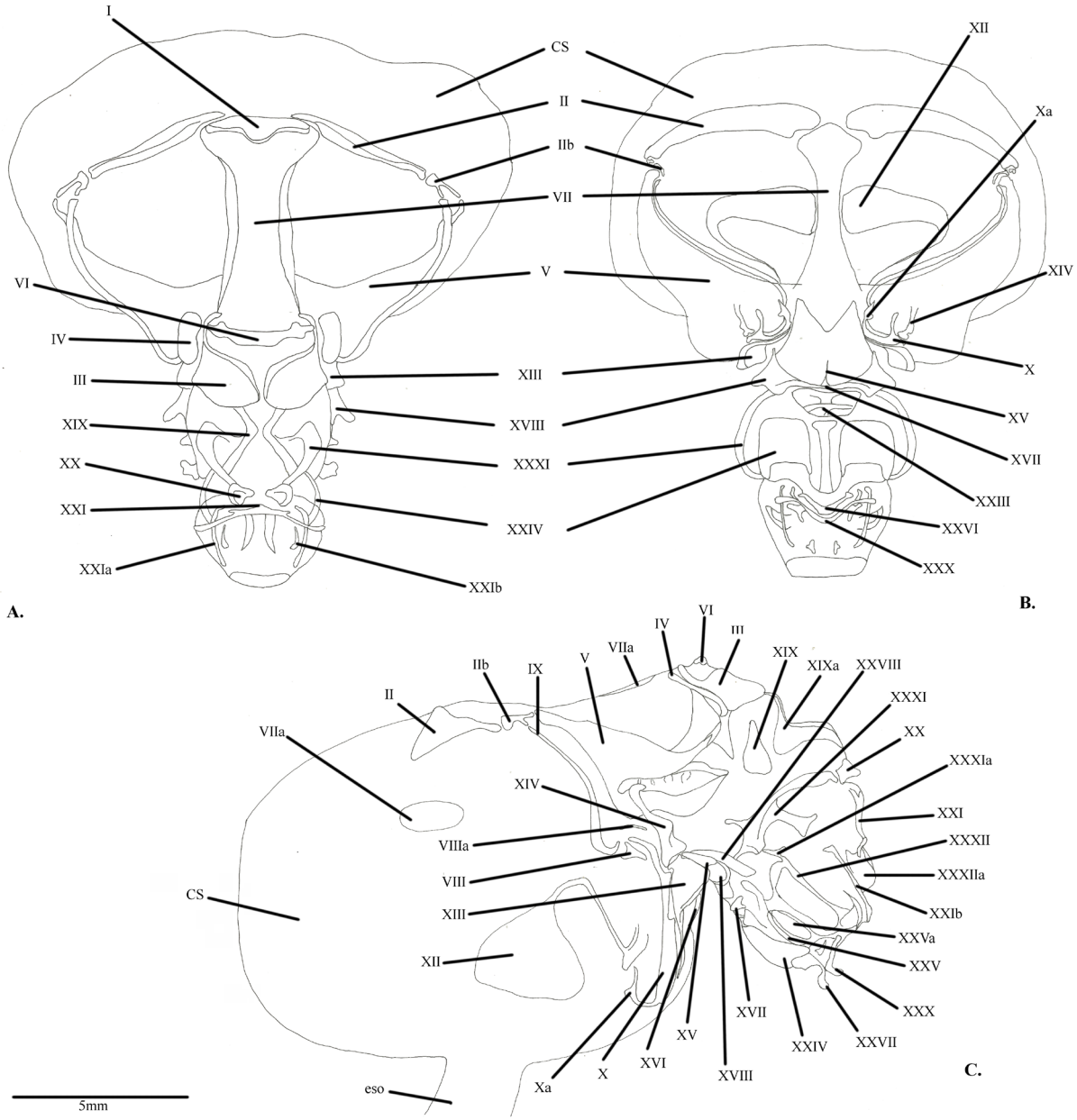

Figure 4. *Moreirocarcinus laevifrons* (Moreira, 1901), cardiac and pyloric chambers of the foregut. A, Dorsal view; B, ventral view; C, lateral view. Abbreviations: CS, cardiac sac; eso, esophagus; see Tab. 2 for the nomenclature of the ossicles.

D. pagei and *D. septemdentatus*, and oblong plate in *M. laevifrons* and *M. emarginatus* (in dorsal view). Mesial margin of the ossicle straight and partially fused to pyloric ossicle (III). Lateral margin of the dorsolateral face convex. Dorsolateral plate reaching a little over half the length of the pyloric ossicle (III) in *D. pagei* and *M. emarginatus*, and reaching more than half the length of the pyloric ossicle (III) in *D. septemdentatus* and *M. laevifrons*.

Zygocardiac ossicle (V): paired, compressed, heavily calcified, ventral face convex, inconspicuous subcircular protuberance in mesial face. Dorsolateral face concave with oblique carina in *D. pagei* and *D. septemdentatus*, and devoid of carina in *M. emarginatus and M. laevifrons*. Anterior margin concave, convex posteriorly. Mesial end armed, with strong teeth; posterior-mesial angle connecting securely to exopyloric ossicle (IV); anterolateral end tapered, connecting to post-pterocardiac ossicle (IIb). Mesial end teeth individualized in *D. pagei*, *D. septemdentatus*, and *M. laevifrons*, and fused in *M. emarginatus*.

Pro-pyloric ossicle (VI): unpaired, compressed, heavily calcified, with conspicuous constriction near inner margin, constituting two subtriangular regions (dorsal-outer and ventral-inner regions). Located dorsally, perpendicular to horizontal plan of the cardiac stomach, connected to pyloric ossicle (III) posteriorly and to urocardiac ossicle (VII) ventraly. Subterminal constriction short in *D. pagei* and *M. emarginatus*, and slightly longer in *D. septemdentatus* and *M. laevifrons*. Upper subtriangular region with three separated concavities; lower subtriangular region with three-star shaped concavity; concavities without communication in *D. pagei*, *D. septemdentatus*, and *M. laevrifrons*, and with communication in *M. emarginatus*. Dorsal edge more than twice as long as ventral edge. The pro-pyloric (VI) is easily distinguished by the median constriction and the concavities of the upper and lower subtriangular regions forming a peculiar asymmetrical "X".

Urocardiac ossicle (VII): unpaired, T-shaped, heavily calcified, located in dorsal part of the cardiac stomach. Completely fused to anterior margin mesocardiac (I). Posterior margin partially fused to pro-pyloric ossicle (VI). Dorsal surface deeply excavated. Ventral side without anterior subterminal bulge. Posterior region with four distinct teeth in *D. pagei* and in *D. septemdentatus*, but with three, partially fused, teeth in *M. laevifrons* and in *M. emarginatus*. Ventrolateral margin without median globular protuberance in *D. pagei*, *M. laevifrons*, and *M. emarginatus*, and with median globular protuberance in *D. semptemdentatus*. Posterior ventrolateral margin with 1 strong subterminal small, acute tooth in *D. pagei* and *M. emarginatus*, 2 strong and short teeth in *M. laevifrons*, and tooth absent in *D. septemdentatus*.

Dorsomedian cardiac plate (VIIa): paired, compressed, located near to posterior portion of urocardiac (VII); anterior region curved laterally, moderately calcified.

Dorsolateral cardiac plate (VIIb): paired, compressed, oblong, dorsally located in the cardiac stomach next to the urocardiac ossicle; devoid calcification.

Lateral supporting cardiac ossicles
(Figs. 1C, 2C, 3C, 4C)

Pectineal ossicle (VIII): paired, weakly calcified on mesial side, moderately calcified on lateral side. Situated laterally in the cardiac stomach, establishing connection between post-pectineal (X) + pre-pectineal ossicles (IX) and suprapectineal lateral ossicle (VIIIb), just below the zygocardiac ossicle. Its mesial portion comb-like, bearing a row of moderately calcified teeth: 4 transverse, spiniform teeth in *D. pagei* and *M. emarginatus*; 3 transverse, spiniform teeth in *D. septemdentatus*, and 1 spiniform tooth in *M. laevifrons*.

Suprapectineal lateral ossicle (VIIIa): paired, compressed, slightly calcified, located laterally in the cardiac stomach, between pectineal (VIII) and subdentate ossicles (XIV).

Pre-pectineal ossicle (IX): paired, heavily calcified, divided into two distinct portions. Anterior portion narrower than posterior portion, thin and subcylindrical, located laterally in the cardiac stomach and connected to the post-pterocardiac ossicle (IIb), Anterior portion is as long as the posterior portion in *D. pagei* and *M. laevifrons*; only one-third the length of the posterior portion in *M. emarginatus*, and almost half the length of the posterior portion in *D. septemdentatus*. Posterior portion connected to the pectineal ossicle (VIII), devoid of lateral process.

Post-pectineal ossicle (X): paired, heavily calcified, located laterally in the cardiac stomach. Mesial margin

with two blades moderately calcified; anterior and posterior blades partially fused to lateral posterior cardiac plate and lateral-inferior cardiac ossicle, respectively; anterior blade with double row of bristles, posterior plate devoid of bristles. Lateral margin subcylindrical and calcified. Distal process connected to pectineal ossicle (VIII) and projected anterolaterally, surpassing distal end of mesial plate.

Quill of the postpectineal ossicle (Xa): paired, heavily calcified, J-shaped, located in lower part of the cardiac stomach; anterior end with compressed, subtriangular process projected dorsally. Anterior portion loosely connected to lower cardiac valve and to lateral posterior cardiac plate; posterior portion subcylindrical, completely fused to post-pectineal ossicle (VIII). Quill of the postpectineal ossicle (Xa) forming obtuse angle with lateral margin of the post-pectineal ossicle (X).

Anterior lateral cardiac plate (XI): paired, moderately calcified, falciform, located in the anterior part of the cardiac sac. Anterior side devoid of longitudinal carina.

Posterior lateral cardiac plate (XII): paired, moderately calcified, subretangular. Lateral side slightly convex, with sinuous, oblique carina projected anterodorsally. Anterior margin tapering, projected dorsally; posterior margin slightly sinuous.

Inferior cardiac valve (XIIa): paired, moderately calcified, tapering, located at lateral part of bottom of cardiac stomach, loosely connected to posterior lateral cardiac plate (XII) and to quill of the postpectineal ossicle (Xa). Mesial part elongated, covered with tufts of long setae.

Inferior-lateral cardiac ossicle (XIII): paired, heavily calcified, located laterally in the cardiac stomach. Lower part with very long process, distinctly larger than width of the ossicle; posterior region strongly convex.

Subdentate ossicle (XIV): paired, heavily calcified, with conspicuous mesial bulge. Inferior part ending in two projections: anterior one acute, posterior one truncated, with clear semicircular mark in *D. septemdentatus* but inconspicuous in *D. pagei*, *M. laevifrons* and *M. emarginatus*. Inferior part of ossicle well tapered in *D. septemdentatus*, and a little tapered in *D. pagei*, *M. emarginatus* and *M. laevifrons*. Posterior margin without laminar expansion.

Lateral cardio-pyloric ossicle (XV): paired, short, curved, heavily calcified, completely fused to the lateral cardio-pyloric valve.

Ossicles of cardio-pyloric valve
(Figs. 1B, C, 2B, C, 3B, C, 4B, C)

Cardio-pyloric valve (vcp): unpaired, heavily calcified. Anterodorsal side without chitinous bristles, posterodorsal side with laminar process projected posteriorly, near fold of lateral ossicle cardio-pyloric valve (XVIII).

Anterior ossicle of cardio-pyloric valve (XVI): unpaired, lateral borders slightly calcified, located ventrally on the border between cardiac and pyloric stomachs, connected laterally to the inferior lateral cardiac ossicle (XIII) and posteriorly to the valve cardio-pyloric (v.c.p.). Posterior mesial part with heavily calcified, elongated process in *D. pagei*, and with short process in *D. septemdentatus*, *M. laevifrons* and *M. emarginatus*; mesial process straight, not bifurcated in any species.

Posterior ossicle of cardio-pyloric valve (XVII): unpaired, heavily calcified, as subcylindrical, transverse bar located immediately after the anterior ossicle of cardio-pyloric valve (XVI), completely fused to lateral ossicle of valve cardio-pyloric (XVIII). Ventral posterior border with 2 laminar processes (better observed in lateral view).

Lateral ossicle of cardio-pyloric valve (XVIII): paired, heavily calcified, located laterally between the cardiac and pyloric stomachs, connected anteriorly to the lateral cardio-pyloric ossicle (XV), and mesially to the cardio-pyloric valve (v.c.p.). Outline subtriangular; fully covering the laminar process of cardio-pyloric valve (v.c.p.) in lateral view.

Supporting ossicles of the dorsal pyloric chamber
(Figs. 1A, C, 2A, C, 3A, C, 4A, C)

Anterior mesopyloric ossicle (XIX): paired, heavily calcified, located dorsally in the pyloric chamber, connected anteriorly to pyloric ossicle (III), laterally to lateral mesopyloric (XIXa) and posterior mesopyloric ossicles (XX). Lateral process tapering, distinctly developed. In dorsal view, it is has a piriform shape in *D. pagei* and *D. septemdentatus,* but elongated in *M. laevifrons* and *M. emarginatus*.

Lateral mesopyloric ossicle (XIXa): paired, mildly calcified, compressed dorsoventrally, slightly concave dorsally, moderately sinuous, located laterally in the upper portion of the pyloric chamber, next to the anterior mesopyloric ossicle (XIX).

Posterior mesopyloric ossicle (XX): paired, heavily calcified, semicircular; dorsolateral face with a crest. Total length of posterior mesopyloric ossicle measures half of the total length of anterior mesopyloric ossicle (XIX) in *D. pagei* and *M. emarginatus*, one third of the length in *M. laevifrons*, and approximately the same length of the anterior mesopyloric ossicle (XIX) in *D. septemdentatus*.

Uropyloric ossicle (XXI): unpaired, arcuate, strongly calcified, located in the dorsal posterior part of the cardiac chamber, connected anteriorly to the posterior mesopyloric ossicle (XIX). Posterior lateral angles connected to infra-uropyloric fragment (XXIa) and posterior uropyloric ossicle (XXIb). Anterior mesial portion convex; posterior mesial portion concave. Anterolateral borders convex, moderately calcified. Posterior region devoid of calcified expansion.

Infra-uropyloric fragment (XXIa): paired, compressed, moderately calcified, less than 0.5 time as long as uropyloric ossicle, shaped as elongate strip located in dorsal posterior part of pyloric stomach next to the uropyloric ossicle (XXI)

Posterior uropyloric ossicle (XXIb): paired, moderately calcified, concave dorso-laterally, located posteriorly and inferiorly to the uropyloric ossicle (XXI). Posterior part uniramous.

Supporting ossicles of the ventral pylorus and ampullae (Figs. 1B, C, 2B, C, 3B, C, 4B, C)

Preampullary ossicle (XXII): paired, slightly calcified, compressed, arrowhead-shaped, located ventrally between the posterior ossicles of cardio-pyloric valve (XII), inferior ampullary ossicle (XXIV) and median supra-ampullary (XXIX), and connected to these ossicles.

Anterior inferior pyloric ossicle (XXIII): unpaired, heavily calcified, trapezoidal, located ventrally between cardio-pyloric valve and ampullae. Margin of anterior portion flat; margin of posterior portion convex.

Inferior ampullary ossicle (XXIV): paired, compressed, heavily calcified, subcircular, convex ventrally, concave dorsally. Borders of mesial region of ampullae dorsally elevated, forming longitudinal fold (= inter-ampullary fold). Terminal portion of inter-ampullary fold ending in acute projection covered with bristles.

Ampullary roof-lower portion ossicle (XXV): paired, compressed, moderately calcified, concave mesially, located in dorsolateral part of ampullary filter. Lateral side with distinct longitudinal ridge.

Ampullary roof-mediun portion ossicle (XXVa): paired, slightly calcified, flat, elipsoid, located between the upper and lower parts of the ampullary roof.

Ampullary roof-upper portion ossicle (XXVI): paired, moderately calcified, concave, oblong, located obliquely in the upper part of the ampullary roof.

Process of the ampullary roof-upper portion (XXVIa): paired, heavily calcified, markedly curved and located laterally in pyloric chamber, connected to the following ossicles: anterior pleuro-pyloric (XXXI) (strong connection), pleuro-pyloric valve (XXXIa) (partially fused), and the ampullary roof-upper portion ossicle (XXVI) (partially fused).

Posterior-inferior pyloric ossicle (XXVII): unpaired, mesially arched, strongly calcified in middle part, moderately so near borders, located in lower end part of pyloric stomach after the ampullae, connected anteriorly to inferior ampullary ossicle (XXIV) and posteriorly to posterior supra-ampullary ossicle (XXX). Lateral borders recurved lateroposteriorly. Maximum width of the posterior-inferior pyloric ossicle (XXVII) as wide as 0.5 time the maximum width of the ampullae.

Lateral inferior post-ampullary plate (XXVIIa): paired, compressed, subtriangular, mildly calcified, located immediately after posterior-inferior ampullary ossicle.

Supra-ampullary supporting ossicles (Figs. 1B, C, 2B, C, 3B, C, 4B, C)

Anterior supra-ampullary ossicle (XXVIII): paired, moderately calcified, narrower and shallower anteriorly, compressed, located vertically on lateral pyloric stomach, behind upper region of lateral ossicle of cardio-pyloric valve. Mesiodorsal margin with well-calcified crest widening medially, near connection with anterior pleuro-pyloric ossicle.

Middle supra-ampullary ossicle (XXIX): paired, situated obliquely in lateral portion of pyloric stomach between anterior supra-ampullary ossicle, posterior cardio-pyloric valve (XVII), and ampullary roof-lower portion ossicle (XVI). Anterolateral portion as

heavily calcified bar, posterior portion laminated with a terminal process.

Posterior supra-ampullay ossicle (XXX): paired, heavily calcified, leaf shaped, located vertically in posterolateral portion of pyloric stomach, after ampullae and above inferior-posterior pyloric ossicle; upper portion fully fused to posterior pleuropyloric ossicle, lower part firmly attached to inferior-posterior pyloric ossicle. Upper part with laminated process projected laterally.

Supporting ossicles of the lateral pylorus (Figs. 1C, 2C, 3C, 4C)

Anterior pleuro-pyloric ossicle (XXXI): paired, heavily calcified, long and arched, positioned vertically beside postero-lateral part of pyloric stomach. Antero-ventral part wider than postero-dorsal, connected to anterior supra-ampullary (XXVIII) and to process of the ampullary roof-upper part (XXVIa); postero-dorsal part connected to posterior mesopyloric ossicle (XX) and to uropyloric ossicle (XXI). Anterior expansion slightly calcified; lateral side with distinctly expanded crest.

Pleuro-pyloric valve's ossicle (XXXIa): paired, anterior part heavily calcified (bar-shaped) and greatly reduced; posterior part expanded as slightly a calcified, translucent membrane, located laterally in pyloric stomach, just after anterior pleuro-pyloric ossicle (XXXI).

Median pleuro-pyloric ossicle (XXXII): paired, compressed, elongated, heavily calcified, dorsally arched, concave laterally, located horizontally on lateral side of pyloric stomach, just above ampullary roof-lower part ossicle (XXV), connected anteriorly and ventrally with process of ampullary roof-lower part ossicle (XXVI), and posteriorly with posterior pleuro-pyloric ossicle (XXXIII).

Lateral pleuro-pyloric plate (XXXIIa): paired, compressed, slightly calcified, located loosely on lateral portion of pyloric stomach, above median pleuro-pyloric ossicle (XXXII).

Posterior pleuro-pyloric ossicle (XXXIII): paired, divided into upper and lower parts: lower part heavily calcified, extended dorsally and beyond posterior supra-ampullary ossicle (XXX), and fully fused with both posterior supra-ampullary ossicle (XXX) and rear upper corner of median pleuro-pyloric ossicle (XXXII); upper part slightly calcified, flatted, with distinct lateral projection.

Discussion

The stomach is composed of seven sets of ossicles that together form 48 ossicles, but the early authors who studied this system did not recognize all of these ossicles (Mocquard, 1883; Cochran, 1935; Maynard and Dando, 1974; Meiss and Norman, 1977; Brösing *et al.*, 2002). Since the studies of Mocquard (1883) only the mesocardiac, exopyloric, pro-pyloric, zygocardiac, and urocardic ossicles have been frequently mentioned. The definition of the remaining ossicles that constitute the foregut ossicles varied depending of each author and, therefore, the nomenclature used in early studies had to be modified. The incorrect identification of the ossicles and the proposals of new ossicles without justification can cause instability in the nomenclature and thus generate difficulties in comparing the results obtained by different authors (Lima, 2010).

Most of the ossicles are very similar in the four species studied. The shape of the ossicles did not vary either in relation to the stage of development or the sex of the individuals, nor did we observe a variation in the number of ossicles in younger individuals, in the same way as in the studies of Lima-Gomes (2013) with Trichodactylidae species. Nevertheless, in some cases the ossicles of young specimens, regardless of sex, were more decalcified than in the adults, but still conspicuous, such as the pyloric ossicles, the anterior inferior-pyloric ossicle and some plaques. This is also the case for *D. septemdentatus*, thus differing from the results of Alves *et al.* (2010), who only recognized 37 ossicles.

According to Mocquard (1883), the pyloric ossicle is an unpaired ossicle, presenting a membranous longitudinal fissure, but Cochran (1935) described it as a paired ossicle. Maynard and Dando (1974), studying lobsters (*Panulirus* White, 1847 and *Homarus* Weber, 1795) and crabs (*Callinectes* Stimpson, 1860), referred to the pyloric ossicle as being an unpaired and paired ossicle, respectively, depending upon the animal under study. In the present study, we considered that the pyloric ossicle is paired because it had a membranous fissure, which would lead to a complete separation of the ossicles (Figs. 1A, 2A, 3A, 4A). The ossicle differed only in relation to the apex of the anterolateral angle among the four species studied, being conspicuous in *D. pagei* and *M. laevifrons*, and inconspicuous in *D. septemdentatus* and M. *emarginatus*.

The dorsomedian and dorsolateral cardiac plates were not mentioned in the studies of Mocquard (1883), Brösing (2010), or Alves *et al.* (2010). In the study of Lima (2010) these plates were observed with distinct calcification in some species of *Uca* Leach, 1814 and *Ucides* Rathbun, 1897 and, according to the author, were recognizable in all the stomachs analyzed, but only slightly calcified. Thus, Lima (2010) considered these ossicles as new. Lima-Gomes *et al.* (2017) also recognized these plates in *V. serrata* and *S. pictus* and, similarly, we observed them in all four species studied herein. Nevertheless, Alves *et al.* (2010) did not recognize these plates in *D. septemdentatus*.

The suprapectineal lateral ossicle was not mentioned by Mocquard (1883), Cochran (1935), Patwardhan (1935), Maynard and Dando (1974), and Brösing *et al.* (2002). Lima (2010), in his study of the foregut ossicles of the Gecarcinidae, recognized this ossicle in all species, but always with low calcification, thus considering it as new. We also recognized this ossicle in the four species of this study (Figs. 1C, 2C, 3C, 4C).

The lateral cardio-pyloric ossicle is known since H. Milne Edwards (1834). Mocquard (1883) also observed it in several groups but did not mention it when it was completely fused to the lateral cardio-pyloric valve, considering only the description of the lateral ossicle of the cardio-pyloric valve. In the present study, it was possible to identify both ossicles, which presented as a fused form in the four species (Figs. 1C, 2C, 3C, 4C). Lima-Gomes *et al.* (2017) observed this ossicle to be partially fused in *V. serrata* and *S. pictus*.

The infra-uropyloric fragment was firstly proposed by Maynard and Dando (1974) when they reviewed the study by Cochran (1935) on the skeletal structure of the stomach of *Callinectes sapidus* Rathbun, 1896. More recently, Brösing *et al.* (2002) recognized this ossicle in species that had already been studied by Mocquard (1883) and Meiss and Norman (1977). Furthermore, Brösing *et al.* (2002) proposed two new ossicles: lateral mesopyloric and posterior uropilic ossicles. Brösing (2010) reported the absence of these ossicles in *Ocypode gaudichaudii* H. Milne Edwards and Lucas, 1843 and *O. cursor* (Linnaeus, 1752). Lima (2010) recognized these ossicles in *Ocypode quadrata* (Fabricius, 1787), but as slightly calcified and considered them as new. Thus, it is probable that the lateral mesopyloric and posterior uropyloric ossicles are indeed present in *O. gaudichaudii* and *O. cursor* and possibly due to their low degree of calcification Brösing (2010) did not regonize them. Lima-Gomes *et al.* (2017) also recognized these ossicles in *V. serrata* and *S. pictus*. These two ossicles that were proposed as new by Lima (2010) are clearly visible in the four species analyzed in this study (Figs. 1C, 4C).

Maynard and Dando (1974) proposed the division of the ossicle of the ampullary roof into two parts: the ampullary roof-lower portion ossicle and the ampullary roof-upper portion ossicle. After a time being considered as two ossicles, a third ossicle was recognized by Lima (2010) and was called ampullary roof-median portion ossicle. The proposal of this ossicle as new was based on the frequency in which this ossicle was found to be moderately calcified and was observed by Lima (2010) in *Cardisoma* Latreille in Latreille, Le Peletier, Serville and Guérin, 1828, *Discoplax* A. Milne-Edwards, 1867, *Ucides* and others. Lima-Gomes *et al.* (2017) also adopted the proposed division of the ossicles into three portions in their study. This ossicle was present in all species studied by us (Figs. 1C, 2C, 3C, 4C).

Lima (2010) observed and proposed as new, the lateral-inferior post-ampullary plate. Lima-Gomes *et al.* (2017) also recognized it in *V. serrata* and *S. pictus*, although always as a slightly calcified piece. This plate is also present in the species studied herein, and always slightly calcificated (Figs. 1C, 3C, 4C).

The ossicles of the crustacean stomachs, in addition to offering a possible indication on the feeding ecology of a species, might provide valuable information on the phylogenetic relationships of a group (Patwardhan, 1935; Brösing *et al.* 2002; Brösing *et al.*, 2007; Lima, 2010; Olguín, 2016). This, however, may not be the case for the trichodactylid crabs, at least among representatives of the Dilocarcinini (this study) and Valdiviini (see Lima-Gomes *et al.*, 2017).

Our results show that the morphological differences among most of the ossicles are very succinct between species, not showing consistent dissimilarities within the same genus, but sometimes being similar between two species of different genera. This is the case, for instance, of the mesocardiac ossicle (I) and the pyloric ossicle (III), which are similar between *D. pagei* and *M. laevifrons*, and between *D. septemdentatus* and *M. emarginatus*, but dissimilar between the species of the same genus. On the other hand, the zygocardiac ossicle (V) has features that are similar between the species of the same genus (*D. pagei* and *D. septemdentatus* bearing dorso-lateral face with oblique carina), and dissimilar across the genera (*M.*

emarginatus and *M. laevifrons* bearing dorso-lateral face devoid of carina). However, this pattern does not hold for another feature of this very same ossicle: the mesial end teeth are separate in *D. pagei*, *D. septemdentatus*, and *M. laevifrons*, but fused in *M. emarginatus*. Other examples of such inconsistent patterns can be found in the morphological description of the ossicles above.

Therefore, the overall resemblance of the ossicles of the stomachs among the species of Dilocarcinini studied herein and those of Valdiviini (see Lima-Gomes *et al.*, 2017) does not seem to provide sufficient support to consider the ossicles as good characters for phylogenetic studies of the Trichodactylidae, since these similarities would generate many homoplasies. Although these characters were useful for corroborating the monophyly of Gecarcinidae (see Lima, 2010) and Lithodidae (see Olguin, 2016), they may not be as useful for separating groups within the trichodactylid crabs. However, a more comprehensive study that includes representatives of the other genera of both subfamilies remains necessary for a conclusive evaluation.

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