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RESEARCH ARTICLE

Description of ten additional ossicles in the foregut of the freshwater crabs *Sylviocarcinus pictus* and *Valdivia serrata* (Decapoda: Trichodactylidae)

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ABSTRACT. The morphology of stomach ossicles of decapod crustaceans provides valuable information on their phylogeny and biology. We herein described ten new ossicles in the foreguts of two trichodactylid crabs, *Sylviocarcinus pictus* (H. Milne-Edwards, 1853) and *Valdivia serrata* White, 1847, in addition to previously described 38 ossicles, which are also recognized and listed. Five specimens each of *S. pictus* and *V. serrata* were selected for morphological analysis of gastric ossicles. The stomachs were obtained after removing the carapace, and they were fixed in 10% formalin for 24 hours. After this procedure, the stomachs were immersed in a solution of 10% Potassium Hydroxide (KOH) and heated to 100 °C during 60 minutes for tissue maceration. At this point, the clean skeletons were colored by adding 1% Alizarin Red to the KOH solution in order to facilitate visualization of the internal structures such as the setae and ossicles. The ten new ossicles are: dorsomedial cardiac plate; dorsolateral cardiac plate; suprapectineal lateral ossicle; inferior cardiac valve; lateral mesopyloric ossicle; ampullary roof-medium portion ossicle; process of the ampullary roof-upper portion; lateral-inferior post-ampullary plate; pleuro-pyloric valve's ossicle; and lateral pleuro-pyloric plate. Some ossicles are thin plates that together with the main ossicles assist in the structure and support of the stomach, which are similar in the two species studied herein. The current knowledge on gastric ossicles will be useful in establishing taxonomic characters, which can evaluate phylogenetic relationships among brachyuran crabs.

KEY WORDS. Amazon, anatomy, foregut, morphology, Neotropical, stomach, Valdiviini.

INTRODUCTION

The stomach of decapod crustaceans is composed of a muscular and nervous complex called gastric mill (Meiss and Norman 1977), where a system of striated muscles performs movements of skeletal elements that work together to break and grind large particles of food in the cardiac chamber. The main skeletal elements consist of the following ossicles: mesocardiac, pterocardiac, pyloric, exopyloric, zygocardiac (that supports the lateral teeth), propyloric and urocardiac (that supports the medial tooth) (Factor 1989). In addition to the support of the gastric skeleton, the ossicles assist in crushing and filtration activities during the feeding process. Many studies have been undertaken to understand how this complex operates in different decapods since the gastric skeleton can provide valuable information on their phylogeny and biology, especially their feeding habits (Felgenhauer and Abele 1983, 1985, Brösing et al. 2002, Abrunhosa et al. 2003, Brösing et al. 2006, Brösing 2010, Alves et al. 2010). The morphology of stomach ossicles can also be an important source for taxonomic characters and is potentially useful for studying phylogenetic relationships in different groups of decapods (Sakai 2005, Sakai et al. 2006, Naderloo et al. 2010, Brösing and Türkay 2011).

Studies on the gastric skeleton of Amazonian decapods are very recent, and only a few have been developed for brackish and freshwater decapods. Alves et al. (2010) published the only

study for the Trichodactylidae. They described 38 ossicles for *Valdivia serrata* White, 1847 and *Sylviocarcinus pictus* (H. Milne Edwards, 1853) (Valdiviini) and 37 ossicles for *Dilocarcinus septemdentatus* (Herbst, 1783) (Dilocarcinini). A total of 48 ossicles have been recognized in Gecarcinidae members (Jô de Farias Lima, unpublished data). In the present work, we describe ten additional ossicles found in the foreguts of *S. pictus* and *V. serrata*, as this complementary information may be useful for studies on the trophic ecology and phylogenic relationships of the trichodactylid crabs.

MATERIAL AND METHODS

Uncatalogued specimens of *S. pictus* and *V. serrata* were obtained from the crustacean collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil. The stomachs of five specimens of each species were analyzed: four males and a female of *S. pictus*, and two males and three females of *V. serrata*. The stomachs of crabs do not differ in regard to gender and so did not matter the number of each gender. The stomachs were obtained after carapace removal and fixed in 10% formalin for 24 hours. For tissue maceration, the stomachs were cooked for 60 minutes in 10% Potassium Hydroxide (KOH) solution and heated to 100 °C (Mocquard 1883, Brösing et al. 2002). The cleaned skeleton was then colored by adding 1% Alizarin Red to the KOH solution to facilitate visualization of the internal structures such as the setae and ossicles (Brösing et al. 2002).

The nomenclature and abbreviations used in the morphological description of the gastric skeleton followed Alves et al. (2010); the degree of calcification of the ossicles was described as: (I) mild calcification (membranous aspect); (II) moderate calcification (cartilaginous aspect); (III) strong calcification (opaque aspect); (IV) free ossicle (when connected to adjacent ossicle by thin and pliable membrane); (V) partially fused ossicles (when connected by rigid membrane cartilaginous aspect or clearly incomplete fusion); and (VI) fused ossicles (indistinct separation) (Jô de Farias Lima, unpublished data). The roman numerals in the table and figures refer to ossicles described by Alves et al. (2010) as well as the ossicles presented herein. The complete list of the names and abbreviations of all the described ossicles is in Table 1.

RESULTS

All the 38 ossicles previously described by Alves et al. (2010) were recognized and listed in Table 1, in addition to the ten new ossicles indentified in the present study. Some are thin plates that together with the main ossicles assist in the structure and support of the stomach.

The stomach ossicles are grouped according to the following regions of the gastric skeleton: 10 ossicles of the gastric mill; 11 ossicles of the lateral supporting cardiac region; 4 ossicles of the cardio-pyloric valve; 6 ossicles supporting the dorsal pyloric stomach; 9 ossicles supporting the ventral pylorus and bulb; 3 ossicles supporting the supra-ampullary region; and 5 ossicles supporting the lateral pylorus region (Table 1). In total, the gastric skeleton consists of 48 ossicles, which are similar in the two species studied herein (Figs 1–8).

The ten new ossicles are described below and illustrated in Figs 1–9.

Ossicles of the gastric mill: Dorsomedial cardiac plate (VIIa) (Figs 3, 4, 7–9) – paired, compressed, devoid of calcification, located close to the posterior portion of the urocardiac, anterior region curved laterally.

Dorsolateral cardiac plate (VIIb) (Figs 3, 4, 7, 8 and 10) – paired, compressed, mildly calcified, oblong, dorsally located in the cardiac stomach next to the urocardiac ossicle.

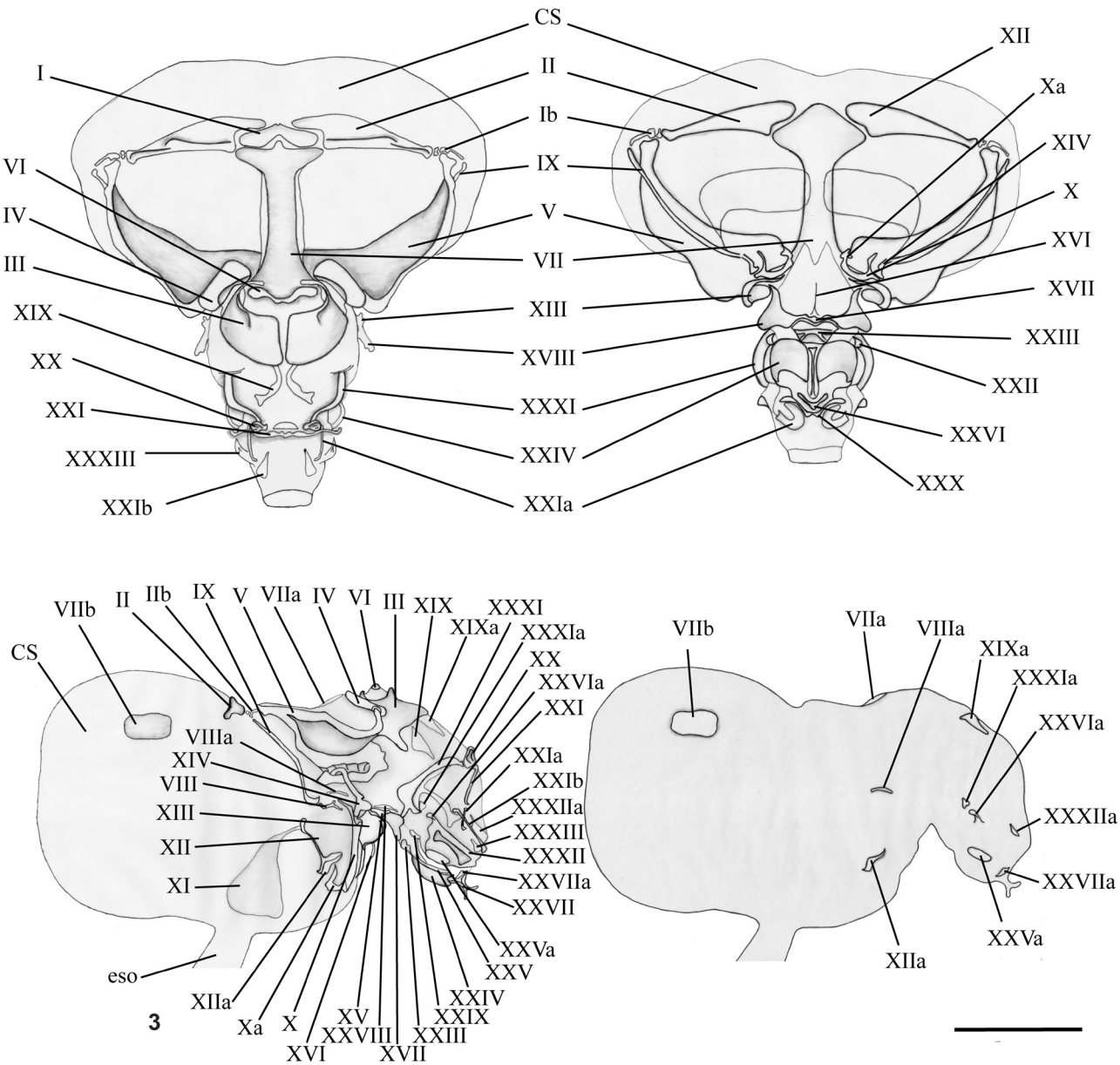
Lateral supporting cardiac ossicles: Suprapectineal lateral ossicle (VIIIa) (Figs 3, 4, 7, 8 and 11) – paired, compressed, mildly calcified, located laterally in the cardiac stomach, between the pectineal and subdentate ossicles. Inferior cardiac valve (XIIa) (Figs 3, 4, 7, 8 and 16) – paired, moderately calcified, tapering, located in the bottom lateral portion of the cardiac stomach, loosely connected to the posterior cardiac plate and to the post-pectineal keel ossicle. Mesial portion elongated, covered with tufts of long setae.

Supporting ossicles of the dorsal pyloric chamber: Lateral mesopyloric ossicle (XIXa) (Figs 3, 4, 7, 8 and 12) – paired, moderately calcified, compressed dorsoventrally, slightly concave dorsally, moderately sinuous, located laterally in the upper portion of the pyloric chamber, next to the anterior mesopyloric ossicle.

Supporting ossicles of the ventral pylorus and ampullae: Ampullary roof-mediun portion ossicle (XXVa) (Figs 3, 4, 7, 8 and 17) – paired, mildly calcified, flat, elipsoid, located between the upper and lower portions of the ampullary roof. Process of the ampullary roof-upper portion (XXVIa) (Figs 3, 4, 7, 8 and 17) – unpaired, heavily calcified consisting of two subtriangular portions obliquely positioned in relation to each other, and located laterally in the pyloric chamber, connected to the following ossicles: anterior pleuro-pyloric (strong connection), pleuro-pyloric valve (partially fused), and the upper ampullary roof (partially fused).

Lateral-inferior post-ampullary plate (XXVIIa) (Figs 3, 4, 7, 8 and 14) – paired, compressed, mildly calcified, subtriangular, located immediately adjacent to the inferior ampullary ossicle.

Supporting ossicles of the lateral pylorus: Pleuro-pyloric valve's ossicle (XXXIa) (Figs 3, 4, 7, 8 and 13) – paired, anterior portion heavily calcified (bar-shaped) and greatly reduced; posterior portion expanded as mildly calcified, translucent membrane, located laterally in the pyloric stomach, just adjacent to the anterior pleuro-pyloric ossicle. Lateral pleuro-pyloric plate (XXXIIa) (Figs 3, 4, 7, 8 and 15) – paired, compressed, mildly calcified, located on the lateral portion of the stomach pyloric, above the median pleuro-pyloric ossicle.

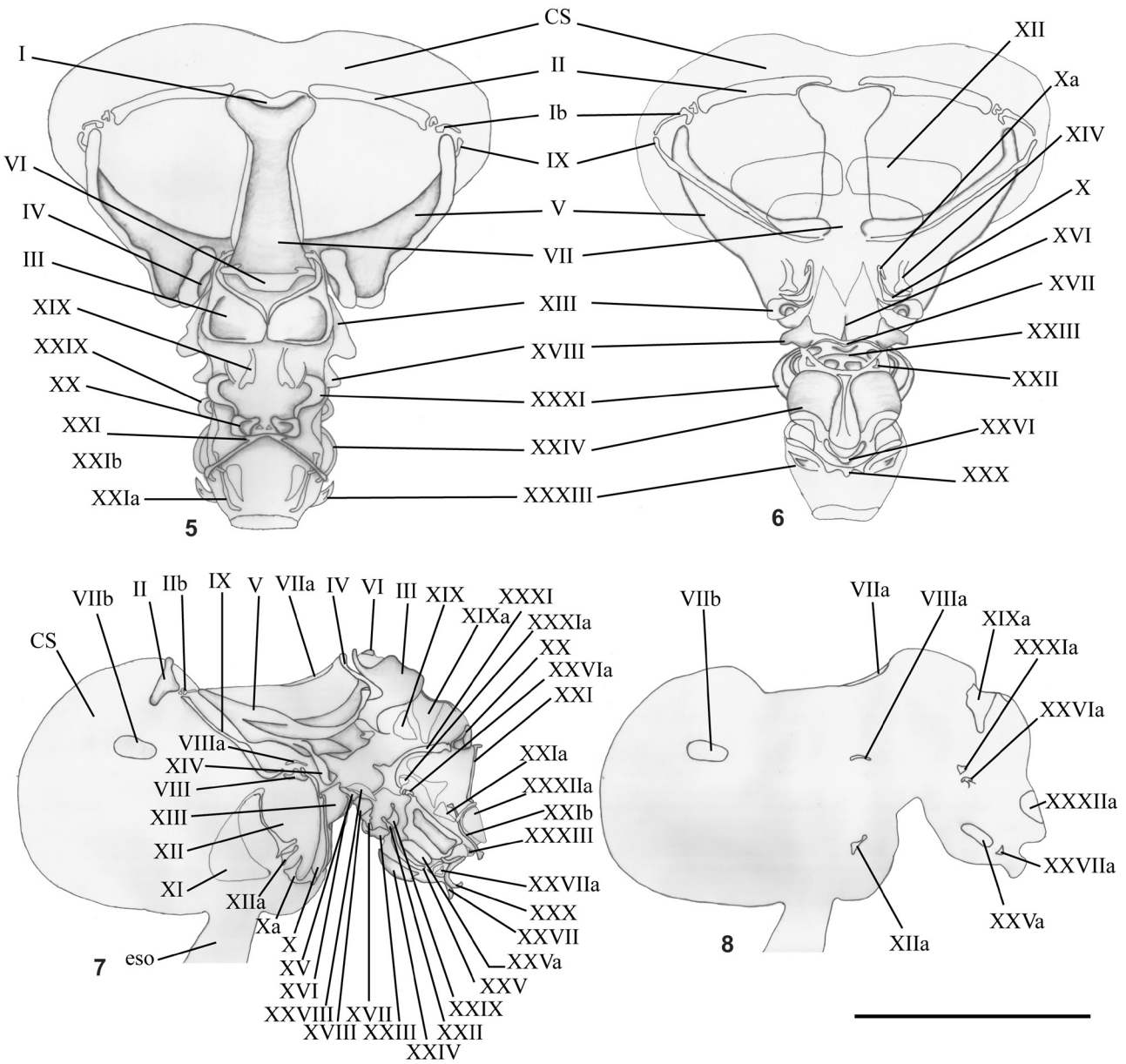


Figures 1–4. Foregut of *Sylviocarcinus pictus*: (1) dorsal view; (2) ventral view; (3) lateral view; (4) lateral view with the additional ossicles found in the present study. (CS) Cardiac sac, (eso) esophagus. Scale bar = 5 mm.

DISCUSSION

We recognized 48 ossicles in the gastric skeletons of *S. pictus* and *V. serrata* whereas Alves et al. (2010) recognized 38 ossicles for the same species (Table 1). This discrepancy may be due to methodological issues or interpretation, as discussed below.

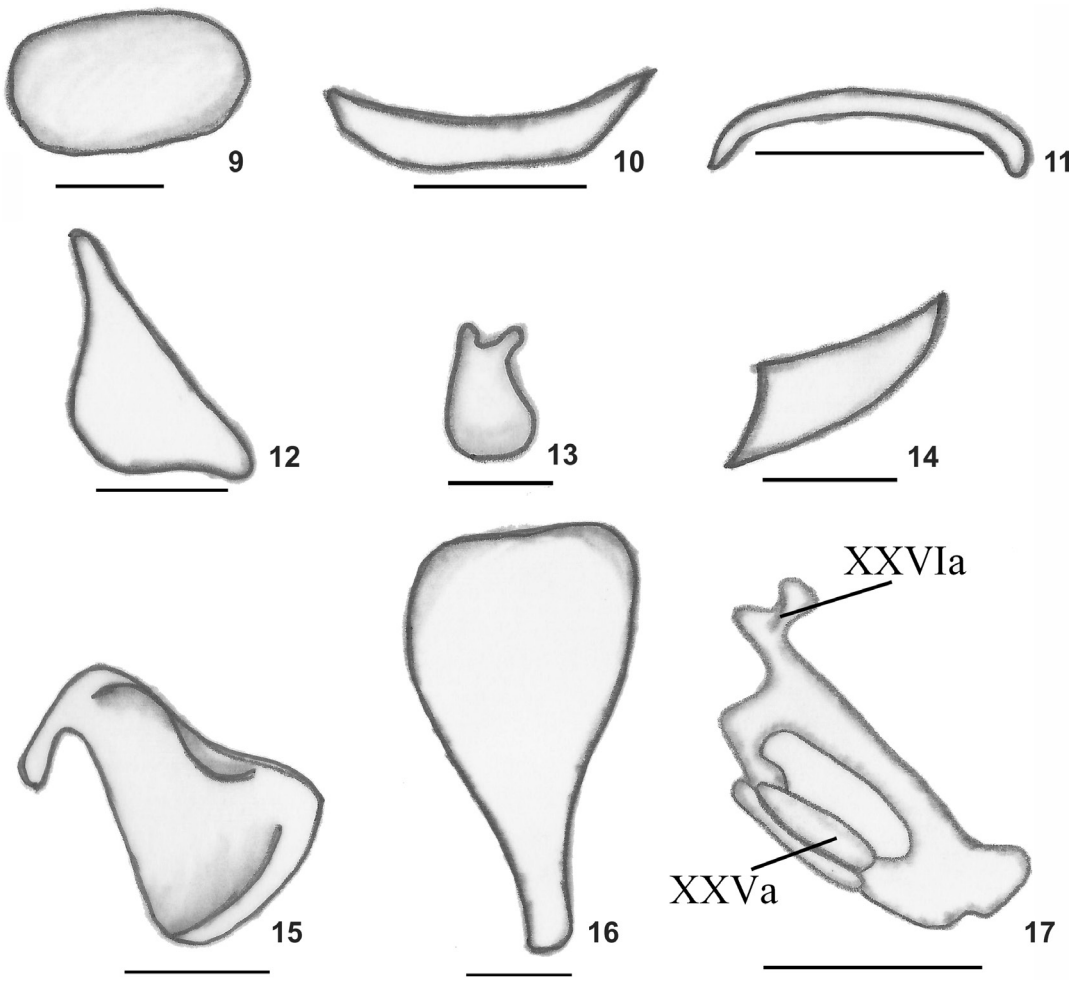
The 'dorsomedial cardiac plate' and the 'dorsolateral cardiac plate', the 'lateral inferior post-ampullary plate' and the 'lateral pleuro-pyloric plate' ossicles were not mentioned by Alves et al. (2010). These structures are very thin due to their low degree of calcification, which makes their visualization and identification somewhat difficult, especially if the cooking time is exceeded, the KOH concentration is higher than the suggested method, or the stomach is not properly colored during the preparation process. The 'lateral supra-pectineal ossicle' was also not described by Alves et al. (2010); this ossicle probably



Figures 5–8. Foregut of *Valdivia serrata*: (5) dorsal view; (6) ventral view (7) lateral view; (8) lateral view with the additional ossicles found in the present study. (CS) cardiac sac, (eso) esophagus. Scale bar = 5 mm.

went unnoticed because it is mildly calcified and translucent. In addition, some ossicles are minuscule, which could have led to them being interpreted as a single entity, rather than individualized structures. This might have been the case for the 'ampullary roof-lower portion ossicle', 'ampullary roof-median portion ossicle' and 'lateral supra-pectineal ossicle'.

The 'lateral mesopyloric ossicle' was first recognized by Brösing et al. (2002) who proposed it as a new ossicle to the foregut ossicle-system of *Dromia wilsoni*, *Dromia personata* and *Lauridormis intermedia*. However, Brösing (2010) reported the absence of this ossicle in *Ocypode gaudichaudi* (H. Milne-Edwards & Lucas, 1843) and *O. cursor* (Linnaeus, 1758). This ossicle was recognized as a poorly calcified structure in *Ocypode quadrata* (Fabricius, 1787) and treated as new (Jô de Farias Lima, unpublished data). Thus, the 'lateral mesopyloric' is most probably present in *O. gaudichaudi* and *O. cursor* as well. Perhaps its low degree



Figures 9–17. The ten additional ossicles of the foregut of *Sylviocarcinus pictus* and *Valdivia serrata*, found in the present study and not previously recognized by Alves et al. (2010): (9) dorsolateral cardiac plate (VIIb); (10) dorsomedial cardiac plate (VIIa); (11) suprapectineal lateral ossicle (VIIIa); (12) lateral mesopyloric ossicle (XIXa); (13) pleuro-pyloric valve's ossicle (XXXIa); (14) lateral-inferior post-ampullary plate (XXVIIa); (15) lateral pleuro-pyloric plate (XXXIIa); (16) inferior cardiac valve (XIIa); (17) process of the ampullary roof-upper portion (XXVIa), ampullary roof-mediun portion ossicle (XXVa) Scale bars: 9-15, 17 = 2 mm, 16 = 1 mm.

of calcification precluded it from being recognized by Brösing (2010). A similar situation could also have occurred during the study of Alves et al. (2010) in which poorly calcified ossicles might have gone unnoticed and hence were not mentioned.

The 'inferior cardiac valve' looks like a very small ossicle from an external perspective coming from the outer side of the stomach, but it is quite distinct and easily recognizable from a point of view coming from the inner surface of the stomach. However, if a disruption of the heart sac occurs during dissection of the stomach, this structure can be lost.

The 'ossicles of the ampullary roof-mediun portion ossicle', the 'ampullary roof-upper portion ossicle', and the 'pleuro-pyloric valve's ossicle', may have been overlooked because they are fused to each other, appearing to be a single piece. The 'ampullary roof-mediun portion ossicle' may have been confused or even not noticed because they are very small and are situated between the 'ampullary roof-upper portion ossicle' and 'ampullary roof-lower portion ossicle'.

A careful recognition of these stomach ossicles and enhanced understanding of the gastric mill complex can provide useful information to establish relevant characters which in turn will be helpful for tracing affinities and evaluating phylogenetic relationships not only in trichodactylid crabs but also among other taxa of Eubrachyura.

Table 1. Nomenclatures and abbreviations used in morphological descriptions ossicles of the stomachs of *Sylviocarcinus pictus* and *Valdivia serrata*, and the comparison between the study of Alves et al. (2010) and the present paper.

Name of the ossicles	Number assigned to the ossicles	Alves et al. (2010)	Present paper
Ossicles of the gastric mill			
Mesocardiac ossicle	(I)	X	X
Pterocardiac ossicle	(II)	X	X
Post-pterocardiac ossicle	(IIb)	X	X
Pyloric ossicle	(III)	X	X
Exopyloric ossicle	(IV)	X	X
Zygocardiac ossicle	(V)	X	X
Propyloric ossicle	(VI)	X	X
Urocardiac ossicle	(VII)	X	X
Pectineal ossicle	(VIII)	X	X
Dorsomedian cardiac plate	(VIIa)	–	X
Dorsolateral cardiac plate	(VIIb)	–	X
Lateral supporting cardiac ossicles			
Suprapectineal lateral ossicle	(VIIIa)	–	X
Prepectineal ossicle	(IX)	X	X
Postpectineal ossicle	(X)	X	X
Quill of the postpectineal ossicle	(Xa)	–	X
Anterior lateral cardiac plate	(XI)	X	X
Posterior lateral cardiac plate	(XII)	X	X
Inferior cardiac valve	(XIIa)	–	X
Inferior lateral cardiac	(XIII)	X	X
Subdentate	(XIV)	X	X
Lateral cardiac-pyloric ossicle	(XV)	X	X
Ossicles of the cardio-pyloric valve			
Anterior ossicle of the cardio-pyloric valve	(XVI)	X	X
Posterior lateral cardiac plate	(XVII)	X	X
Lateral ossicle of the cardio-pyloric valve	(XVIII)	X	X
Supporting ossicles of the dorsal pyloric chamber			
Anterior mesopyloric ossicle	(XIX)	X	X
Lateral mesopyloric ossicle	(XIXa)	–	X
Posterior mesopyloric ossicle	(XX)	X	X
Uropyloric ossicle	(XXI)	X	X
Infra-uropyloric fragment	(XXIa)	X	X
Posterior uropyloric ossicle	(XXIb)	X	X
Supporting ossicles of the ventral pylorus and ampullae			
Preampullary ossicle	(XXII)	X	X
Anterior inferior pyloric ossicle	(XXIII)	X	X
Inferior ampullary ossicle	(XXIV)	X	X
Ampullary roof ossicle, lower portion	(XXV)	X	X
Ampullary roof-mediun portion ossicle	(XXVa)	–	X
Ampullary roof ossicle, upper portion	(XXVI)	X	X
Process of the ampullary roof-upper portion	(XXVIa)	–	X
Posterior inferior pyloric ossicle	(XXVII)	X	X
Lateral-inferior post-ampullary plate	(XXVIIa)	–	X
Supporting ossicles of the supra-ampullary			
Anterior supra-ampullary ossicle	(XXVIII)	X	X
Middle supra-ampullary ossicle	(XXIX)	X	X
Posterior supra-ampullary ossicle	(XXX)	X	X
Supporting ossicles of the lateral pylorus			
Anterior pleuropyloric ossicle	(XXXI)	X	X
Pleuro-pyloric valve's ossicle	(XXXIa)	–	X
Middle pleuropyloric ossicle	(XXXII)	X	X
Lateral pleuro-pyloric plate	(XXXIIa)	–	X
Posterior pleuropyloric ossicle	(XXXIII)	X	X
Cardiac-pyloric valve (v.c.p.)	(v.c.p.)	X	X

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