# Evolution of carnivorous gastropods of the family Costellariidae (Neogastropoda) in the framework of molecular phylogeny

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ABSTRACT. On the basis of the molecular sequences of 12S r-RNA gene of 16 species of Costellariidae the phylogenetic hypothesis is proposed. The anatomy of the foregut and radula of 15 species of the family was studied. The anterior foregut is the most rich in phylogenetically informative characters in Costellariidae and Neogastropoda in general. 6 types of anatomy of the mid-oesophagus have been recognized. They differ in the anatomy of the gland of Leiblein and in the presence/absence and state of development of the glandular tube, formed by stripping off and closure of the glandular folds of mid-oesophagus. On the basis of molecular phylogeny it was demonstrated that the most basal underived type of anterior foregut was found in still unnamed species of the family. It is characterized by unmodified gland of Leiblein opening directly into glandular mid-oesophagus. It is proved that the process of the formation of the glandular tube and transformation of the gland of Leiblein into muscular organ occurred in Costellariidae independently from other lineages of Neogastropoda. The functional morphology of the digestive system is discussed. The most probable source of toxins in costellariids is despite expectations not the gland of Leiblein, but accessory or primary salivary glands.

The family Costellariidae MacDonald, 1860 (= Vexillidae Thiele, 1929) is a large family of tropical Neogastropods, encompassing about 320 Recent species [Robin, Martin, 2004]. The group is relatively well defined conchologically, and is characterized by elongated fusiform shell with strong columellar folds and with normally well developed axial ribs (*"costae"*), that gave the name to the family.

Being popular group among shell collectors, the family attains significant interest from conchological point of view. The latest most comprehensive guide to the family (as well as to the family Mitridae) was published in 2004 [Robin, Martin, 2004], there is a WEB cite dedicated exclusively to "Mitroidea", including Costellariidae (http://mitroidea.eurasiashells.net/). The Philippine representatives of the family have been treated in Poppe [2008]. The family is often erroneously considered as closely related to Mitridae (and included in the same superfamily Mitroidea or Mitracea), although Ponder [1972] convincingly demonstrated the great disparity in anatomy between two families.

The family remains poorly studied in terms of anatomy and ecology and just a few contributions have been published [Azuma, 1965; Ponder, 1972; Maes, Raeihle, 1975].

An interesting peculiarity of Costellariidae is the mode of feeding and diet, although very few observations have been published. According to Maes and Raeihle [1975], *Mitromica foveata* (Sowerby, 1874) [as *Thala floridana* (Dall, 1883)] feeds on a number of gastropod species, killing the prey with the toxic secretion, which is acting through the undamaged integuments of the prey. Feeding of an unidentified species of *Vexillum* on a gastropod has been observed by YuK in New Guinea. Finally, Morton and Miller [1968] recorded feeding of *Austromitra rubiginosa* (Hutton, 1873) on various species of tunicate, both solitary and compound. Nevertheless, Taylor *et al.* [1980] marked only Gastropoda as a prey item in the diet of Costellariidae.

Taxonomy of the family is in the state of flux. The following genera and subgenera are usually recognized within Costellariidae:

Costellaria Swainson, 1840 Mitromica S. S. Berry, 1958 Nodicostellaria Petuch, 1987 Pusia Swainson, 1840 Thala H. et H. Adams, 1853 Thaluta Rosenberg et Callomon, 2003 Tosapusia Azuma, 1965 Vexillum Röding, 1798 Visaya Poppe, Guillot de Suduiraut et Tagaro, 2006

Zierliana Gray, 1847

Except few species (attributed to genera *Thaluta, Zierliana* and *Visaya*) the generic position of species

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FIG. 1. Shells of studied specimens of the family Costellariidae.
A — Vexillum plicarium, 42 mm; B — V. rugosum, 37 mm; C — V. vulpecula, 43.2 mm; D — V. albofulvum, 57.5 mm; E — V. subdivisum, 22.7 mm; F — V. modestum, 17.4 mm; G — V. echinatum, 23.1 mm; H — Costellariidae gen. sp., 32.5 mm; I — V. militare, 13.3 mm; J — V. virgo, 14.0 mm; K — Vexillum costatum, 44.0 mm.



FIG. 2. Shells of studied specimens of the family Costellariidae A — Vexillum tusum, 12.0 mm; B — V. noduliferum, 7.3 mm; C — V. aureolatum, 8.1 mm; D — V. interruptum, 5.5 mm; E — V. diutenerum, 6.6 mm; F — Pusia salisburyi, 9.0 mm; G — Pusia rubra, 5.0 mm.

FIG. 2. Раковины исследованных экземпляров семейства Costellariidae A — Vexillum tusum, 12,0 мм; B — V. noduliferum, 7,3 мм; C — V. aureolatum, 8,1 мм; D — V. interruptum, 5,5 мм; E — V. diutenerum, 6,6 мм; F — Pusia salisburyi, 9,0 мм; G — Pusia rubra, 5,0 мм.

as well as the status of some taxa of the genus group is often arbitrarily changed. This occurs mostly due to lack of morphological data not to mention the absence of the available molecular data.

The purpose of the current publication is to examine the foregut anatomy (the most variable in Neogastropoda) of a number of species and on the basis of the molecular sequences to reconstruct the phylogeny of some selected species of Costellariidae. This will allow to trace the the major tendencies of the morphological transformations of the foregut.

#### Materials and methods

#### Specimens examined

Material for the studies was collected during several fieldworks in Philippines (AF) and Vietnam (YK) by SCUBA diving as well as from commercial sources — fishermen and from "lumun-lumun" collecting nets [Seronay *et al.*, 2010], and in Madagascar (YK) by dredging (Figs. 1, 2). Specimens were relaxed in isotonic solution of MgCl<sub>2</sub> and tissue clip (usually the part of the foot) was taken and preserved

РИС. 1. Раковины исследованных экземпляров семейства Costellariidae.

A — Vexillum plicarium, 42 мм; B — V. rugosum, 37 мм; C — V. vulpecula, 43,2 мм; D — V. albofulvum, 57,5 мм; E — V. subdivisum, 22,7 мм; F — V. modestum, 17,4 мм; G — V. echinatum, 23,1 мм; H — Costellariidae gen. sp., 32,5 мм; I — V. militare, 13,3 мм; J — V. virgo, 14,0 мм; K — Vexillum costatum, 44,0 мм.

Generic and specific identification	Sampling location (or authors for outgroup's sequences)	12S sequence GB accessing N
Vexillum plicarium	Olango Is, Philippines	HQ665033
Vexillum rugosum	Olango Is, Philippines	HQ665032
Vexillum vulpecula (dark form)	Nucnucan Is, Philippines	HQ665035
Vexillum albofulvum	Nucnucan Is, Philippines	HQ665034
Vexillum modestum	Marinduque Is, Philippines	HQ665038
Vexillum militare	Balicasag Is, Philippines	HQ665044
Vexillum echinatum	Olango Is, Philippines	HQ665040
Vexillum virgo	Marinduque Is, Philippines	HQ665042
Vexillum subdivisum	Dam Bay, Che Is, Vietnam	HQ665036
Vexillum costatum	Hansa Bay, New Guinea	
Vexillum tusum	Punta Engania, Mactan Island, Philippines	HQ665045
Vexillum nodiliferum	Punta Engania, Mactan Island, Philippines	HQ665037
Vexillum aureolatum	Punta Engania, Mactan Island, Philippines	HQ665039
Vexillum interruptum	Punta Engania, Mactan Island, Philippines	HQ665046
Vexillum diutenerum	Olango Is, Philippines	HQ665043
Pusia salisburyi	Balicasag Island, Philippines	HQ665041
Pusia rubra	Punta Engania, Mactan Island, Philippines	
Costellariidae gen. sp.	Madagascar, Mozambique Channel, 15°34'S, 45°43'E, 396 m	HQ665047; HQ665048
Outgroups		
Cancellaria cancellata	Oliverio M., Modica M.V., 2010	FM999074
Harpa sp.	Holford M., Puillandre N., Terryn Y., Cruaud C., Olivera B. Bouchet P., 2009	EU685491
Mitra lens	Oliverio M., Modica M.V., 2010	FM999079
Trophon plicatus	pphon plicatus Barco A., Claremont M., Reid D.G., Houart R., Bouchet P.,Williams S.T., Cruaud C., Couloux A. Oliverio M., 2010	
Latiromitra sp.	Oliverio M., Modica M.V., 2010	FM999085

Table 1. Examined material and outgroups used in the phylogenetic analysis.

in 96% ethanol. The body with the shell was preserved in 75% ethanol for anatomical studies. In the case of minute species entire body of some specimens was preserved in 96% ethanol.

In laboratory the preserved bodies were dissected for anatomical studies. The radula was cut out of the body and cleaned in diluted solution of bleach (NaOCl), rinsed in distilled water and mounted on clear glass slides, that were glued to the SEM stubs. The radulae were photographed on the TesSCAN TS5130 scanning electron microscope in the A.N.Severtzov Institute of Ecology and Evolution.

#### DNA extraction, amplification and sequencing

DNA was extracted from the peace of foot preserved in 96% ethanol, using the Gentra Puregene Tissue (QIAGEN) extraction kit. A fragment of 545 bp of mitochondrian 12S rRNA gene has been amplified using the standard primers [Oliverio, Mariottini, 2001]. Fw: 12SI — 5' TCG CAG CAG YCG CGG TTA Rw: 12SIII — 5' AGA GYG RCG GGC GAT GTG T

All PCR reactions were set up in the 25ul tubes with the Advanced Tag-polymerase (5-prime). The PCR thermal profile was set up as follows: initial denaturation — 3 min at 95°, 35 cycles of denaturation (30 sec at 95°), annealing (45 sec at 55°) and extension (45 sec at 68°) with the final elongation phase (5 min at 68°).

The PCR products were purified with QIAGEN gel extraction kit. All genes were sequenced in both directions. The GenBank accession numbers of the sequences are summarized in Table 1.

#### Phylogenetic analysis

A set of additional 12S sequences of the close families members [Oliverio, Modica, 2010] has been accessed in Genbank (see Table 1). The 12S sequences have been automatically aligned using Clustal

W2 algorithm available online (http://www.ebi.ac.uk/Tools/msa/clustalw2/), the result of alignment has been inspected by eye and, if needed, manually modified in BioEdit, version 7.0.5.3. The best fitting model of nucleotide substitution has been calculated via Modeltest 7.3 [Posada, Crandall, 2001]. All phylogenetic reconstructions were performed with the Bayesian analysis with the following parameters: two Marcov chains of 3,000,000 generations with the sampling frequency of one tree each 100 generations were run in 8 parallel analyses [Huelsenbeck et al., 2001]. When the log-likelihood scores got stable, a set of strict consensus trees has been constructed, the different proportion from 0 to 20% of trees has been omitted while the consensus tree construction.

Abbreviations used in the text and figure captions:

af — additional flap of the penis; AL — aperture length; aoe — anterior oesophagus; asg — accessory salivary gland; bm — buccal mass; bt — buccal tube; cbcw — cut wall of the buccal cavity; cpw cut proboscis wall; CTW — central tooth width; dgL - duct of gland of Leiblein; gL - gland of Leiblein; gmo — glandular mid-oesophagus; gpoe — glandular posterior oesophagus; gt — glandular tube; nr — nerve ring; od — odontophore; odr - odontophore retractor; ord - opening to the radular diverticulum; pggL - poorly glandular gland of Leiblein; poe — posterior oesophagus; pr - proboscis; prr — proboscis retractors; prw proboscis wall; rad - radular membrane; RL radular membrane length; rnh — rhynchodaeum; RW—radular membrane width; sd—salivary duct; sg — salivary gland; SL — shell length; vd — vas deferens; vL - valve of Leiblein.

#### Results

#### Molecular data analysis

Alignment of 12S sequences for 16 species of Costellariidae and 5 outgroups, gave a dataset of 510 positions in total, of which 234 were constant and 191 variable and parsimony informative. GTR+I+G was found to be the best fitting model of nucleotide substitution.

The phylogenetic tree, shown (Fig. 3) is a strict consensus tree, obtained after omitting of 5% of elementary trees. The tree is characterized by relatively low support of most nodes; however some are supported enough that allows some rather general judgments on the phylogeny and relationships of the family Costellariidae.

The family Costellariidae — clade 1, was shown to be monophyletic with the high reliability (the basal node of the family has a PP value of 1). *Latiromitra* sp. (Ptychatractidae) came out as a closest to the Costellariidae outer taxon, with the PP value of 0.96. Oppositely the family Mitridae, (represented here by *Mitra lens*) traditionally supposed to be closely related to Costellariidae, appeared far distanced from the costellariid clade.

Well supported clade 2 generally corresponds to the genus *Vexillum* in broad sense and includes 14 of studied species with the type species of the genus, *Vexillum plicarium* (Linnaeus, 1758). Three costellariid, not included into the clade 2, form two distinct lineages, which represent genus *Pusia* (*P. salisburyi*) and one more, likely still undescribed genus, members of which in contrast to all others costellariids studied, inhabit bathyal depths of South African area.

#### Morphology of the foregut

Costellariidae gen. sp. (Fig. 1H, 4, 5 A-B)

**Material examined:** Madagascar, Mozambique Channel, 15°34'S, 45°43'E, 396 m, F/V *Miriky* CP 3266, MNHN IM-2009-7427, two specimens dissected, one specimen sequenced (SL 32.5 mm, AL 10.5 mm).

Anterior foregut (Fig. 4). Proboscis in contracted state short, comprising ca. 2 mm (0.19 AL, 0.06 SL), cylindrical, slightly tapering. Rhynchodaeum rather thick and muscular. Proboscis occupies about  $1/_2$  of rhynchodaeum length. Paired very powerful retractors attached symmetrically to anteriormost ventral part of rhynchodaeum and columellar muscle. Very thin, extremely convoluted buccal tube leads from mouth opening to muscular buccal cavity. Thick walled radular diverticulum branches off buccal cavity and contains deeply retracted odontophore. Loops of buccal tube interconnected with connective tissue fibers, forming rather rigid structure.

Powerful branching odontophoral retractor leaves proboscis and follows along oesophagus to pass through nerve ring. It is probably attached to columellar muscle. Anterior oesophagus medium thick within proboscis and attached by numerous muscle fibers to proboscis walls. After leaving proboscis oesophagus sharply narrows and becomes very thin and convoluted, forms rather long loop and follows anteriorly to anteriormost part of rhynchodaeum. It is attached to rhynchodaeum by few connective tissue fibers.

Odontophore short (1.22 mm in length), bulbous, situated mostly within proboscis in its contracted state, deeply retracted into radular diverticulum. Subradular cartilages short, fused anteriorly.

Radula (Fig. 5 A-B) consists of 60 rows of teeth, 15 nascent, alary processes well developed, about  $\frac{1}{2}$  of radular membrane length. Radular length 2.2 mm (0.21 AL) width 220 mkm (2.1% AL). Central



0.1

FIG. 3. Strict consensus tree inferred from the 12S gene with Bayesian analysis after 5% of trees have been omitted. The values of posterior probabilities are indicated for each node.

РИС. 3. Строго консенсусное дерево, полученное Бэйесовским анализом по последовательностям нуклеотидов гена 128 после. 5% деревьев проигнорировано при построении консенсусного дерева. Значение постериорных вероятностей указано для каждого узла.

tooth medium broad (ca 80 mkm, or 0.76% AL), tricuspid, with strongly curved anterior margin and long lateral flaps. Cusps broad and relatively short, closely spaced, triangular, central being smallest. Lateral tooth sub-triangular, with slightly curved and blunt tip. No signs of teeth wear observed on radular bending plane.

Valve of Leiblein well developed, much broader than oesophagus, pyriform. Mid-oesophagus abruptly narrows to pass through small nerve ring. Pos-



FIG. 4. Foregut anatomy of Costellariidae gen. sp. A, B — organs of the body haemocoel: A — in natural position, B — extended, salivary gland removed, rhynchocoel open to show the proboscis. C — opened proboscis. Abbreviatons see p. 121.

РИС. 4. Анатомия переднего отдела пищеварительной системы Costellariidae gen. sp. А, В — органы туловищного гемоцеля: А — в естественном положении, В — в расправленном положении, слюнные железы удалены, полость ринхоцеля вскрыта, чтобы показать хобот. С — вскрытый хобот. Сокращения: см. стр. 121.

terior to ring mid-oesophagus slightly broadens and is obviously glandular and slightly convoluted.

Salivary glands large, massive and bulky, fuse together without visible border. Salivary ducts narrow, enter oesophagus walls shortly after leaving gland in front of valve of Leiblein. Paired large bulbous accessory salivary glands adjoin salivary glands and gland of Leiblein. Their very narrow ducts follow along odontophoral retractor to proboscis base.

Gland of Leiblein is large, broadly tubular, bulky, opens via very short duct into mid-oesophagus significantly posterior to nerve ring (position of duct opening is marked by arrow on drawing). Gland whitish in proximal part and light brownish along most of its length.

## Pusia salisburyi (Cernohorsky, 1976) (Fig. 2G; 5 C-D; 6 A-C)

Material examined: Balicasag Island, Philippi-

nes, lumun-lumun net, one specimen sequenced, one specimen dissected (SL 9.0 mm, AL 4.3 mm).

Anterior foregut (Fig. 6 A-B). Proboscis in contracted state medium long, comprising ca. 1.35 mm (0.31 AL, 0.15 SL), occupies entire rhynchocoel, tapering to tip. Rhynchodaeum thin, transparent. Paired very long but thin proboscis retractors attached to anteriormost part of rhynchodaeum. Thin and nearly straight buccal tube (ca 0.35 mm in length, or 0.25 of proboscis length) leads from mouth opening to muscular buccal cavity.

Odontophoral retractor leaves proboscis and follows partially along oesophagus to pass through nerve ring and is probably attached to columellar muscle. Anterior oesophagus relatively thick, after leaving proboscis coiled, forms very long loop and follows anteriorly to middle part of rhynchodaeum, free from rhynchodaeum.

Radular diverticulum muscular, thick, occupies posterior  $^{2}/_{3}$  of proboscis length, lies entirely within proboscis. Odontophore short, deeply retracted into radular diverticulum.



FIG. 5. Radular (A, B, C, E) and jaw (D, F) morphology. A, B — Costellariidae gen. sp.; C, D — Pusia salisburyi; E, F — P. rubra.

РИС. 5. Строение радулы (А, В, С, Е) и челюсти (D, F). А, В — Costellariidae gen. sp.; C, D — Pusia salisburyi; E, F — P. rubra.



FIG. 6. Foregut anatomy of *Pusia salisburyi* (A-C) and *Pusia rubra* (D).
A, D — organs of the body haemocoel in natural position, B — organs of the body haemocoel extended, salivary gland removed, gland of Leiblein and posterior oesophagus not shown. C — penis. Abbreviatons see p. 121.

РИС. 6. Анатомия переднего отдела пищеварительной системы *Pusia salisburyi* (A-C) и *Pusia rubra* (D). A, D — органы туловищного гемоцеля в естественном положении. В — органы туловищного гемоцеля в расправленном положении, слюнные железы удалены, железа Лейблейна и задний пищевод не изображены. С — пенис. Сокращения: см. стр. 121.

Radula (Fig. 5C) consists of 46 rows of teeth, 2 nascent. Radula short, length 450 mkm (0.10 AL), width 125 mkm (2.9% AL). Central tooth broad (ca 60 mkm, or 1.4% AL), with curved anterior margin and numerous (12-13) thin, pointed cusps, gradually elongating from shortest lateral ones to most long, occupying more central position. Central cusp slightly shorter than adjoining cusps. Slight teeth wear observed on anterior rows (lateral cusps broken off). Lateral tooth sub-triangular, with slightly curved and blunt tip.

Small horseshoe-shaped jaw (Fig. 5D), rounded in profile, is situated in anterior part of radular diverticulum. Valve of Leiblein well defined, relatively large, pyriform.

Salivary glands small, fuse together without visible border, cover valve of Leiblein and nerve ring. Accessory salivary gland small, cone-shaped, adjoins nerve ring. Mid-oesophagus after passing through nerve ring expands and becomes obviously glandular.

Gland of Leiblein large, long, broadly tubular, brownish, opens into posterior portion of glandular part of mid-oesophagus significantly posterior to nerve ring. Posterior to opening of duct of gland of Leiblein oesophagus narrows and becomes nonglandular. Penis is very different from other Costellariidae in being club-shaped rather then simple narrowing towards the tip (as in other species) and having additional flap (Fig. 6C).

### *Pusia rubra* (Broderip, 1836) (Fig. 2G; 5 E-F; 6D)

**Material examined**: Punta Engania, Mactan Island, Philippines, lumun-lumun net, one specimen sequenced, one specimen dissected (SL 5.0 mm, AL 2.25 mm).

Anterior foregut (Fig. 6D). Proboscis in contracted state narrow, short, ca. 0.36 mm in length (0.16 AL, 0.07 SL). Proboscis with moderately folded walls, diameter at its base ca. 1.15 mm, at tip 0.3 mm. Rhynchodaeum rather thick, muscular. Paired thin proboscis retractors attached to anteriormost ventral part of rhynchodaeum.

Odontophore large, lying entirely outside proboscis. Radular membrane is clearly seen through walls of radular diverticulum by transparency.

Radula (Fig. 5E) consists of 37 rows of teeth, 2 nascent. Radula short, length 240 mkm (0.10 AL), width 60 mkm (2.6% AL). Central tooth medium broad (ca. 35 mkm, or 1.5% AL), with slightly curved anterior margin and numerous (12) thin pointed cusps, gradually elongating from shortest lateral ones to most long, occupying more central position. Slight teeth wear can be observed on anterior rows (some cusps broken off). Lateral tooth sub-triangular, with long curved and blunt tip.

Small horseshoe-shaped jaw (Fig. 5F), rounded in profile is situated in anterior part of radular diverticulum.

Anterior oesophagus thin, convoluted after leaving proboscis, follows anteriorly along rhynchodaeum, and opens into relatively very large valve of Leiblein. Mid-esophagus very thick, convoluted. Gland of Leiblein medium sized, glandular, light brownish, opens into oesophagus by short narrowed duct.

Salivary glands medium-sized, separate, not covering valve of Leiblein.

# Vexillum subdivisum (Gmelin, 1791) (Fig. 1E; 7A; 8A)

**Material examined**: Dam Bay, Che Island, southern Vietnam, mangroves, one specimen sequenced, 2 specimen dissected (SL 20.5 mm, AL 10.7 mm — radula illustrated), SL 22.7 mm, AL 11.7 mm — anatomy illustrated).

Anterior foregut (Fig. 7A). Proboscis in contracted state narrow, medium long, about 3.7 mm in length (0.31 AL, 0.16 SL), gradually narrowing toward tip, with moderately folded walls. Rhynchodaeum rather thick and muscular. Paired very powerful retractors attached to anteriormost dorsal part of rhynchodaeum. Buccal tube not pronounced.

Powerful branching odontophoral retractor follows along oesophagus to pass through nerve ring. Anterior oesophagus thin, leaving proboscis and following anteriorly along rhynchodaeum, to which it is attached by numerous connective tissue fibers.

Radular diverticulum splits from anterior oesophagus near proboscis tip. Odontophore deeply retracted, ca. 2 mm in length. Cartilages separate, connected anteriorly by muscular sheath, shorter or slightly longer than radular membrane.

Radula (Fig. 8A) consists of 46 rows of teeth, 2 nascent. Radula short, length 1.25 mm (0.11 AL), width 290 mkm (2.7% AL). Central tooth medium broad (ca. 175 mkm, or 1.6% AL), with slightly curved anterior margin and numerous (15-16) thin pointed cusps, gradually elongating from shortest lateral ones to most long and thick central ones. Rather strong teeth wear can be observed on anterior rows (some cusps on central teeth broken off and tips of lateral ones worn off). Lateral tooth hook-like, with narrow base and long curved pointed tip.

Valve of Leiblein small, distinct, with circle of mucous cells seen through its walls.

Salivary glands large, massive and bulky, fused, covering nerve ring. Salivary ducts narrow, enter oesophagus walls shortly after leaving gland, convoluted. Accessory salivary glands not found.

Gland of Leiblein medium-sized, tubular and convoluted, narrow, with semi-transparent walls, brownish, tapering to narrow tip. Gland duct opens into glandular tube formed by stripped off dorsal folds of mid-oesophagus. Glandular tube whitish, moderately convoluted, forming funnel-shaped expansion, embracing oesophagus. Tube opens into oesophagus at some distance posterior to small nerve ring.

### Vexillum noduliferum (A.Adams, 1853) (Fig. 2B; 9A; 10C)

**Material examined**: Punta Engania, Mactan Island, Philippines, lumun-lumun net, one specimen sequenced, one specimen dissected (SL 7.3 mm, AL 3.6 mm).

Anterior foregut (Fig. 9A). Proboscis in contracted state bended within rhynchodaeum, ca. 0.8 mm in length (0.22 AL, 0.10 SL), tapering to its tip, 0.09 mm in diameter at base and 0.05 mm at tip. Rhynchodaeum very thin and transparent. Paired thin proboscis retractors attached to anteriormost dorsal part of rhynchodaeum.

Odontophoral retractor follows along oesophagus to pass through nerve ring, and attached to columellar muscle. Odontophore occupies posterior  $1/_5$ of radular diverticulum, radular membrane slightly protruding beyond proboscis. Anterior oesophagus



- FIG. 7. Foregut anatomy of Vexillum subdivisum (A) and V. virgo (B).
  A organs of the body haemocoel extended, left salivary gland removed, B organs of the body haemocoel in natural position, rhynchocoel open to show the proboscis.
  Abbreviatons see p. 121.
- РИС. 7. Анатомия переднего отдела пищеварительной системы Vexillum subdivisum (A) и V. virgo (B). А — органы туловищного гемоцеля в расправленном положении, левая слюнная железа удалена, полость ринхоцеля вскрыта, чтобы показать хобот. В — органы туловищного гемоцеля в естественном положении. Сокращения: см. стр. 121.

thin, convoluted after leaving proboscis, forms a loop and follows anteriorly along rhynchodaeum.

Radula (Fig. 10C) consists of 53 rows of teeth, 3 nascent. Radula short, length 290 mkm (0.08 AL), narrow, width 55 mkm (1.5% AL). Central tooth medium narrow (ca 35 mkm, or 1.0% AL), with slightly curved anterior margin and 7 triangular pointed cusps of sub-equal length. No signs of teeth wear observed. Lateral tooth hook-like, with narrow base and long strongly curved pointed tip. Alary processes of membrane long, comprising about  $V_2$ of the radular length.

Valve of Leiblein obsolete.

Salivary glands large, massive and bulky, fused, covering nerve ring. Accessory salivary not found.

Gland of Leiblein small, conical, with muscular walls, opens into thick, relatively short and strongly convoluted glandular tube. Tube opens into oesophagus immediately posterior to massive nerve ring.

### Vexillum modestum (Reeve, 1845) (Fig. 1F; 10B)

Material examined: Marinduque Island, Philip-

pines, from fishermen, one specimen sequenced, one specimen dissected (SL 17.4 mm, AL 6.5 mm).

Anterior foregut. In general morphology rather similar to *V. noduliferum*. It differs in having much longer, straight, rather thick proboscis (2.8 mm in length, 0.43 AL, 0.16 SL), only slightly tapering to its tip, 0.9 mm in diameter at base and 0.3 mm at tip. Gland of Leiblein relatively slightly larger, sausage-shaped rather then conical, opens into slightly longer glandular tube.

Odontophore lies within proboscis, about 0.95 mm in length, deeply retracted into radular diverticulum, which itself 1.65 mm in length. Subradular cartilages paired, fused anteriorly and free posteriorly. Buccal tube medium thin, slightly convoluted, and not long, in extended state about 1/4 of the proboscis length.

Radula (Fig. 10B) consists of 66 rows of teeth, 14 nascent. Radula short, length ca. 750 mkm (0.12 AL), width 145 mkm (2.2% AL). Central tooth medium broad (ca. 80 mkm, or 1.2% AL), with slightly and evenly curved anterior margin and 10 thin pointed cusps of sub-equal length. No teeth wear can



FIG. 8. Radular morphology. A — Vexillum subdivisum; B, C — V. albofulvum; D — V. rugosum; E — V. plicarium; F — V. vulpecula. РИС. 8. Строение радулы. A — Vexillum subdivisum; B, C — V. albofulvum; D — V. rugosum; E — V. plicarium; F — V. vulpecula.



FIG. 9. Foregut anatomy of Vexillum noduliferum (A), V. militare (B) and V. plicarium (C). A — organs of the body haemocoel in natural position, left salivary gland removed. B, C — organs of the body haemocoel extended, in B rhynchocoel open to show the proboscis. Abbreviatons see p. 121.

РИС. 9. Анатомия переднего отдела пищеварительной системы Vexillum noduliferum (A), V. militare (B) и V. plicarium (C). А — органы туловищного гемоцеля в в естественном положении. В, С — органы туловищного гемоцеля в

A — органы туловищного темоцеля в в естественном положении. В, С — органы туловищного темоцеля в расправленном положении, на рис. В полость ринхоцеля вскрыта, чтобы показать хобот. Сокращения: см. стр. 121.

be observed on anterior rows. Lateral tooth hooklike, with narrow base and long pointed tip curved near the summit. Alary processes of the membrane long, comprising about  $\frac{1}{2}$  of the radular length.

### Vexillum militare (Reeve, 1845) (Fig. 1I; 9B; 10A)

**Material examined**: Balicasag Island, Philippines, lumun-lumun net, one specimen sequenced and dissected (SL 13.3 mm, AL 6.6 mm).

Anterior foregut (Fig. 9B). Proboscis in contracted state long, comprising ca. 2.25 mm (0.34 AL, 0.17 SL), occupies entire rhynchocoel. Rhynchodaeum thin, transparent. Paired thin retractors attached to middle ventral part of rhynchodaeum. Thin and strongly convoluted buccal tube leads from mouth opening to muscular buccal cavity. It forms not long rigid structure, impossible to extend.

Odontophoral retractor leaves proboscis and follows along oesophagus to pass through nerve ring. Anterior oesophagus relatively thick, coiled after leaving proboscis, forms short loop and follows anteriorly to middle part of rhynchodaeum and attached to rhynchodaeum by numerous connective tissue fibers.

Radular diverticulum muscular and thick, lies entirely within the proboscis in its posterior half. Odontophore short, bulbous. Subradular cartilages short, fused anteriorly.

Radula (Fig. 10A) consists of 38 rows of teeth, 6 nascent. Radula short, length ca. 600 mkm (0.09 AL), width 155 mkm (2.3% AL). Central tooth medium broad (ca. 90 mkm, or 1.3% AL), with slightly and evenly curved anterior margin and short lateral flaps. Central tooth with 9 thin, pointed cusps of sub-equal length. Additional much smaller cusp appear as most lateral on some teeth. Although two central teeth had broken cusps, this is probably artifact, since anteriormost rows, exposed on bending plane, were intact. Lateral tooth hook-like, with relatively broad base and blunt tip curved near summit. Alary processes of membrane long, comprising more than  $\frac{1}{2}$  of radular length.

Valve of Leiblein well defined although small, pyriform.

Salivary glands medium-sized, fuse together without visible border. Accessory salivary gland not found. Mid-oesophagus after passing through nerve ring greatly expands and becomes obviously glandular.

Gland of Leiblein small, narrow, conical, with muscular walls. It opens into rather short, slightly coiled glandular tube. Tube opens into posterior portion of glandular part of mid-oesophagus significantly posterior to nerve ring. Posterior to opening of glandular tube oesophagus sharply narrows.

### Vexillum virgo (Linnaeus, 1767) (Fig. 1J; 7B; 10E)

**Material examined**: Marinduque Island, Philippines, from fishermen, one specimen sequenced, one specimen dissected (SL 14.0 mm, AL 6.0 mm).

Anterior foregut (Fig. 7B). Proboscis in contracted state medium long, comprising ca. 1.75 mm (0.29 AL, 0.13 SL), conical, tapering towards tip. Rhynchodaeum thin-walled, transparent. Paired powerful retractors attached to anteriormost dorso-lateral part of rhynchodaeum. Very thin and convoluted buccal tube leads from mouth opening to buccal cavity. When extended, tube nearly equals in length to proboscis.

Powerful odontophoral retractor leaves proboscis and follows along oesophagus to pass through nerve ring. Anterior oesophagus thin. It is convoluted after leaving proboscis and forms long loop that follows anteriorly to anteriormost part of rhynchodaeum.

Odontophore medium long, ca. 0.7 mm in length, deeply retracted into radular diverticulum, extends backward from proboscis in its contracted state.

Radula (Fig. 10E) consists of 42 rows of teeth, 2 nascent. Radula short, length ca. 510 mkm (0.09 AL), width 150 mkm (2.5% AL). Central tooth medium broad (ca. 90 mkm, or 1.5% AL), with slightly and evenly curved anterior margin and short lateral flaps directed posteriorly. Central teeth with 14-15 thin pointed cusps, lateral being slightly shorter than centrals. Some cusps vary in length from row to row. No wear of teeth observed. Lateral tooth hook-like, with relatively narrow base and sharp tip strongly curved near summit. Alary processes of membrane long, comprising slightly more than 1/2 of radular length.

Valve of Leiblein well pronounced, mediumsized, pyriform.

Salivary glands medium-sized, bulky, fuse together without visible border, cover nerve ring and valve of Leiblein. Gland of Leiblein large, convoluted, with partitions seen through its walls, terminating in small ampulla. It opens into long and massive convoluted glandular tube, uniform yellowish tan. Tube opens into oesophagus immediately posterior to nerve ring.

### Vexillum diutenerum (Hervier, 1898) (Fig. 2E; 10D)

**Material examined**: Olango Island, Philippines, from fishermen, one specimen sequenced, one specimen dissected (SL 6.6 mm, AL 2.4 mm).

**Anterior foregut.** In general morphology very similar to *V. noduliferum.* It differs in having much longer straight, rather thick proboscis (1.15 mm in length, 0.48 AL, 0.17 SL), only slightly tapering to tip.

Odontophore lies within proboscis, about 0.27 mm in length, deeply retracted into radular diverticulum, itself about 0.5 mm in length. Buccal tube medium thin, slightly convoluted and not long, in extended state about  $1/_2$  of proboscis length.

Radula (Fig. 10D) consists of 40 rows of teeth, 3 nascent. Radula not long, length ca. 250 mkm (0.10 AL), width ca. 55 mkm (2.3% AL). Central tooth medium broad (ca. 35 mkm, or 1.5% AL), with strongly curved arched anterior margin and rather long lateral flaps. Central teeth with 6-7 broad and large pointed cusps. Three central cusps of subequal length more closely spaced. Lateral cusps smaller. Cusps shape and size differ from row to row. No wear of teeth observed. Lateral tooth hooklike, with relatively narrow base and long cusp with sharp tip curved near summit. Alary processes of membrane long, comprising slightly more than  $V_2$ of radular length.

### *Vexillum rugosum* (Gmelin, 1791) (Fig. 1B; 8D; 11A-B)

**Material examined**: Olango Island, Philippines, from fishermen, one specimen sequenced, one specimen dissected (SL 37.0 mm, AL 21.2 mm),

Anterior foregut (Fig. 11A-B). Proboscis in contracted state short, comprising ca. 5 mm (0.24 AL, 0.14 SL). Rhynchodaeum rather thick and muscular. Paired very powerful retractors attached to anteriormost ventral part of rhynchodaeum. Very thin and convoluted buccal tube leads from mouth opening to buccal cavity. When extended, tube comprises nearly half of proboscis length.

Powerful odontophoral retractor leaves proboscis and follows along oesophagus to pass through nerve ring. Anterior oesophagus medium thick convoluted, forms a very long loop directed anteriorly to anteriormost part of rhynchodaeum and attached to rhynchodaeum by numerous connective tissue fibers.



- FIG. 10. Radular morphology. A Vexillum militare; B V. modestum; C V. noduliferum; D V. diutenerum; E V. virgo; F V. costatum.
- FIG. 10. Строение радулы. A Vexillum militare; B V. modestum; C V. noduliferum; D V. diutenerum; E V. virgo; F V. costatum.



FIG. 11. Foregut anatomy of *Vexillum rugosum* (A-B), *V. vulpecula* (C-D) and *V. costatum* (E). A, C, E — organs of the body haemocoel extended, salivary gland removed. C-E — rhynchocoel open to show the proboscis. B — enlarged fragment of the nerve ring and oesophagus. D — anterior part of the proboscis opened. Abbreviatons see p. 121.

РИС. 11. Анатомия переднего отдела пищеварительной системы Vexillum rugosum (A-B), V. vulpecula (C-D) and V. costatum (E).

А, С, Е — органы туловищного гемоцеля в расправленном положении. С-Е — полость ринхоцеля вскрыта, чтобы показать хобот. В — увеличенный фрагмент нервного кольца и пищевода. D — вскрытая передняя часть хобота. Сокращения: см. стр. 121.

Odontophore very short, bulbous, situated outside proboscis in its contracted state. Subradular cartilages short, not fused, broadly spaced and connected by muscular sheath.

Radula (Fig. 8D) consists of 56 rows of teeth, 3 nascent. Radula not long, length ca. 1.9 mm (0.08 AL), width ca. 420 mkm (2.0% AL). Central tooth medium narrow (ca. 230 mkm, or 1.1% AL), with strongly curved arched anterior margin and short lateral flaps. Central teeth with 19-20 thin, pointed

cusps, central ones being slightly broader and longer. No wear of teeth observed. Lateral tooth sub-triangular, with concave in profile curved cusp with pointed tip. Alary processes of membrane long, comprising about  $\frac{1}{2}$  of radular length.

Valve of Leiblein hardly discernible.

Salivary glands very large, massive and bulky fuse together without visible border. Salivary ducts narrow, enter oesophagus walls shortly after leaving gland. Small tubular convoluted accessory salivary gland embedded into salivary gland. Duct narrow and follows along odontophoral retractor to proboscis base, and then ventrally along odontophore towards its opening, which was not traced.

Gland of Leiblein small, conical, with muscular walls, opens into very long and massive convoluted glandular tube that forms structure occupying most of body haemocoel. Tube grayish in proximal part close to gland of Leiblein and more creamy anteriorly. Tube opens into oesophagus immediately posterior to nerve ring.

### Vexillum plicarium (Linnaeus, 1758) (Fig. 1A; 8E; 9C)

**Material examined**: Olango Island, Philippines, from fishermen, one specimen sequenced, one specimen dissected (SL 37.0 mm, AL 23.7 mm)

Anterior foregut (Fig. 9C). Proboscis in contracted state very narrow and thus seemingly long, ca. 6.2 mm in length (0.26AL, 0.17 SL), with strongly folded walls. Diameter/length ratio ca. 0.15. Rhynchodaeum rather thick and muscular. Paired very powerful retractors attached to anteriormost ventral part of rhynchodaeum. Very thin (about 0.1 mm in diameter) and convoluted buccal tube leads from mouth opening to buccal cavity. In non-extended position it comprises about 0.15 of proboscis length.

Powerful odontophoral retractor leaving proboscis and follows along oesophagus to pass through nerve ring. Anterior oesophagus medium thick, convoluted, and after leaving proboscis forms long loop that follows anteriorly to anteriormost part of rhynchodaeum. It is attached to rhynchodaeum by numerous connective tissue fibers.

Odontophore very small, lies in anterior part of proboscis.

Radula (Fig. 8E) consists of 62 rows of teeth, 3 nascent. Radula short and narrow, length ca. 0.8 mm (0.03 AL), width ca. 175 mkm (0.7% AL). Central tooth narrow (ca. 115 mkm, or 0.5% AL), with strongly curved anterior margin and short lateral flaps. Central teeth with 11 slightly variable in width thin pointed cusps, most lateral slightly shorter than others. No wear of teeth observed. Lateral tooth sub-triangular, with concave in profile curved cusp. Alary processes of membrane long, comprising about 1/2 of radular length.

Valve of Leiblein small, distinct, with glandular pad seen through its walls.

Salivary glands large, massive and bulky, separate, although tightly adjoin each other. Salivary ducts narrow, enter oesophagus walls shortly after leaving gland. Accessory salivary glands not found.

Gland of Leiblein small, conical, muscular, opens into relatively short convoluted glandular

tube. Tube opens into oesophagus immediately posterior to massive nerve ring.

# Vexillum vulpecula (Linnaeus, 1758) (Fig. 1C; 8F; 11 C-D)

**Material examined**: Nucnuckan Island, Philippines, from fishermen, one specimen sequenced and dissected (SL 43.2 mm, AL 24.1 mm) (dark form).

Anterior foregut (Fig. 11 C-D). Proboscis in contracted state narrow, not long, ca. 5.5 mm in length (0.23 AL, 0.13 SL), gradually narrowing toward tip with moderately folded walls. Proboscis diameter ca. 1.15 mm at base, 0.3 mm at tip. Rhynchodaeum rather thick and muscular. Paired very powerful retractors attached to anteriormost ventral part of rhynchodaeum. Very thin (about 0.1 mm in diameter) and convoluted buccal tube (2.7 mm in extended position, or half of proboscis length) leads from mouth opening to buccal cavity.

Powerful odontophoral retractor follows along oesophagus to pass through nerve ring. Anterior oesophagus thin, convoluted after leaving proboscis, forms loop that follows anteriorly along rhynchodaeum, attached to it by numerous connective tissue fibers.

Radular diverticulum situated in posterior 1/3 of proboscis length. Odontophore deeply retracted and protruding backward from proboscis, ca. 1.7 mm in length. Cartilages separate, connected anteriorly by muscular sheath.

Radula (Fig. 8F) consists of 62 rows of teeth, 13 nascent. Radula not long, length ca. 1.5 mm (0.06 AL), width ca. 320 mkm (1.3% AL). Central tooth broad (ca 175 mkm, or 0.7% AL), with slightly curved anterior margin and short lateral flaps. Central teeth with 11 slightly variable in width, thin pointed cusps. No wear of teeth observed. Lateral tooth sub-triangular, with concave in profile curved cusp. Alary processes of membrane long, comprising about 1/2 of radular length.

Valve of Leiblein small, distinct, with glandular pad seen through its walls.

Salivary glands large, massive and bulky, fused, covering valve of Leiblein and nerve ring. Salivary ducts narrow, enter oesophagus walls shortly after leaving glands. Accessory salivary gland not found.

Gland of Leiblein small, cone shaped, with muscular walls, opens into very long, convoluted glandular tube. Tube opens into oesophagus immediately posterior to massive nerve ring.

# Vexillum albofulvum Herrmann, 2007 (Fig. 1D; 8 D-C)

**Material examined**: Nucnucan Island, Philippines, from fishermen, one specimen sequenced and dissected (SL 57.5 mm, AL 32.5 mm).

Anterior foregut. Only part of body retrieved. Proboscis long, 15 mm in length (0.46 AL, 0.26 SL), with strongly contracted walls, suggesting possibility of great expansion. Buccal tube short and comparatively broad, straight, ca. 1.1 mm in length. Radular diverticulum short, occupies anterior position within proboscis, ca. 3 mm in length. Odontophore very short (1.65 mm in length), broadly oval, cartilages fused only in anteriormost part. Anterior oesophagus thick and convoluted. Valve of Leiblein well defined. Gland of Leiblein opens into convoluted glandular tube.

Radula (Fig. 8 D-C) consists of 65 rows of teeth, 11 nascent. Radula short, length ca. 1.4 mm (0.04 AL), width ca. 320 mkm (1.3% AL). Central tooth broad (ca. 210 mkm, or 0.65% AL), with slightly curved arched anterior margin and short lateral flaps. Central teeth with 16 slightly variable in width thin pointed cusps. Several anterior rows of teeth with some cusps broken off. Lateral tooth sub-triangular, concave in profile, with thickened edge. Cusp not long, with pointed and curved at summit tip. Alary processes of membrane long, comprising more than 1/2 of radular length.

# Vexillum costatum (Gmelin, 1791) (Fig. 1L; 10F; 11E)

**Material examined**: New Guinea, Hansa Bay, one specimen dissected (SL 44 mm, AL 15.8 mm).

Anterior foregut. Proboscis in contracted state not long with moderately folded walls, comprising ca. 4.5 mm (0.28 AL, 0.1 SL) cylindrical, occupies around  ${}^{3}\!/_{4}$  of rhynchodeal length. Paired powerful proboscis retractors attached to anteriormost ventral part of thick and muscular rhynchodaeum. Rhynchodaeum occupies right side of body haemocoel, while rest of foregut structures lies in its left part.

Anterior oesophagus is medium thick within proboscis and attached by numerous muscle fibers to proboscis walls. After leaving proboscis it forms a long loop that follows anteriorly along rhynchodaeum, attached to it by few connective tissue fibers. Anterior oesophagus of same diameter throughout its length, non convoluted. Thin odontophore retractor follows along oesophagus to pass through nerve ring.

Salivary glands not large, separate, compact. Salivary ducts enter esophagus walls right in front of nerve ring.

Odontophore lies at mid-length of proboscis, slightly shifted to its base.

Radula (Fig. 10 F) narrow, width ca. 210 mkm (0.6% AL). Central tooth not broad (ca 120 mkm, or 0.75% AL), with strongly curved arched anterior margin and long lateral flaps. Central teeth with 7 pointed cusps, lateral being much smaller than others. Lateral tooth hook-like, with narrow base

and long curved cusp pointed and curved near summit.

Valve of Leiblein visually not pronounced.

Gland of Leiblein small, cone shaped, muscular, opens into thick and strongly convoluted glandular tube. It occupies all dorsal space, backward from nerve ring covering posterior oesophagus and opens into oesophagus immediately posterior to nerve ring.

#### Discussion

#### Summary of morphology of the foregut in a framework of molecular phylogeny

The foregut anatomy appeared to be very variable in studied Costellariidae. Several common characters can be mentioned.

Proboscis retractors are always paired and are attached usually to the anteriormost part of the rhynchodaeum. There is always a narrow buccal tube (which can be from very short to usually long and convoluted) leaving from mouth opening to muscular buccal cavity. Odontophore is usually in posterior position, often protruding backward from the rear of the proboscis. Odontophoral retractor is very long and passes through the nerve ring. Salivary glands are usually fused without visible border or at least tightly adjoin each other. Valve of Leiblein usually present, although can be reduced in some species.

None of the mentioned characters are outapomorphic for the family.

The most variable is the anatomy of the midoesophagus and the gland of Leiblein. Several morphological types can be segregated.

The first, most underived type (Type 1A) of midoesophagus is found in Costellariidae gen. sp. and Pusia salisburyi (Figs. 12A). In these species the gland of Leiblein is broadly tubular, may be slightly coiled (in the former), obviously glandular. It has usual for the Neogastropoda greenish-grey colour, that persists in alcohol preserved specimens, although fading with time. It opens through the short narrow duct in the glandular mid-oesophagus significantly posterior to the nerve ring. This type of mid-oesophagus is found in most neogastropods, including the outgroups used in our analysis, Trophon and Latiromitra [Ponder, 1974; Bouchet, Kantor, 2000]. Both mentioned species came out as the most basal branches in our phylogenetic analysis (Fig. 3).

Rather similar arrangement of the mid-oesophagus (which we recognize as type *IB*) was found in *Pusia rubra*. In this species the gland of Leiblein is relatively small, glandular and opens via very short duct. The mid-oesophagus is very long, strongly glandular and coiled (Figs. 6D, 12 B). It differs from type 1A in relatively longer glandular mid-oesophagus. Similar arrangement was found in *Mitromica*  *foveata* [Maes, Raeihle, 1975] and in *Austromitra rubiginosa* [Ponder, 1972]. Unfortunately none of the mentioned species have been sequenced.

In the next branching taxon, *Vexillum subdivisum* the gland of Leiblein is still glandular, coiled tubular, but the glandular dorsal folds of the oesophagus are stripped off from the mid-oesophagus and form the coiled glandular tube, that opens into mid-oesophagus posterior to the nerve ring. We recognize this as type 2A (Fig. 12 C). The stripping of the glandular folds from the mid-oesophagus with formation of the glandular tube is a common process in Neogastropoda and occurs besides Costellariidae independently in several families, such as Volutidae, Marginellidae, some Muricidae [Ponder, 1974].

Very similar type (which we consider as type 2B — Fig. 12 D) is found in *Vexillum virgo*. It differs from type 2A in the degree of stripping of the glandular folds from the oesophagus, which occurred to the level of the nerve ring. It should be noted, that in our molecular tree V. virgo is in the same clade with V. diutenerum, which possesses different sub-type of mid-oesophagus anatomy. But the node is characterized by low posterior probability support (0.66), which is not considered as sufficient for being reliable.

The third type is found in the clade 2 (except *Vexillum virgo*). This type is characterized by the transformation of the gland of Leiblein, which becomes much less glandular, greatly reduced in size and have thick muscular walls [Ponder, 1972]. Externally it is reflected in shining appearance of the gland. The glandular function is taken over by the tube, formed by the stripped glandular folds of the mid-oesophagus. In type *3A* (*Vexillum militare*) (Fig. 12 E) the stripping of the folds is not complete and the glandular tube is opening through the constricted duct in the posterior part of the glandular elongated and very thick mid-oesophagus.

In type 3B (Fig. 12 F) the stripping off of the glandular folds occurred to the level of the nerve ring. This type is rather variable in terms of the relative size of the gland of Leiblein and the glandular tube. The glandular tube can be very thick and relatively short, forming few loops (e.g. in V. noduliferum and V. diutenerum) to extremely long and very convoluted, although thin (V. vulpecula, V. rugosum) to thin and relatively short in V. plicarium. Although not supported by the molecular tree, type 3A seems more primitive than type 3B.

Basing on the molecular tree and taking into account the nodes with high support, it is becoming obvious that the plesiomorphic condition is the "normal" glandular tubular gland of Leiblein, opening directly into oesophagus. The process of stripping off of the glandular folds of mid-oesophagus with the formation of the glandular tube is the prominent tendency in the evolution of Costellariidae. Since



- FIG. 12. Diagram of the mid-oesophagus anatomy in studied species of Costellariidae. A — type 1A; B type 1B; C — type 2A; D — type 2B; E type 3A; F — type 3B.
- РИС. 12. Схема строения среднего пищевода у изученных видов Costellariidae. А тип 1А; В тип 1В; С тип 2А; D тип 2В; Е тип 3А; F тип 3В.

the molecular tree suggests that this is an apomorphic condition in the family, our data confirmed that it occurred in Costellariidae independently from other lineages of Neogastropoda. And, finally, the transformation of the gland of Leiblein into muscular poorly glandular sac, opening into glandular tube, is an apomorphy of the clade 2 of our tree.

The radulae appeared also rather variable in Costellariidae (Table 2). According to the molecular tree the most underived type (found in Costellariidae gen. sp., occupying the basal position in the tree) is characterized by tricuspidate central tooth. Such morphology is widely distributed in Neogastropods and very similar radulae were found, eg. in Olividae [Kantor, Bouchet, 2007], although this is clearly homeomorphy. Among Costellariidae the similar radulae were recorded in *Tosapusia isaoi* (Kuroda et

Species	RL/AL	RW/AL,%	CTW/AL,%	Number of cusps on the	Number of rows of teeth
				central tooth	
Costellariidae gen.sp.	0.21	2.1	0.76	3	60
Pusia salsburyi	0.10	2.9	1.4	12-13	46
Pusia rubra	0.10	2.6	1.5	12	37
Vexillum subdivisum	0.11	2.7	1.6	15-16	64
Vexillum noduliferum	0.08	1.5	1.0	7	53
Vexillum modestum	0.12	2.2	1.2	10	66
Vexillum militare	0.09	2.3	1.3	9	38
Vexillum virgo	0.09	2.5	1.5	14-15	42
Vexillum diutenerum	0.10	2.3	1.5	6-7	40
Vexillum rugosum	0.08	2.0	1.1	19-20	56
Vexillum plicarium	0.03	0.7	0.5	11	62
Vexillum vulpecula	0.06	1.3	0.7	11	62
Vexillum albofulvum	0.04	1.3	0.65	16	65
Vexillum costatum*	?	0.6	0.75	7	?

Table 2. Summary of radular measurements.

\* - only part of the radula was available for study.

Abbreviations: RL - radular membrane length; RW - radular membrane width; CTW - central teeth width.

Sakurai in Kuroda, 1965) and some species of *Pusia* (*P. hizenensis, P. simulans, P. australis*) [Azuma, 1965]. Ponder [1998] illustrated similar radula for *Pusia patriarchalis*, but rather different, with multicuspid central tooth for *Pusia luculentum*. In studied species, that we attributed to *Pusia (P. salisburyi* and *P. rubra)* on the conchological characters, the radula (Fig. 5 C,E) is similar to that, illustrated for *P. luculentum*.

An interesting peculiarity of our species of *Pusia* is the presence of the horseshoe-shaped structure in the radular divericulum, which we consider here as a jaw. The jaw is very rarely present in Neogastropoda, so far reported only in Cancellariidae [eg. Harasewych, Petit, 1982] and Volutomitridae [Kantor, Harasewych, 1992]. In these families the jaw is situated close to the mouth opening in the anterior part of the buccal cavity. In our species the described structure is also positioned in the buccal cavity or in the radular diverticulum, but due to the presence of the buccal tube the jaw is shifted far backward from the mouth opening.

Among species belonging to clade 2, the radular morphology is also rather variable. In the clade (which PP support — 1.00), including *Vexillum plicarium* (type species of *Vexillum*), *V. rugosum*, and *V. vulpecula* radula is rather similar, as well as in *V. albofulvum*, which is grouping with the clade without significant support. It is characterized by medium-sized or small radula (in relation to the aperture length) — the ratio RW (radular width)/AL, % differs from 0.7 in *V. plicarium* to 2.0 *V. rugosum*. The central teeth are also small in relation to AL (from 0.5% in *V. plicarium* to 1.1% in *V. rugosum*), but looks broad, since they are short and bear numerous small subequal cusps — 10-16. It should be noted, that even among closely related species the variation can be remarkable. Thus, in *V. plicarium* the radula is nearly 3 times smaller, that in *V. rugosum*. These species also differs in proboscis size and in the size of glandular tube and gland of Leiblein.

#### Functional morphology of foregut

Maes and Raeihle [1975] examined in details the feeding process of *Mitromica foveata* [as *Thala floridana*]. They wrote: "Prey is subdued by a venom which initially causes no signs of trauma but causes ataxia, paralysis and death usually within 4 minutes. The venom penetrates unbroken epidermis. *Thala*'s pink buccal mass and radula are well back from the mouth during attack and the tip of proboscis is unarmed" (p. 54).

Maes and Raeihle specifically mentioned that there were no signs of teeth wear in radula of *Mitromica foveata* and that the massive odontophore can not possibly pass through the narrow oral tube to the mouth and must function within the buccal cavity.

We examined the anterior working part of the radulae in our species. The clear signs of teeth wear (some of the cusps on the central teeth were broken off) were recorded in some species, mostly strongly in *Vexillum subdivisum, Vexillum albofulvum* (Fig. 8C), *Pusia salisburyi*. In these species the buccal tube is either short and poorly convoluted, or even hardly discernible (*V. subdivisum*). In species with very long and convoluted tube no signs of teeth wear were found.

It is not always easy to evaluate the possibility of expanding of the tubular structures basing on dissections of preserved and even live specimens. Recent observations on feeding and swallowing the prey by molluscivorous species of *Conus* [Kantor, 2007] demonstrated that the oral sphincter in *Conus textile* is able to hold a single radular tooth, a fraction of a millimeter in diameter. At the same time, it can expand up to 50 times (to 9 mm) to allow the prey to pass into the oesophagus. Therefore we considered that at least in a species with short and not too convoluted buccal tube the odontophore can pass through the tube to the mouth and used in the prey capture and feeding.

The presence of the toxin in Mitromica foveata was demonstrated by the observations on feeding process. So far this is the only record for the family. Maes and Raeihle [1975] suggested that the source of the toxins is accessory salivary gland, whose duct opens in the close proximity to the mouth. Later Andrews, Elphick and Thorndyke [1991] demonstrated that secretion of the accessory salivary gland in another Neogastropod — Nucella lapillus (Linnaeus, 1758) (Muricidae) indeed cases flaccid paralysis in bivalves. Besides, in all studied neogastropods there is a layer of circular muscle fibers in the wall of the gland. It seems likely, that contraction of the muscle fibers causes the propulsion of the secretion through the duct. Accessory salivary gland (or paired glands) are present in studied costellariids (they were not found in some smaller species on a dissection, which can be explained by the minute size of the glands) and the suggestion of Maes and Raeihle seems plausible.

Another potential source of the toxins can be the salivary glands, which are generally rather large in Costellariidae. Recently it was demonstrated that salivary glands in *Conus pulicarius* probably produce toxins similar to those, secreted in the venom gland [Biggs *et al.*, 2008].

The final conclusion might be done after the examination of the properties of the secretion of both glands, which in itself is a very complicated task.

The function of the most prominent gland of the anterior foregut in Costellariidae — the gland of Leiblein and in many species associated with it glandular tube (originated by stripping off and closing of the glandular folds of mid-oesophagus) remains unclear. Although superficially it is very similar to the venom gland of Conoidea, which most probably originated in a similar way [Ponder, 1970], it can not produce any toxic secretion used for envenomation of the prey. This is confirmed by the fact, that the gland (or the glandular tube) opens *posterior* to the valve of Leiblein (and the nerve ring), which precludes passing of the secretion or content of the mid-oesophagus into anterior oesophagus or proboscis. In Conoidea the valve is absent in most species

and the gland producing toxins passes through the nerve ring and opens into the buccal cavity. Even in the few species, that possess the valve of Leiblein, the gland bypasses it and opens anterior to it [Kantor, Taylor, 2002].

The posterior oesophagus in studied species is usually very narrow (much narrower that the midoesophagus, especially in species, in which the stripping off of the glandular folds is not complete). This may suggest, that the food is passing into stomach (which is very small and simple itself — Ponder, 1972; Maes, Raeihle, 1975; our ovservation) being already significantly digested. This was also suggested by Maes and Raeihle [1975] who thought that *Mitromica foveata* feeds by sucking tissue from the prey rather then consuming large fragments of food. This also explains the presence of a very narrow buccal tube and rather muscular buccal cavity that can act as a pump.

#### Taxonomic implications

Although our dataset of sequenced species is very limited in comparison with overall volume of Costellariidae, some provisional taxonomic conclusions can be drawn.

One of the unexpected outcomes of the molecular tree is suggested close relationships between Latiromitra (Ptychatractidae) and Costellariidae. Latiromitra was in a similar position (with 100% posterior probability support) in a molecular tree of Neogastropoda, obtained ion the basis of five genes and published by Oliverio and Modica [2010]. The anatomy of Latiromitra [Bouchet, Kantor, 2000] is also rather similar to our basal branch of costellariids Costellariidae gen. sp. Although it should be mentioned, that no synapomorphies for Costellariidae and Ptychatractidae in the character of the foregut can be mentioned and their morphology is very basic and underived for Neogastropoda. Previously Ptychatractidae were considered as rather basal branch of Neogastropoda, placed together with Pseudolividae in a separate superfamily Pseudolivoidea [Bouchet, Rocroi, 2005]. Our newly obtained data are clearly opposing the basal position of Ptychatractidae.

Our examined species Costellariidae gen. sp. is constituting the basal branch and according to anatomical characters (including the radular characters) is clearly separate from any other recognized genera of the family. Its radula is very similar to the radula of *Tosapusia isaoi*, the type species of the monotypical genus. Although there are some conchological similarities between two species, without examination of the anatomy of *T. isaoi* the generic position of Costellariidae gen. sp. can not be resolved with certainty.

Pusia has been treated in two different ways, either

as a subgenus within the genus *Vexillum*, or as a distinct genus. Two species examined by us (*Pusia rubra* and *P. salisburyi*) that we attribute to the genus on conchological basis, constitutes a separate genus, although their congenerity with type species of *Pusia* — *P. microzonias* (Lamarck, 1811) needs confirmation.

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Эволюция хищных брюхоногих моллюсков семейства Costellariidae (Neogastropoda) в рамках молекулярной филогении

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На основании исследования последовательности нуклеотидов гена 12S r-PHK шестнадцати видов Costellariidae построена филогенетическая гипотеза. Исследована анатомия переднего отдела пищеварительной системы и радула 15 видов семейства. Передний отдел пищеварительной системы оказался наиболее богат филогенетически значимыми признаками у Costellariidae и у Neogastropoda в целом. Выделено 6 типов организации среднего пищевода, отличающиеся анатомией и характером развития железы Лейблейна, а также наличием и степенью развития железистой трубки, в которую открывается железа. Железистая трубка появляется в результате отслаивания и смыкания дорсальных железистых складок пищевода. На основе молекулярного дерева показано, что наиболее базальный тип строения переднего отдела пищеварительной системы найден у неописанного пока вида и характеризуется неспециализированной железистой железой Лейблейна, открывающейся непосредственно в средний пищевод. Доказано, что образование железистой трубки и трансформация железа Лейблейна в мускулистый мешок происходит у Costellariidae независимо от других ветвей неогастропод. Обсуждается функциональная морфология пищеварительной системы. Наиболее вероятной железой, вырабатывающей токсины, вопреки ожиданиям, является не железа Лейблейна, а дополнительная слюнная или первичные слюнные железы.



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