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Life on a leaf: 3D-reconstruction and description of a new limapontiid sacoglossan (Gastropoda: Heterobranchia: ‘Opisthobranchia’) living on the seagrass *Halophila ovalis*

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ABSTRACT

Sap-sucking sea slugs, Sacoglossa, are almost all suctorial herbivores feeding on siphonalean green algae. *Ercolania* is probably the most species-rich genus in the family Limapontiidae, containing at least 25 species, most of which feed on filamentous green algae of the order Cladophorales. Here we describe a new *Ercolania* species from the Andaman Sea coast of Thailand. The species is very small, generally less than 1 mm long in sexually mature animals. It is found exclusively on the leaves of the seagrass *Halophila ovalis* and accordingly we name it *Ercolania halophilae* n. sp. Anatomy and histology have been studied by serial sectioning and 3D-reconstruction. This is the first limapontioid species studied in full 3D-microanatomical detail. *Ercolania halophilae* shows several special features. A heart and pericardium are absent, as is also seen in a few other species of Sacoglossa. Salivary glands are very prominent and unequal on left and right sides. There is a system of highly vacuolated cells situated along the main digestive gland tubules. Those cells are apparently associated with the kidney and we assume that they are involved in excretion. Radular teeth are sabot-shaped and more similar in shape to those of the enigmatic Japanese *Alderiopsis nigra* than to any known species of *Ercolania*. The reproductive system is typical limapontioid, with a closed seminal receptacle, a penetrant penial stylet and a bilobed prostate gland. Branches of the albumen gland enter the cerata, as seen in some but not all species presently referred to *Ercolania*. We therefore place the species in *Ercolania* rather than the poorly defined *Alderiopsis*. We briefly compare limapontioid genera and show that revision is needed.

INTRODUCTION

With the exception of two species that feed on gastropod eggs (Crane, 1971; Lemche, 1974), all sacoglossan sea slugs are suctorial herbivores. The majority of species feed on the cell sap of siphonalean green algae (Jensen, 1980, 1983a, 1993a, c), but a few species have more specialized diets, feeding on diatoms (Williams & Gosliner, 1973; Jensen, 1981), red algae (Miller, 1961; Lance, 1962; Vogel, 1971; Jensen, 1993b; Trowbridge, Hirano & Hirano, 2009) or seagrasses (Clark, 1975; Jensen, 1982, 1983a, b). Only two species, *Elysia catulus* (Gould, 1870) and *E. serca* Marcus, 1955, both living on the Atlantic coast of North and South America, have been shown to feed on seagrasses. In the present paper a third species, a member of the family

Limapontiidae, is described. It feeds on the cell sap of the seagrass *Halophila ovalis*. This is the first record of seagrass feeding in a limapontioid sacoglossan, and the first record outside the Western Atlantic Ocean. Adult specimens are mostly less than 1 mm in body length and they are therefore easily overlooked in the field. Due to the small size we have used 3D-reconstruction from serial sections. This method has been shown to be a suitable tool to provide reliable morphological data, especially in small heterobranchs, in addition to microdissection and SEM to describe the internal anatomy of the species (Neusser *et al.*, 2006; DaCosta *et al.*, 2007). The genera of the Limapontiidae are defined by combinations of external and internal characters (Jensen, 1996), many of which are variable within a genus and/

or overlapping between genera. We place the new species in the genus *Ercolania* and discuss its relation to other genera.

MATERIAL AND METHODS

Individual leaves of the seagrass *Halophila ovalis* were collected intertidally from four localities on the Andaman Sea coast of Thailand between September 2010 and May 2011, and at Shelly Beach near Townsville, Queensland, Australia in 2002. Leaf blades were 1.0–2.5 cm long. They were carefully examined with a hand-lens or under a dissecting microscope. Even with those aids this miniature sacoglossan is difficult to find. Most of the leaf damage observed on *H. ovalis* was caused by a neritid snail, *Smaragdia souverbiana* (Montrouzier, 1863), so leaf damage could not be used as an indicator of the presence of the new species. Specimens were never found on any other seagrass species.

Specimens

Ten specimens were collected from Ko Phra Thong, Kuraburi District, Phang Nga Province (9°10'06"N, 98°16'50"E) on 12 September 2010 (sample spec. 1), 9 December 2010 (samples spec. 2 and 3) and 22 March 2011 (samples spec. 18–23). Four of these have been dissected. Four specimens were collected at Paklok Bay, Phuket Island (8°1'00"N, 98°25'10"E) on 22 January 2011 (samples spec. 5–8). Nine specimens (three dissected) were collected at Ban Mod Tanoi, Kantang District, Trang Province (7°18'35"N, 99°24'30"E) on 6 February 2011 (samples spec. 9–16). The remaining (*c.* 80) specimens were collected at Ko Mook, Kantang District, Trang Province (7°22'56"N, 99°18'15"E) between 5 and 8 May 2011 (samples spec. 24–56); 10 of those were dissected and 2 serially sectioned for 3D-reconstruction and histological investigation. Specimens were fixed in formalin with the *Halophila* leaf they were collected on. Some of the animals were observed under a dissection microscope for several days before being preserved in formalin and shipped to the Zoological Museum in Copenhagen, Denmark (ZMUC). In addition three specimens from Shelly Beach near Townsville, Australia were externally examined.

Microdissection and SEM

Specimens were dissected and digital photos were taken at different stages of organ removal. Pharynx and penis were dissected out and photographed under light microscopy. Pharynx musculature was dissolved in NaOH and digital photos taken before the tissues were completely dissolved. The radulae were rinsed in demineralized water, transferred to 96% ethanol and air-dried before placing on SEM-stubs. The penis was transferred to 80% ethanol and dehydrated in a series of acetone (see below). It was then air-dried and placed on a SEM-stub. Stubs were palladium-coated and observed in a JEOL JSM-6335F SEM microscope.

Histology

Two paratypes (ZSM Mol 20310524: 0.99 mm long; ZSM Mol 20130525: 0.82 mm long) selected for serial sectioning were decalcified in 1% ascorbic acid overnight and subsequently dehydrated in a graded (30, 50, 70, 90 and 100%) acetone series. Afterwards, specimens were embedded in Epon (Luft, 1961) epoxy resin blocks. Serial sectioning of the embedded specimens was performed on a Microm HM 360 rotation microtome using a Histo Jumbo diamond knife (Diatome, Biel, Switzerland). Ribboned serial sections of 1.5 µm thickness were obtained by adding contact cement to the knife-facing side of the resin block (Ruthensteiner, 2008). Sections were transferred to microscopy slides and stained with methylene blue/azur II (Richardson,

Jarett & Finke, 1960). Slides were sealed with araldite and cover slips (Romeis, 1989) for preservation.

3D-reconstruction

Sections of both paratypes were photographed using a ProgRes C3 digital camera (Jenoptik, Jena, Germany) mounted on a Leica DMB-RBE microscope (Leica Microsystems, Wetzlar, Germany). Images were transformed to an 8-bit greyscale format and adjusted for contrast and brightness. The resulting picture stacks of both series were aligned and specimen ZSM Mol 20310524 was chosen for reconstruction with AMIRA v. 5.2 (Visage Imaging GmbH, Germany) software according to the procedure described by Ruthensteiner (2008). All major organ systems were reconstructed. Anatomical features were compared with the aligned images of the second paratype ZSM Mol 20130525.

SYSTEMATIC DESCRIPTION

Order Sacoglossa von Ihering, 1876

Limapontiidae Gray, 1847

Ercolania Trinchese, 1872

Type species: *Ercolania sioyii* Trinchese, 1872, by subsequent designation (Iredale & O'Donoghue, 1923).

Ercolania halophilae new species

(Figs 1–13)

Type material: A specimen from Ko Mook (6 May 2011), 0.97 mm long and 0.53 mm wide preserved, has been selected as the holotype (ZMUC GAS-330). The remaining dissected and sectioned specimens are paratypes. These have not been given museum registration numbers because very little remained after dissection, but we have referred to the original field collecting numbers to identify specimens in text and illustrations. Also four adult and one juvenile specimens from Ko Mook (7 May 2011) were selected as paratypes and deposited at the Zoological Museum (Natural History Museum) in Copenhagen, Denmark (ZMUC GAS-331). Serially sectioned paratypes have been deposited at Zoologische Staatssammlung München (ZSM Mol 20310524, series 5W3/ ZSM Mol 20130525, series 5W7).

ZooBank registration: urn:lsid:zoobank.org:act805BE935-5EDB-48EA-B4FC-3979BBC4BC0A.

Colour of live animals (Fig. 1): Body mostly transparent with thin green lines from contents of digestive gland (Fig. 1A). In most specimens a white triangular patch, corresponding to kidney, visible middorsally, surrounded by green main branches of digestive gland (Fig. 1A, C). Two main digestive tubules run parallel in posterior part of body. Some dark brown pigment found in variable amounts on body; some specimens with middorsal irregular patch of brown pigment covering white triangular patch, but most with several to numerous tiny specks (Fig. 1C). Green contents of digestive gland in cerata not evenly distributed, but forming reticulate pattern inside digestive gland tubule. White dots scattered over body, especially at tips of cerata. A pair of black dots or short lines on either side of mouth (Fig. 1D). Foot sole unpigmented, though sometimes with fine network of black lines centrally. All epidermal pigmentation disappeared when animals transferred to ethanol for permanent storage.

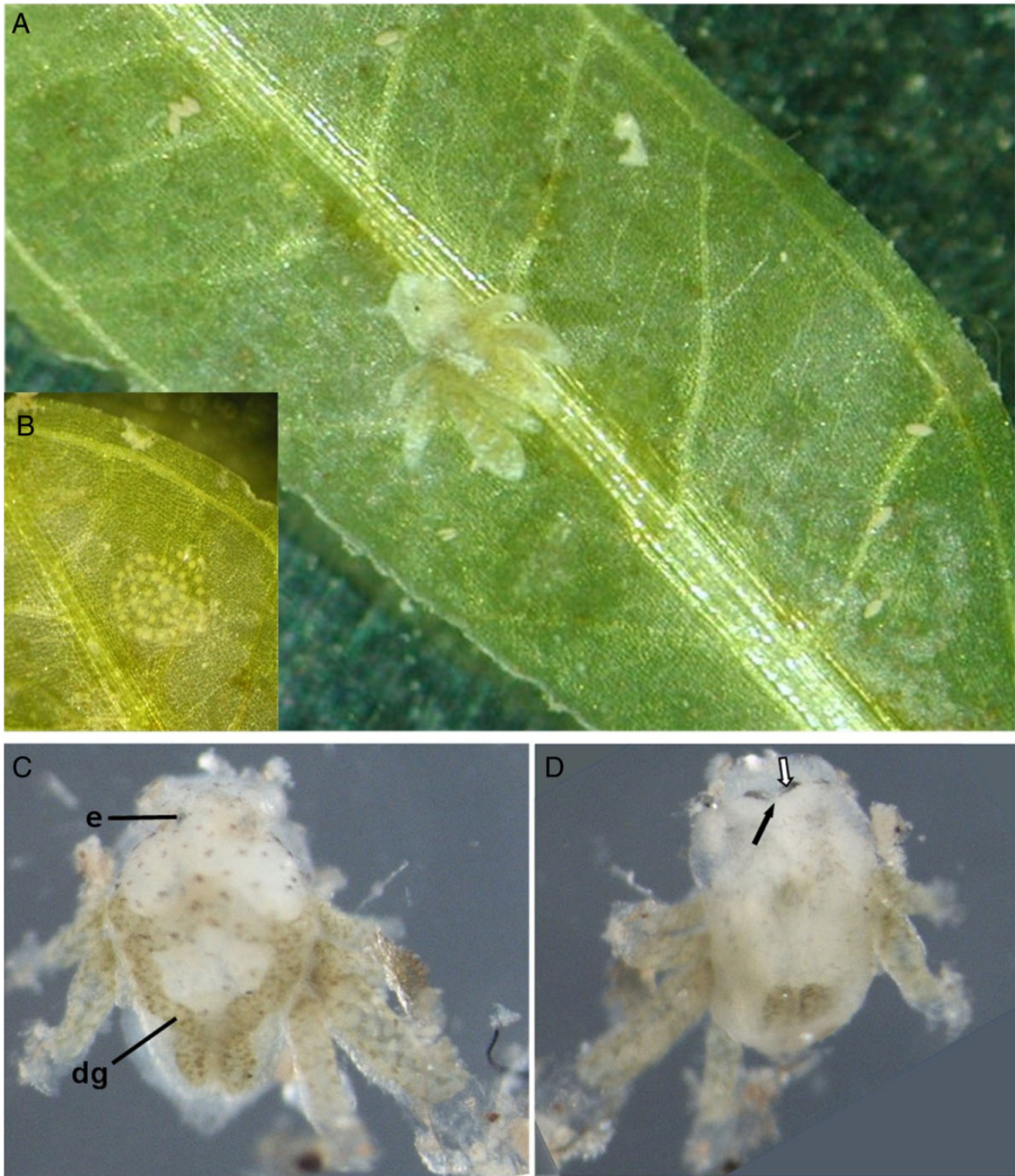


Figure 1. *Ercolania halophilae* n. sp. **A.** Live animal from Australia, on leaf of *Halophila ovalis* (Photo: J. Collins). **B.** Egg mass of animal from Australia (Photo: J. Collins). **C.** Preserved holotype (ZMUC GAS-330), dorsal view. **D.** Preserved holotype, ventral view. Black arrow pointing to median notch in anterior pedal margin; white arrow pointing to black line flanking mouth opening. **C** and **D** were photographed shortly after fixation when content of digestive gland retained greenish brown colouration and epidermal pigment dots were still visible. Abbreviations: dg, digestive gland; e, eye.

External features (Fig. 1): Most specimens less than 1 mm long preserved; maximum length 1.24 mm. Rhinophores short, slightly flattened with rounded tips. Comma-shaped eyes behind rhinophores. Eyes movable; sometimes appear circular with diameter

of about 45 μm . Anterior margin of head broadly rounded, without median notch. Anterior pedal corners rounded, with slight median notch (Fig. 1D). Mouth ventrally in front of notch in foot margin. Up to five cerata on each side in two irregular

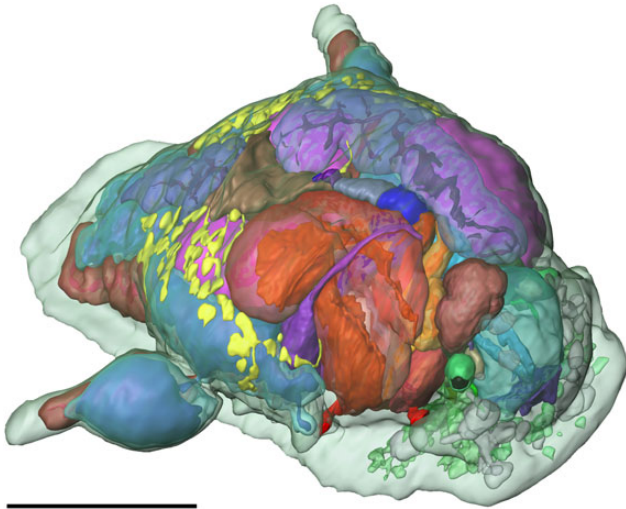


Figure 2. 3D-reconstruction of all major organ systems of *Ercolania halophilae* n. sp. Scale bar: 200 μm .

rows; fourth ceras longest (longer than body). Juveniles 0.27–0.5 mm long have only one fully formed ceras on either side and one or two budding cerata; no rhinophores, only low crests in front of eyes. Main tubules of digestive gland visible through epidermis, forming diamond-shaped pattern dorsally between left and right side cerata (Fig. 1C). Anus indistinct, without anal papilla, flanked by a tiny black dot (probable nephropore). Foot sole broad, posterior end with two branches of digestive gland rounded with only slightly demarcated tip. Male genital opening just behind rhinophore, below eye on right side. Oviduct opening not seen in preserved specimens; no vaginal opening visible.

Internal anatomy: An overview of the 3D arrangements of internal organs is given in Figure 2.

Digestive system (Figs 3–5): Folded oral tube receives ducts from at least two pairs of differently stained oral glands. Pharynx almost spherical, though height often greater than length (Fig. 3A). Length 160–200 μm in dissected specimens. Ascus muscle as long as or longer than pharynx, broad, apparently attached to ventral surface of pharynx over most of its length. Radula of 0.8 mm (preserved length) specimen with three teeth in ascending limb, four fully formed teeth plus three preradular teeth in descending limb. First preradular tooth just a short base, following two with short pointed cusp, remaining teeth sabot (clog)-shaped (Figs 3A, C, 4A, B). Length of leading tooth 60–70 μm . Some specimens with heap of used teeth in ascus (Fig. 3B), descending limb much longer than ascending one, containing seven to nine fully formed teeth; in these specimens ascus muscle separated from ventral surface of pharynx for about half its length. Descending limb of radula surrounded by thin layer of longitudinal muscles, outside that a thick layer of muscles (Fig. 8A) runs obliquely to function as protractors of descending limb of radula.

Massive, sac-like salivary glands (*c.* 300 μm long in reconstructed specimen) paired, but differing in position within animal. Flattened, left gland situated more anteriorly directly under dorsal epidermis. Anterior part of right gland located posterodorsally, continuing ventrally, distally wedged between follicles of gonad, directly above epidermis of foot (Fig. 5). Very narrow, thin-walled salivary ducts emerging from anterior tip of respective gland, connecting to salivary reservoirs, located laterally on each side of pharynx (Fig. 5B, C). Salivary reservoirs

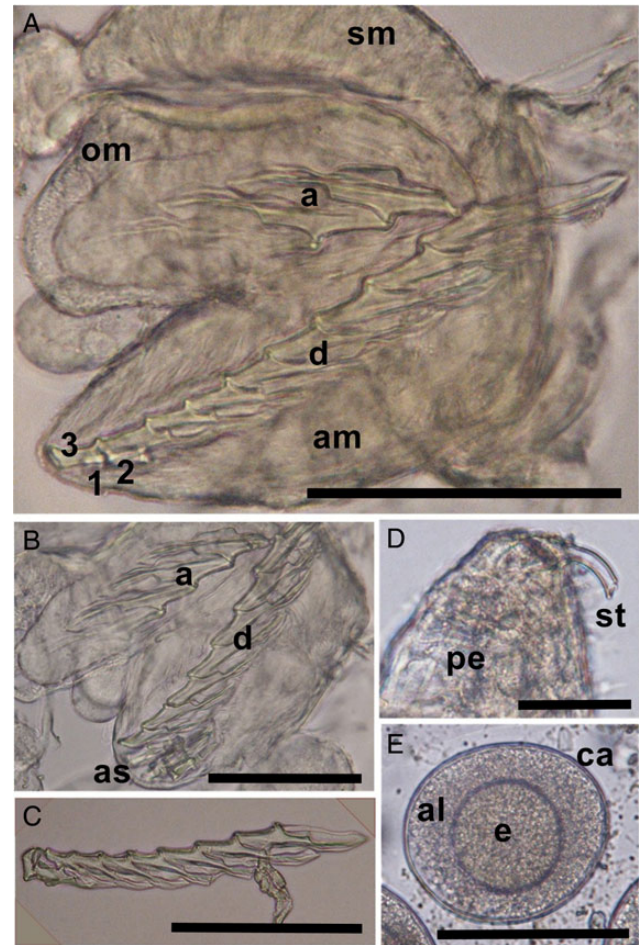


Figure 3. Anatomy of *Ercolania halophilae* n. sp. **A.** Pharynx (specimen 48), partly dissolved in NaOH to make radula visible. **B.** Pharynx of different specimen (spec. 55) with teeth heaped in ascus. **C.** Descending limb of radula of third specimen (spec. 39-2). **D.** Penis (specimen 44-1) with stylet. **E.** Egg from preserved egg mass (5 May 2011) from Andaman Sea, Thailand. Abbreviations: a, ascending limb; al, albumen; am, ascus muscle; as, ascus; ca, egg capsule; d, descending limb; e, uncleaved egg; om, odontophore muscle; pe, penis; sm, dorsal septate muscle; st, penial stylet; 1, 2, 3, preradular teeth. Scale bars: **A, B, C, E** = 100 μm ; **D** = 50 μm .

flattened, disc-like (diameter 30 μm) with muscular wall. Right salivary duct slightly coiled, 130 μm long, with glandular enlargement in its middle part. Short oesophagus emerging from posterodorsal end of pharynx, connecting the latter to small stomach. Columnar, ciliated epithelium of oesophagus and stomach almost identical, therefore transition is gradual not distinct. From stomach, shortly behind pharynx, two digestive gland tubules extend to sides of body; lateral branches enter cerata, main branches run towards midline in tail; digestive gland tubule in cerata unbranched (Fig. 5A, C). From posterodorsal end of stomach, a short, straight, ciliated intestine rises to mediadorsal anus.

Genital system (Figs 6–8): Based on 3D-reconstruction genital system is androdiaulic (Figs 6, 7). Three spherical follicles of hermaphroditic gonad contain oocytes and spermatocytes in various stages of development (Fig. 8B, C, E). Each follicle connects to preampullar gonoduct, which widens to form ventral ampulla extending anteriorly to posterior end of pharynx. In both specimens examined histologically, thin-walled ampulla was tightly packed with spermatozoa (Fig. 8B, C). Densely

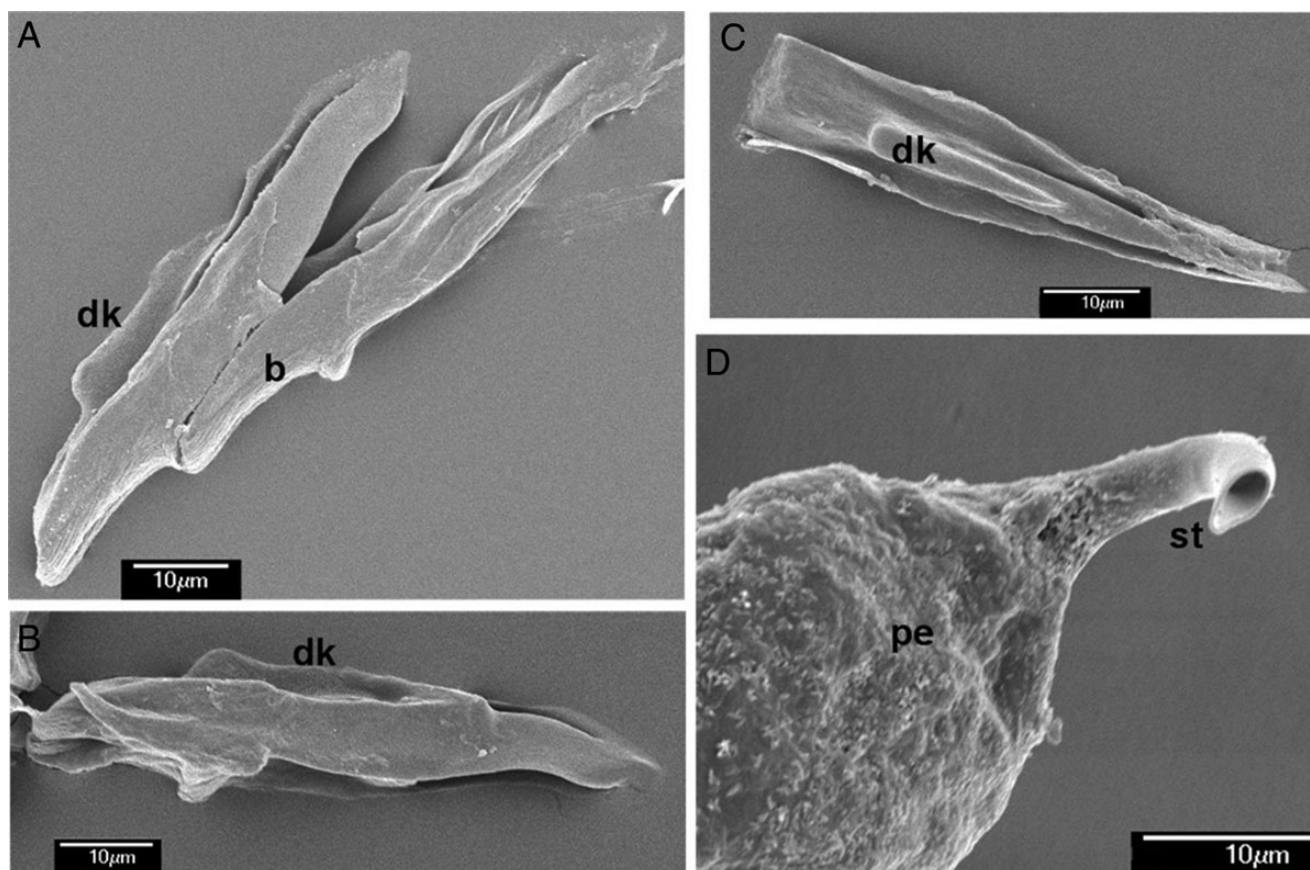


Figure 4. *Ercolania halophilae* n. sp., SEM photos. **A.** Radular teeth (spec. 38-1) from ascending limb, lateral view. **B.** Single tooth from descending limb of same specimen, lateral view. **C.** Single tooth from descending limb of same specimen, back-side view. **D.** Tip of penis with stylet (spec. 55). Abbreviations: b, base; dk, dorsal keel; pe, penis; st, penial stylet.

ciliated postampullar gonoduct splits into vas deferens and wide fertilization area. Vas deferens receives ciliated duct of bipartite prostate gland shortly after separation from postampullar gonoduct (Fig. 6). Prostate gland consists of voluminous cells containing equally sized dark stained granules surrounding central unbranched lumen (Fig. 8B, C). Vas deferens continues anteriorly, entering penis on right side of head (Figs 6, 7). Penis conical with short, curved stylet at tip (Figs 3D, 4D); penis of 1.24 mm (preserved length) specimen 208 μm long; stylet 30 μm long; penis surrounded by penial sheath, opening to outside through male genital opening directly behind right rhinophore (Fig. 7A).

Fertilization area (oviduct loop; Rückert, Altnöder & Schrödl, 2008) receives duct from seminal receptacle and combined duct from paired albumen gland. Anteriorly, genital receptacle (terminology prevalent in Sacoglossa, following Gascoigne, 1976; Jensen, 1996 and others; bursa *sensu* Wägele & Willan, 2000; Kohnert *et al.*, 2013 and others) connects to fertilization area. Genital receptacle located immediately behind pharynx, its content a dense ball of sperm plus unidentifiable material. Dark blue staining albumen gland forms two main tubules running along digestive gland tubules, sending unbranched tubules into larger cerata (Figs 6, 7A, D). No albumen gland in cerata of juvenile specimens. Seminal receptacle on right side of body between anteriormost part of digestive gland and mucus gland (Fig. 8C, G).

Nervous system (Figs 9–11): Central nervous system (CNS) of circumoesophageal, postpharyngeal nerve ring of paired cerebropleural and pedal ganglia (including cerebropedal and

pleuropedal connectives) and visceral loop with two distinct, unpaired ganglia (Fig. 9B, C, F). Left visceral ganglion bigger than right one. We interpret the left ganglion as fused suboesophageal and abdominal ganglia due to its size and number of dispatched nerves, and the right one as the supraoesophageal ganglion, but we did not see signs of fusion in either of the two ganglia. Paired buccal and rhinophoral ganglia present. All ganglia subdivided into outer cortex where perikarya of neurons are situated, and inner medulla exclusively containing nerve fibres. Most paired ganglia (except for rhinophoral ganglia) interconnected via short but strong commissures. No giant neuronal cells present within ganglia.

Most prominent ganglia of CNS are roundish, slightly elongated, totally fused cerebropleural ganglia (*c.* 60 μm long, 29 μm wide, 69 μm high) roofing posterior end of pharynx. Buccal ganglia wedged between ascending and descending limbs of radula. Buccal nerves run laterally alongside pharynx, entering the latter shortly anterior to salivary reservoirs. Distinct statocysts containing one statolith dorsally on pedal ganglia (Figs 9C, F, 11E).

Excretory system (Figs 9, 12): Heart and pericardium absent, although tiny remnant of pericardium may be wedged between kidney and gonad (Fig. 12C). Kidney roughly triangular with narrow end anteriorly, 160 μm long in sectioned specimens, composed of highly vacuolated translucent cells surrounding wide lumen. Short, narrow, straight, ciliated nephroduct emerging dorsally from kidney about one third of its length, connecting latter to nephropore, which opens beside middorsal anus. An enigmatic structure, composed of giant, rhogocyte-like cells

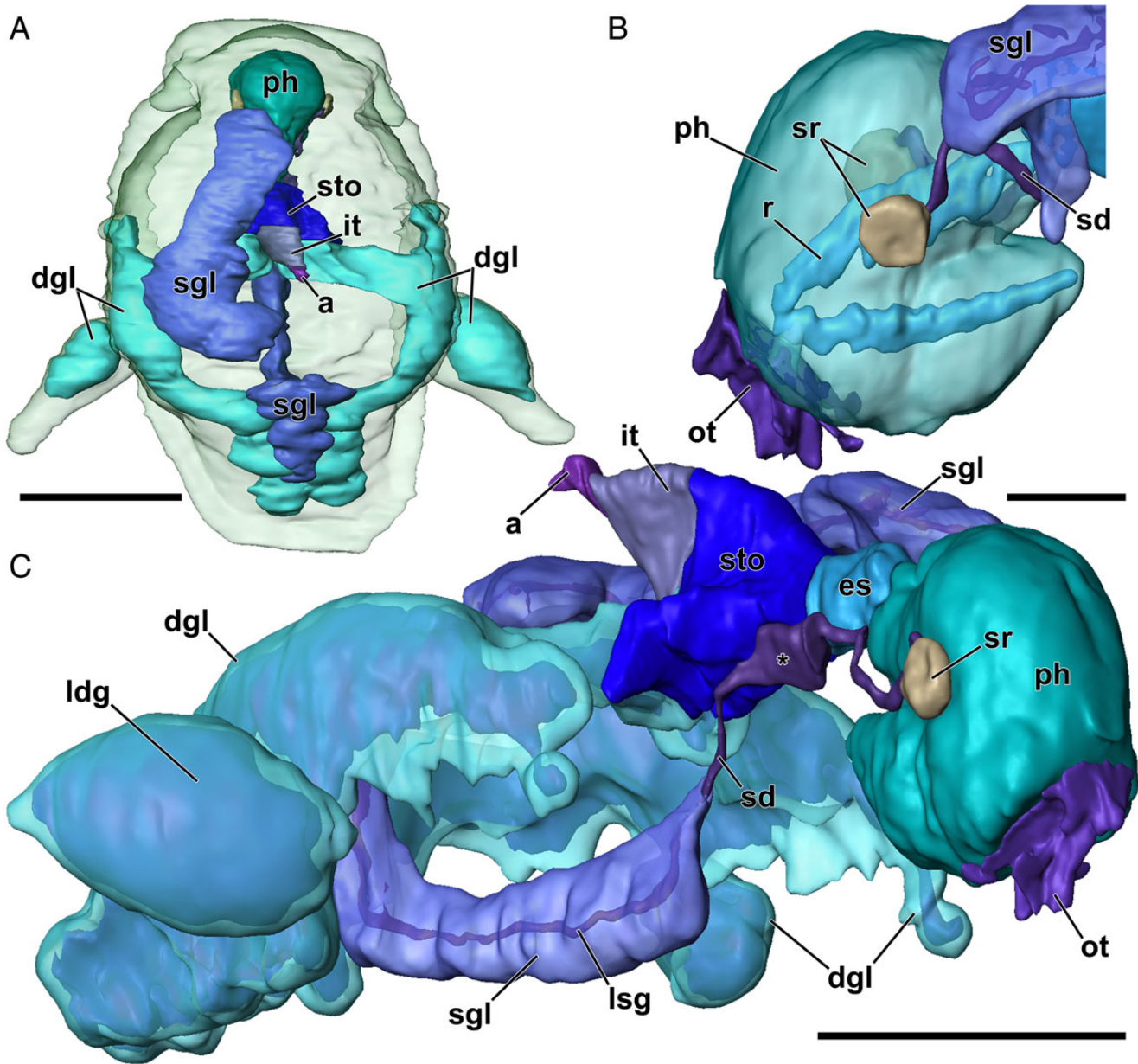


Figure 5. 3D-reconstruction of the digestive system of *Ercolanina halophilae* n. sp. **A.** Localization of digestive system within animal, dorsal view. **B.** Left lateral view of pharynx. **C.** Right anterolateral view of the complete digestive system (*note glandular swelling of right salivary duct). Abbreviations: a, anus; dgl, digestive gland; es, oesophagus; it, intestine; ldg, lumen of digestive gland; lsg, lumen of salivary gland; ot, oral tube; ph, pharynx; r, radula; sd, salivary duct; sgl, salivary gland; sr, salivary reservoir; sto, stomach. Scale bars: **A** = 200 μm ; **B** = 50 μm ; **C** = 150 μm .

(25 μm diameter) with large, light blue stained nucleus and numerous, predominantly nonstained vacuoles, located left and right of kidney (Figs 9D, E, 12). Indefinable, extremely thin structures interconnect these cells forming reticulate structure overlying gonad and main branches of digestive gland, directly under dorsal epidermis.

Subepidermal glands and special features (Fig. 13): Irregularly shaped, voluminous (diameter to 50 μm), subepidermal glands concentrated laterally and dorsally inside head area, and inside lateral foot margin (Fig. 13A: gl1); content homogeneously stained light grey. Long, narrow ductules open to epidermal surface. Pedal gland with numerous subepithelial glandular follicles located in foot area posterior to mouth. Pedal gland

follicles connected to densely ciliated epidermis on underside of foot via separate ductules (Fig. 13D). Follicle cells with big, light blue stained nuclei and dark blue stained, granular content. Epidermis of cerata with two equally sized but differently stained, glandular cell types, always in direct vicinity of one another (Fig. 13B); type I with dark violet stained and granular content; type II with a basal, light blue nucleus and single, unstained voluminous vacuole. Apical ductules of both cell types open to outside close to one another.

An enigmatic feature only found in reconstructed specimen (ZSM Mol 20310524) is a paired, roundish structure (diameter 40 μm) of unclear function and identity; it is located at same level as statocysts beneath epidermis in fold between foot and body; flat epithelium with dark stained, spherical structure

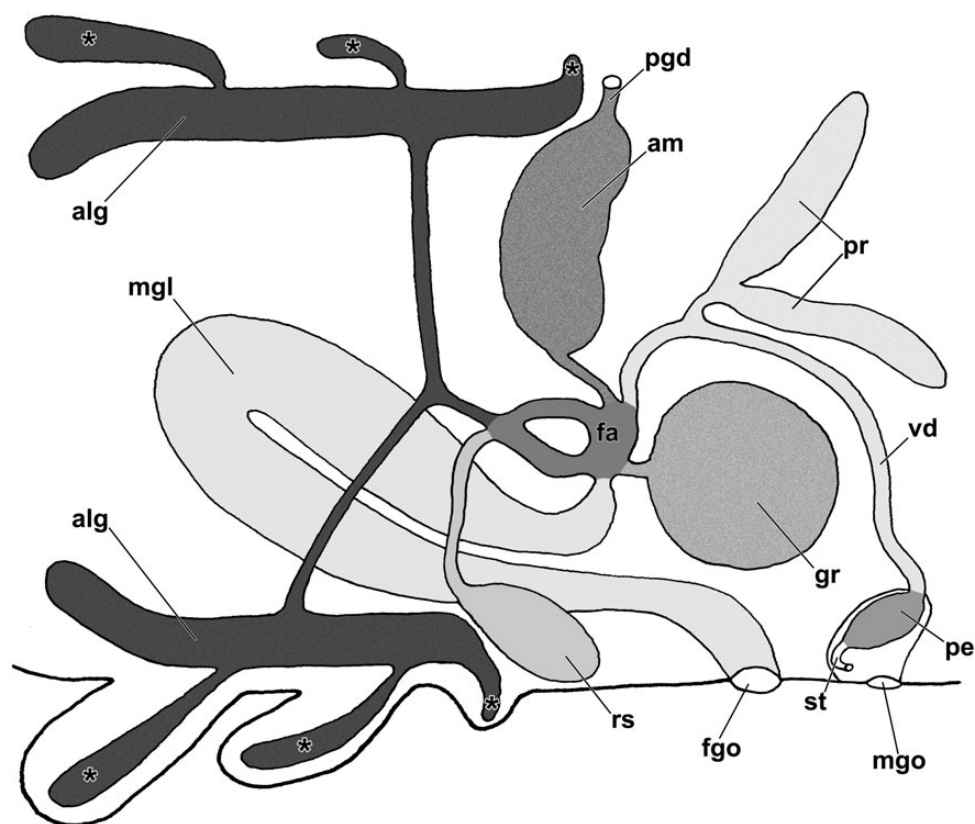


Figure 6. Schematic overview of the genital system of *Ercolania halophilae* n. sp. (modified after Schmekel & Portmann, 1982). Asterisks indicate the portions of the albumen gland entering the cerata. Abbreviations: alg, albumen gland; am, ampulla; fa, fertilization area; fgo, female genital opening; gr, genital receptacle; mgl, mucus gland; mgo, male genital opening; pe, penis; pgd, proximal gonoduct; pr, prostate; rs, seminal receptacle; st, stylet; vd, vas deferens.

attached to inner wall surrounds nonstained lumen (Fig. 13A, C); no innervation, ciliation or opening to outside was detected.

Observations of living animals: Feeding seems to be extremely fast. Apparently only one cell layer is penetrated, and the feeding-tracks are not very distinct. The intestine is emptied by a strong contraction of one of the large cerata. The cerata pulsate regularly and contents of the digestive gland move around. Pulsation may also serve as substitution for the absence of a heart. The green colour of the digestive gland tubules turns brown soon after collecting the animals, indicating that no functional chloroplasts are taken up by this species.

Spawn: Egg masses coiled with eggs usually in single row (Fig. 1B); eggs pale yellow. Egg capsules of uncleaved eggs contain granular albumen (Fig. 3E). Most egg masses contain 10–30 eggs. An egg mass containing 18 uncleaved eggs had capsule diameters of $117 \times 102 \mu\text{m}$ and egg diameter of $66 \mu\text{m}$ ($n = 10$). In another egg mass containing 34 embryos at early veliger stage, capsules measured $97 \times 80 \mu\text{m}$ ($n = 12$).

DISCUSSION

Taxonomic placement

The new species belongs to the family Limapontiidae. It has fusiform cerata, a dorsal anus and rhinophores, but no oral or pedal tentacles. Also, the reproductive system is the typical limapontiid type (Gascoigne, 1976) with a genital receptacle

connected only to the central fertilization area, never to the distal oviduct or vagina or opening directly to the outside. Generic placement is more complicated. None of the limapontiid genera can be characterized by a single apomorphic character (Jensen, 1996). Many morphological characters of the limapontiid genera, e.g. the shape of rhinophores, presence of an anal papilla, branching of digestive and albumen glands, are highly variable within genera and there is also some overlap of characters between genera (Table 1). The sabot-shaped radular teeth of the present species could place the latter in any of the genera *Ercolania*, *Limapontia*, *Alderia* and *Alderioopsis*, though *Limapontia* species have no cerata and it can therefore be excluded. The two currently recognized species of *Alderia* lack rhinophores, have numerous cerata and a long posterior anal papilla. The genus *Alderioopsis* is rather poorly defined (see below) and hence we place the new species in the genus *Ercolania* and name it *E. halophilae* for its association with the seagrass *Halophila ovalis*.

In the original description of the genus *Ercolania*, Trinchese (1872) did not distinguish between generic and specific characters and for many years *Ercolania* was considered a junior synonym, or at the most a subgenus, of *Stiliger* Ehrenberg, 1831 (e.g. Baba, 1938, 1959; Marcus & Marcus, 1956, 1960, 1970). Trinchese's description included an elongate renopericardial ridge, a middorsal anus at the anterior edge of the pericardium, long, thin rhinophores grooved externally, branched digestive gland tubules in inflated cerata, curved penial stylet and radular teeth without denticles but with rounded tips [later called spoon-shaped (Eliot, 1904) or sabot-shaped (Gascoigne, 1977)]. Following the redescription by Baba & Hamatani (1970a) of the

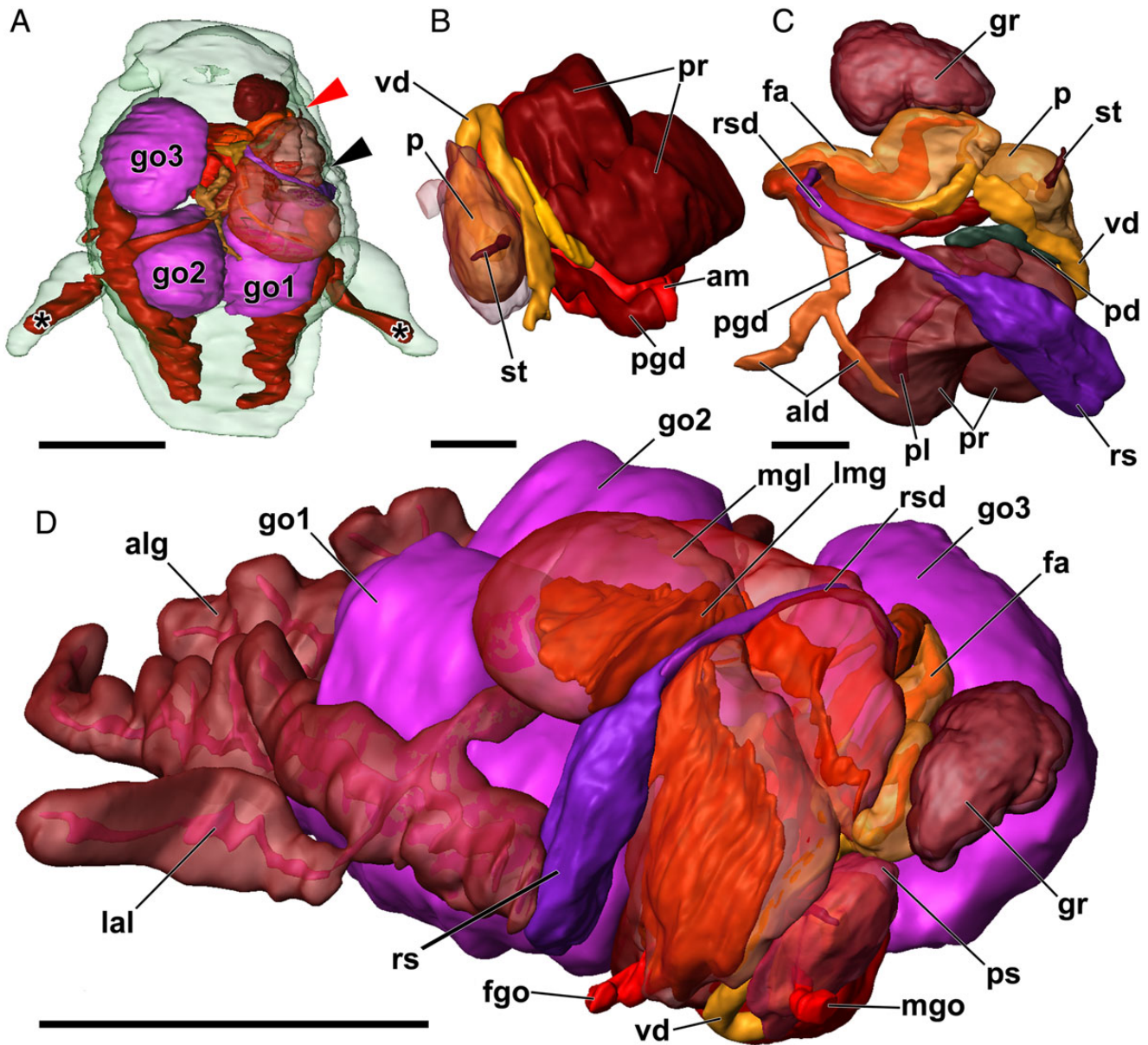


Figure 7. 3D-reconstruction of the genital system of *Ercolania halophilae* n. sp. **A.** Localization of the genital system in the specimen, dorsal view. Note albumen glands entering the cerata (asterisk), arrowheads mark the positions of genital openings (red, male genital opening; black, female genital opening). **B.** Copulatory apparatus. **C.** Glands and ducts in direct vicinity of the copulatory apparatus. **D.** Complete genital system, right anterolateral view. Abbreviations: ald, albumen gland duct; alg, albumen gland; am, ampulla; fa, fertilization area; fgo, female genital opening; go1-3, three gonad follicles; gr, genital receptacle; lal, lumen of albumen gland; lmg, lumen of mucus gland; mgl, mucus gland; mgo, male genital opening; p, penis; pd, prostate duct; pgd, postampullary gonoduct; pl, prostate lumen; pr, prostate; ps, penial sheath; rs, seminal receptacle; rsd, receptaculum seminis duct; st, stylet; vd, vas deferens. Scale bars: **A, D** = 200 μm ; **B, C** = 50 μm .

type species of *Stiliger*, *S. ornatus* Ehrenberg, 1831 with blade-shaped teeth and no penial armature, *Ercolania* was reinstated at full generic rank and all cerata-bearing limapontiids with simple or flattened rhinophores and smooth sabot-shaped teeth were transferred to this genus (Baba & Hamatani, 1970b). Although this was accepted by most subsequent authors, some maintained *Ercolania* as a junior synonym (Thompson, 1973) or a subgenus (Marcus, 1982) of *Stiliger*.

The diagnosis of *Ercolania* has been discussed and amended every time a new species has been added (e.g. Jensen, 1985, 1993b). Most recently Wägele *et al.* (2010) gave a very broad definition: presence of cerata, which are fusiform or inflated; digitiform rhinophores, which are circular or slightly flattened in

cross-section; elongate or inconspicuous renopericardial prominence; presence of sabot-shaped teeth; presence of a curved penial stylet; probable absence of albumen gland in the cerata. *Ercolania halophilae* described in this study is only consistent with three of these features, namely the presence of fusiform cerata, sabot-shaped teeth and a curved penial stylet. The rhinophores are short, in preserved specimens reduced to small triangular prominences, and the pericardium is (almost) absent. Reduced or absent rhinophores also occur in *Limapontia*, *Alderioopsis* and *Alderia* (Table 1), and heart and pericardium appear absent in the two species of *Alderia* and in *Placida viridis* (Trinchese, 1873) (Evans, 1953; Gascoigne & Sordi, 1980; Krug *et al.*, 2007) and also in the basal plakobranchoid *Platyhedyle denudata* Salvini-

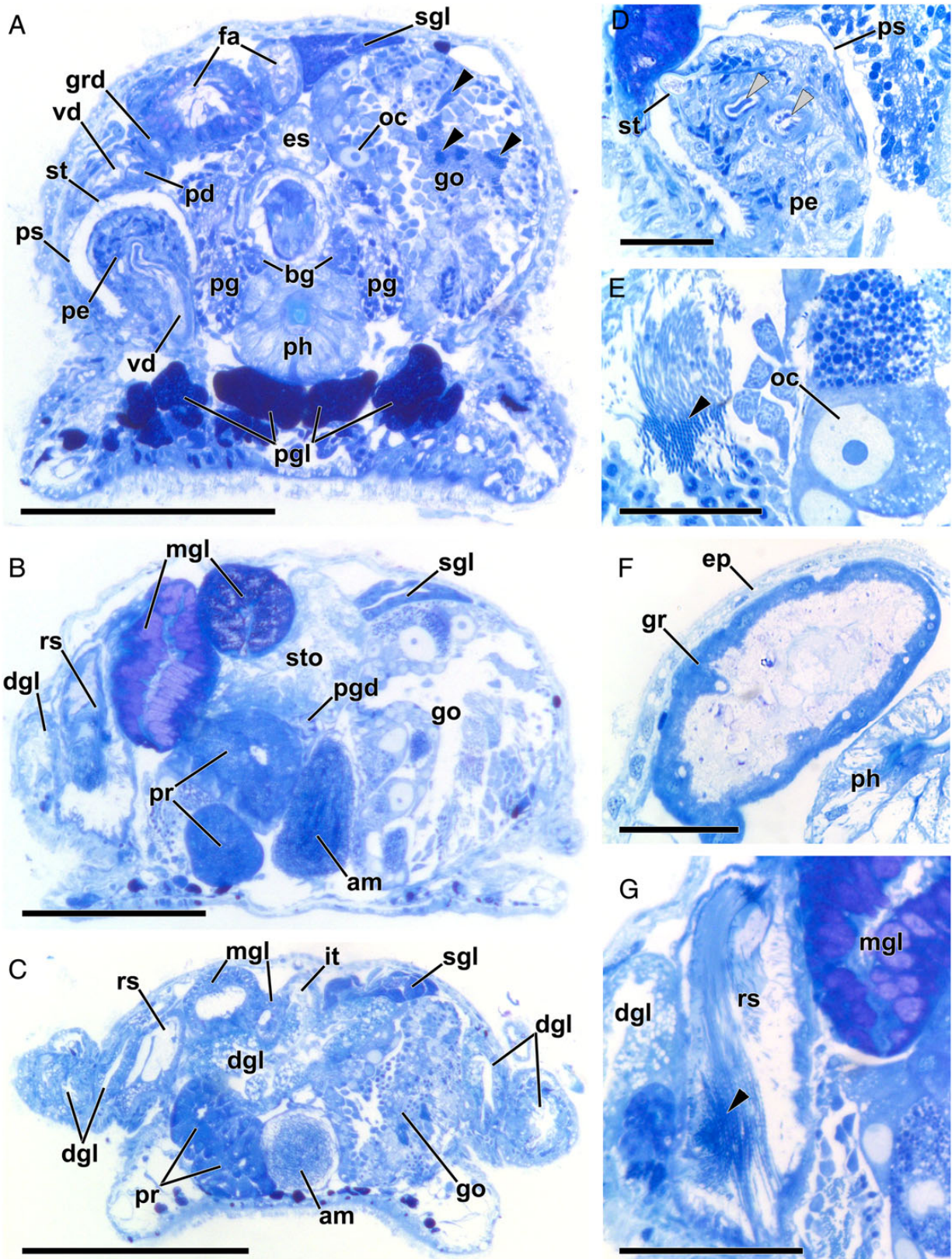


Figure 8. Histological cross-sections of *Ercolania halophilae* n. sp. **A.** Anterior genital organs. Arrowheads indicate spermatozoa. **B, C.** Cross-section of posterior genital organs of different specimens. **D.** Copulatory organ. Arrowheads pointing to vas deferens coils. **E.** Spermatozoa (arrowhead) and oocytes inside gonad follicle. **F.** Genital receptacle. **G.** Seminal receptacle. Arrowhead pointing to spermatozoa. Abbreviations: am, ampulla; bg, buccal ganglion; dgl, digestive gland; ep, epidermis; es, oesophagus; fa, fertilization area; go, gonad; gr, genital receptacle; it, intestine; mgl, mucus gland; oc, oocyte; pe, penis; pd, prostate duct; ph, pharynx; pg, pedal ganglion; pgl, pedal gland; pr, prostate; ps, penial sheath; seminal receptacle; sgl, salivary gland; st, stylet; sto, stomach; vd, vas deferens. Scale bars: **A–C** = 200 μm ; **D–F** = 50 μm ; **G** = 100 μm .

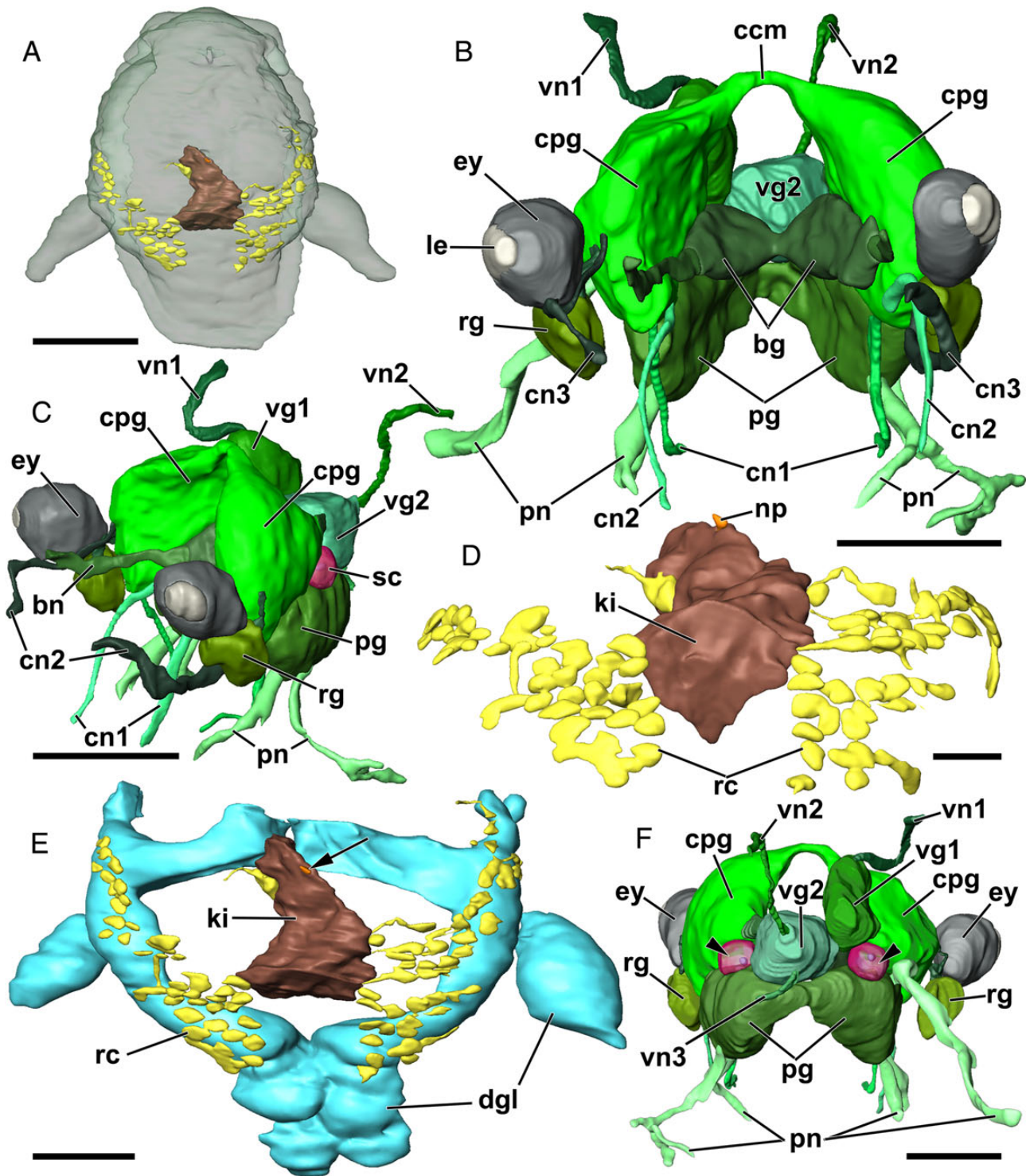


Figure 9. 3D-reconstructions of the nervous and excretory systems of *Ercolania halophilae* n. sp. **A.** Location of the excretory system within the animal, dorsal view. **B.** Frontal view of the central nervous system (CNS). **C.** Left anterolateral view of the CNS. **D.** Kidney and potential rhogocytes, posterior view. **E.** Overlay of reconstructions of excretory system and digestive gland, dorsal view. Arrow indicates position of nephropore. **F.** CNS, posterior view. Arrowheads indicate statocysts. Abbreviations: bg, buccal ganglion; bn, buccal nerve; ccm, cerebropleural commissure; cn1, cerebral nerve 1; cn2, cerebral nerve 2; cn3, cerebral nerve 3; cpg, cerebropleural ganglion; dgl, digestive gland; ey, eye; ki, kidney; le, lens; np, nephropore; pg, pedal ganglion; pn, pedal nerve; rc, potential rhogocytes; rg, rhinophoral ganglion; sc, statocyst; vg1, right visceral ganglion; vg2, left visceral ganglion; vn, visceral nerve. Scale bars: **A** = 200 μm ; **B–D, F** = 50 μm ; **E** = 100 μm .

Plawen, 1973 (see Rückert *et al.*, 2008). The albumen gland of *E. halophilae* definitely enters the cerata and even extends further distally than the digestive gland. The albumen gland in cerata is

found in several species presently included in the genus *Ercolania*, e.g. *E. boodleae* (Baba, 1938), *E. gopalai* (Rao, 1937) and *E. evelinae* (Marcus, 1959) (Baba & Hamatani, 1970b).

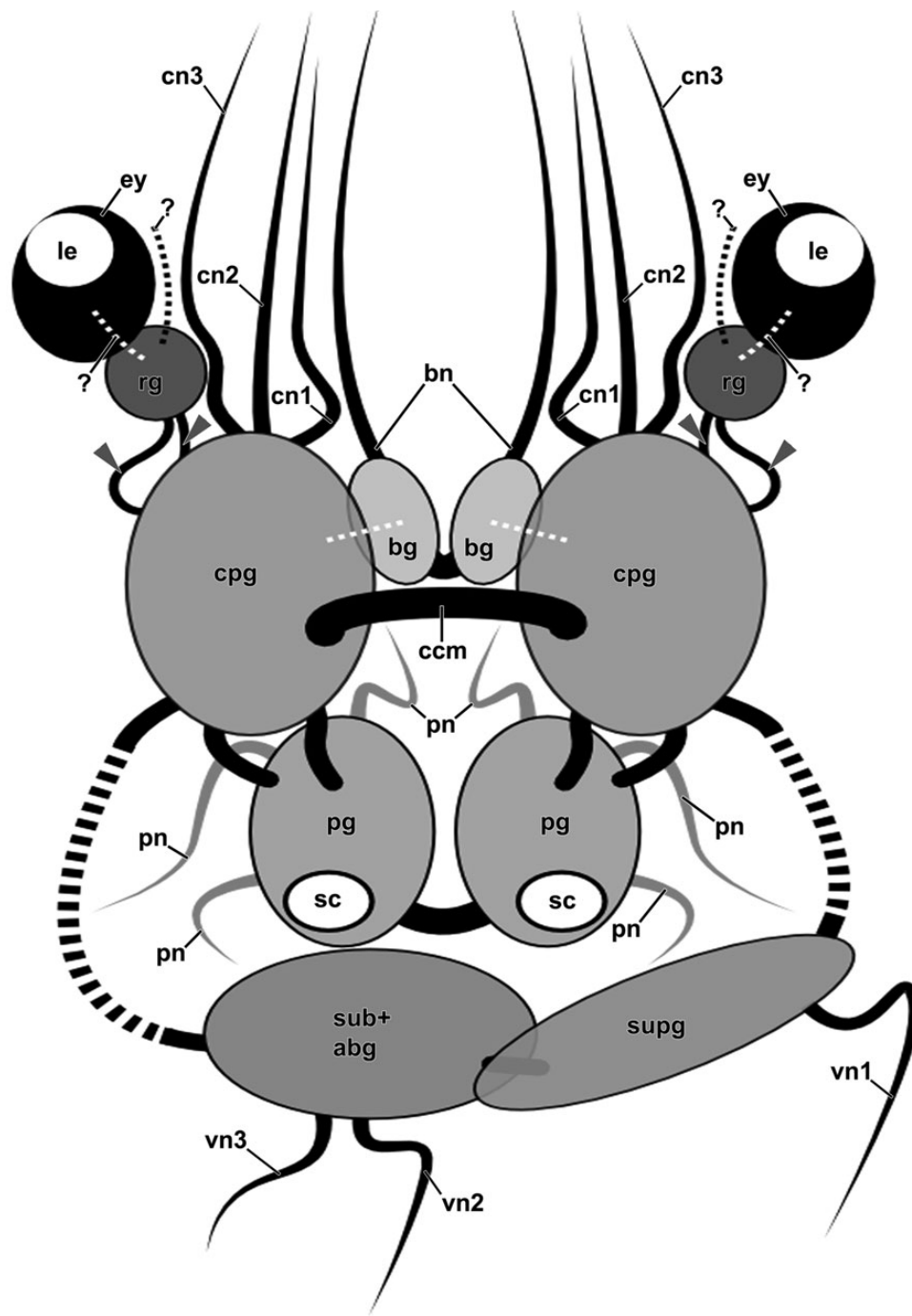


Figure 10. Schematic overview of the CNS of *Ercolania halophilae* n. sp., dorsal view. Short dotted lines: assumed connections, not detectable. Connectives of cerebroplesural ganglia and visceral ganglia not to scale (long, heavy dotted lines). Note double root of rhinophoral ganglion (arrowheads). Abbreviations: bg, buccal ganglia; bn, buccal nerves; ccm, cerebral commissure; cn1, cerebral nerve 1; cn2, cerebral nerve 2; cn3, cerebral nerve 3; cpg, cerebroplesural ganglion; ey, eye; le, lens; pg, pedal ganglion; pn, pedal nerves; rg, rhinophoral ganglion; sc, statocyst; sub+abg, fused suboesophageal and abdominal ganglion; supg, supraoesophageal ganglion; vn1, visceral nerve 1; vn2, visceral nerve 2; vn3, visceral nerve 3.

Baba (1968) created the genus *Alderopsis* for the Japanese species *Alderia nigra* Baba, 1937. This species is similar to the present new *Ercolania* species in a number of characters, but also differs in several characters. *Alderopsis nigra* has a broad foot and relatively few cerata with an unbranched tubule of digestive gland. Also, the anus is not on a papilla. However, the size is somewhat bigger, 2–3 mm for adult specimens, and the albumen gland does not enter the cerata. Furthermore *A. nigra* has a heart and many small hermaphroditic follicles (Baba, 1968). The

radular teeth were originally described as blade-shaped (Baba, 1937). Judged from the drawings in the redescription (Baba, 1968), they seem to be of a shape similar to those of *E. halophilae*, although more numerous and bigger in *A. nigra* than in *E. halophilae*. There are discrepancies between the original description (Baba, 1937), according to which the teeth are about 83–100 μm , and the subsequent redescription, according to which they are over 200 μm long (Baba, 1968). *Alderopsis* was not included in the morphology-based phylogenetic analysis of

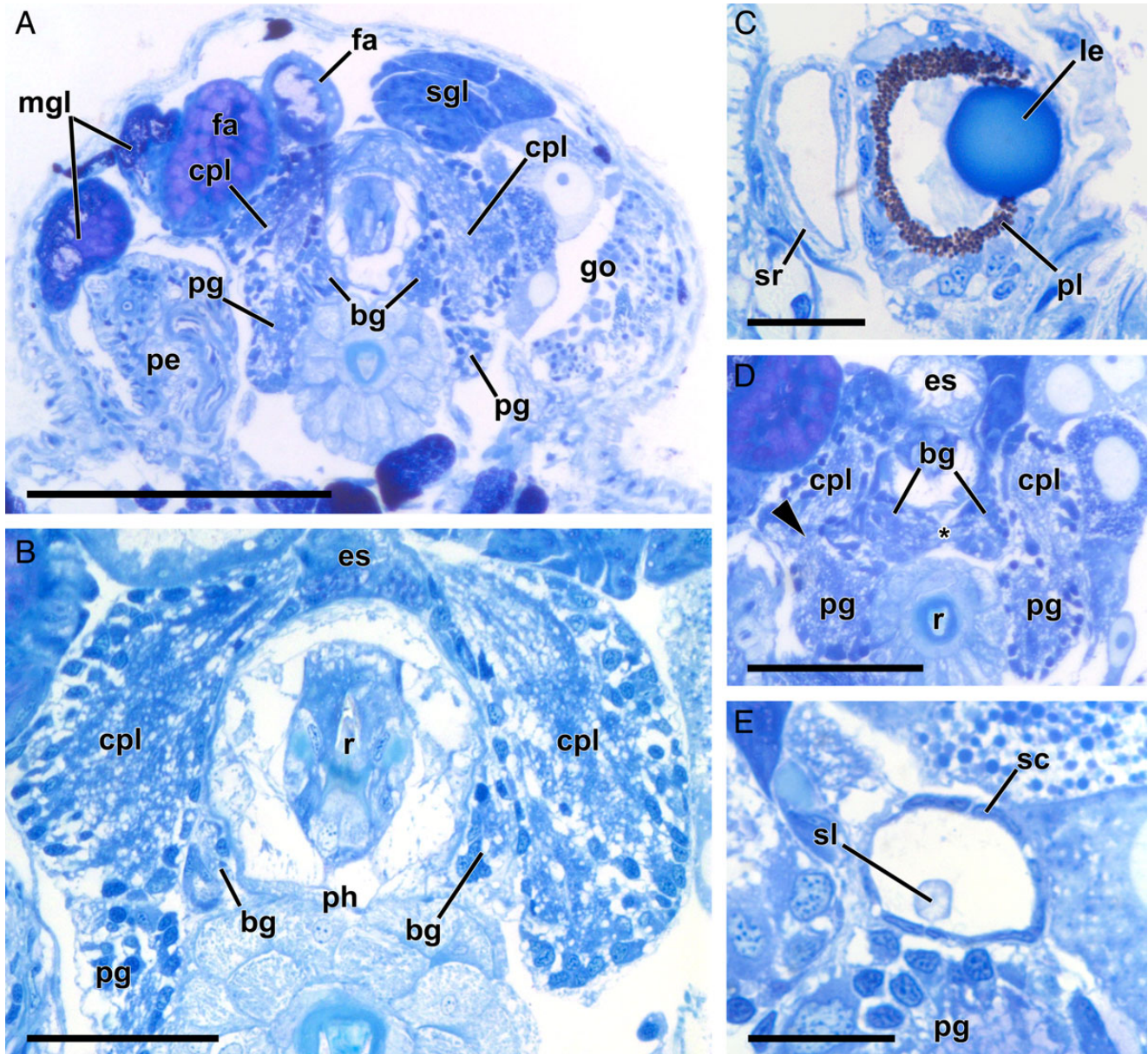


Figure 11. Semithin cross-sections of the CNS and sensory organs of *Ercolania halophilae* n. sp. **A.** Cross-section of the anterior body showing CNS and surrounding organs. **B.** Cerebroleural ganglia roofing posterior end of pharynx. **C.** Cross-section of the eye. **D.** Ganglia of the postpharyngeal nerve ring; note cerebropedal connective (arrowhead) and buccal commissure (asterisk). **E.** Statocyst on top of pedal ganglion. Abbreviations: bg, buccal ganglion; cpl, cerebroleural ganglion; es, oesophagus; fa, fertilization area; go, gonad; le, lens; mgl, mucus gland; pe, penis; pg, pedal ganglion; ph, pharynx; pl, pigmented layer; r, radula; sc, statocyst; sgl, salivary gland; sl, statolith; sr, salivary reservoir. Scale bars: **A** = 200 μ m; **B** = 50 μ m; **C**, **E** = 25 μ m; **D** = 100 μ m.

sacoglossan genera (Jensen, 1996), due to lack of information on many characters. If the teeth are really sabot-shaped, *A. nigra* falls within the current definition of *Ercolania*, and its status as a separate genus is questionable. It should be noted that *A. nigra* and its egg masses have been found on the seagrass *Zostera* (Baba, 1937, 1968). The salivary glands, which are extremely prominent in *E. halophilae* (present study), may constitute a diet-related similarity like those of *A. nigra*, in which salivary glands were described as ‘exceedingly elongated’ (Baba, 1968). Caballer, Ortea & Espinosa (2006) described a new species as *A. garfio* from Cuba. However, this has little resemblance to either *A. nigra* or *E. halophilae* and the species was even called *Stiliger garfio* in the figures of the original description.

Comparison with congeners

Although sacoglossans are generally small animals, few species reach sexual maturity at a size of only 1 mm as in *E. halophilae*. *Ercolania talis* (Marcus & Marcus, 1956) was described as being sexually mature at 1.2 mm (Marcus & Marcus, 1956; as *S. talis*). That species was questionably synonymized with the more common West Atlantic species *E. fuscata* (Gould, 1870) (Jensen & Clark, 1983). Also, *E. translucens* Jensen, 1993 can reach sexual maturity at less than 2 mm body length (Jensen, 1997). *Ercolania irregularis* (Eliot, 1904) is known from only two specimens collected from Zanzibar (western Indian Ocean), both less than 2 mm, but it is not known whether they were sexually mature, and only a

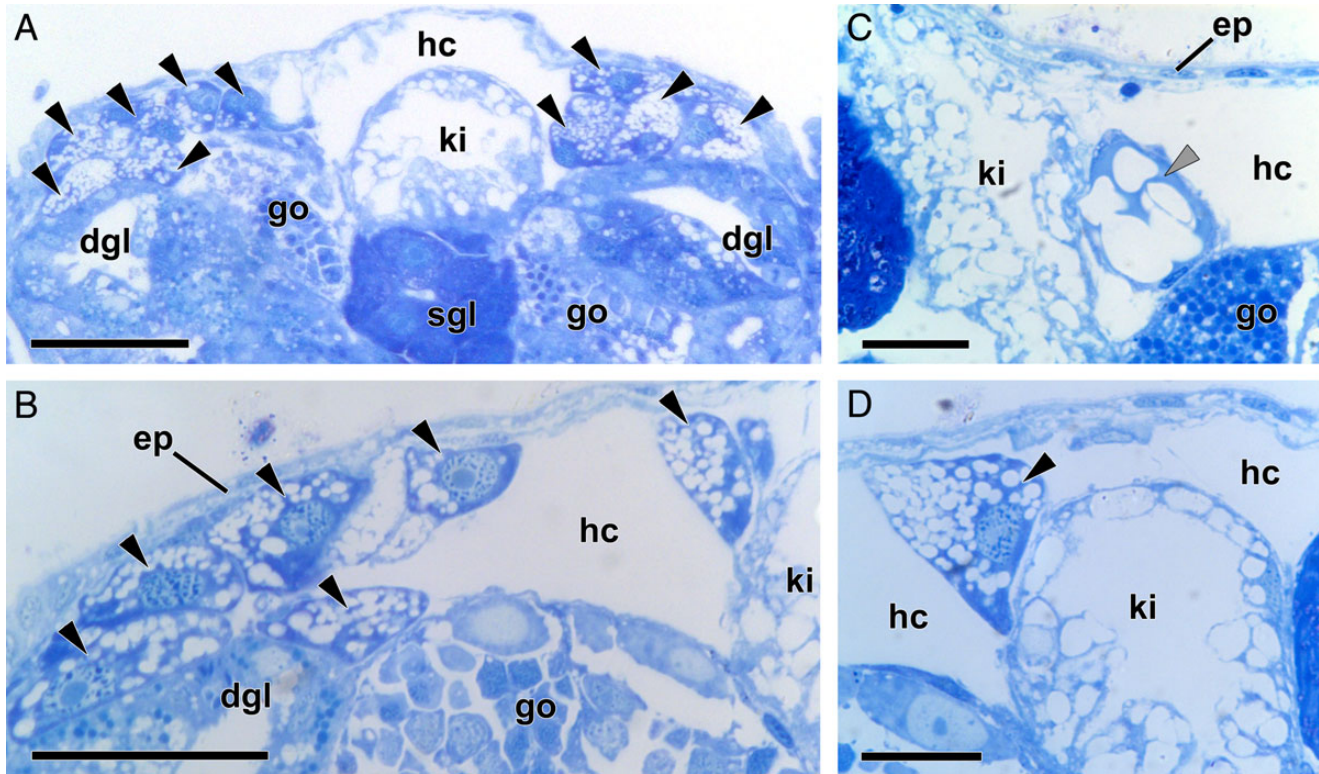


Figure 12. Semithin cross-section of the kidney and adherent potential rhogocytes (black arrowheads) of *Ercolania halophilae* n. sp. **A.** Cross-section through posterior half of the body of spec. 5W7. **B.** Dorsal cells in spec. 5W3. **C.** Putative leftover of pericardium (grey arrowhead) in spec. 5W3. **D.** Kidney and dorsal cells in specimen 5W3. Abbreviations: ep, epidermis; hc, haemocoel; dgl, digestive gland; go, gonad; ki, kidney; sgl, salivary gland. Scale bars: **A** = 100 µm; **B** = 50 µm; **C, D** = 25 µm.

sabot-shaped radular tooth has been illustrated. The description mentions that the cerata are cylindrical and that the posterior ones are twice as long as the anterior ones (Eliot, 1904; as *S. irregularis*). In *E. halophilae* the fourth ceras out of five is the longest and may be longer than the body. A relatively broad foot as seen in *E. halophilae* is also found in *E. coerulea* Trinchese, 1892. This has been related to feeding on algae that are broader than the body of the slug (Jensen, 1981). A broad foot also occurs in *Alderia* species and in *A. nigra*. The latter has been found on seagrass, whereas the former are found on intertidal mud-flats.

As the current definition of *Ercolania* is very broad we will discuss some characters that have not been used for species delimitation before. One such character is the branching pattern of digestive gland tubules. In species like *Placida dendritica* (Alder & Hancock, 1843) the digestive gland branches throughout the body, including head, rhinophores and pericardial hump as well as into the cerata (Schmekel & Portmann, 1982). There are no digestive gland tubules entering the head and rhinophores in *E. halophilae*. The same is seen in several other species of *Ercolania*, e.g. *E. fuscata* (see Gascoigne, 1978), *E. gopalai* and probably *E. boodleae* (judged from figures in Baba & Hamatani, 1970b), whereas other species of the genus, e.g. *E. coerulea* and *E. viridis* (Costa, 1866), have more or less finely branched tubules of digestive gland in the anterior part of the head and in the rhinophores (Schmekel & Portmann, 1982). In *E. translucens* and *E. annyleorum* Wägele *et al.*, 2010, the main branches of the digestive gland enter the head, but do not reach into the rhinophores (Jensen, 1993b; Wägele *et al.*, 2010). This character unfortunately has not been included in many species descriptions, but it should be further analysed in the future.

All species of *Ercolania* have a dorsal anus. However, there is considerable variation in the position of the anus relative to the

renopericardium. In *E. coerulea* and *E. viridis* the anus is on a papilla on the anterior edge of the pericardium (Schmekel & Portmann, 1982); in *E. boodleae*, *E. gopalai* and *E. translucens* the anus is not on a papilla, but is located at the anterior right border of the pericardium, and in *E. margaritae* Burn, 1974, the anus is on a papilla on the right posterior side of the pericardial hump (Gascoigne, 1976). As the pericardium is absent in *E. halophilae* it is not possible to give its relative position. However, it is approximately middorsal, which is where the heart and pericardium would be expected.

Sabot-shaped smooth teeth are considered apomorphic in the limapontiid genera *Ercolania*, *Limapontia*, *Alderia* and *Alderiopis* (see Jensen, 1996). Details in the shape of these teeth differ among species. This has been attributed to differences in diet. The present study indicates that these differences in details should be analysed in relation to phylogeny as SEM pictures of more species become available. The shape of the radular teeth of *E. halophilae* is sabot-like, but slightly different from the shape of other species of *Ercolania*, e.g. *E. nigra* (Lemche, 1935) and *E. annyleorum* (see Jensen, 1993c: fig. 12; Wägele *et al.*, 2010: fig. 3). In those species there is a cavity in front of the dorsal keel for accommodating the tip of the neighbouring tooth, but no cavity surrounding the dorsal keel. In *E. endophytophaga* Jensen, 1999, the cavity is very big, corresponding to the inflated tip of the teeth. The keel is very small and there is a narrow cavity along the anterior part of the keel (Jensen, 1999). The arrangement of the dorsal keel in *E. halophilae* (Fig. 4A, B) seems more similar to that of *Alderia modesta* (Lovén, 1844) (see Bleakney, 1988). However, the teeth of *A. modesta* are much larger than those of *E. halophilae* and also more numerous. Unfortunately the back-side of the teeth of *A. willowi* Krug *et al.*, 2007, is indistinct in the figures accompanying the original description (Krug *et al.*,

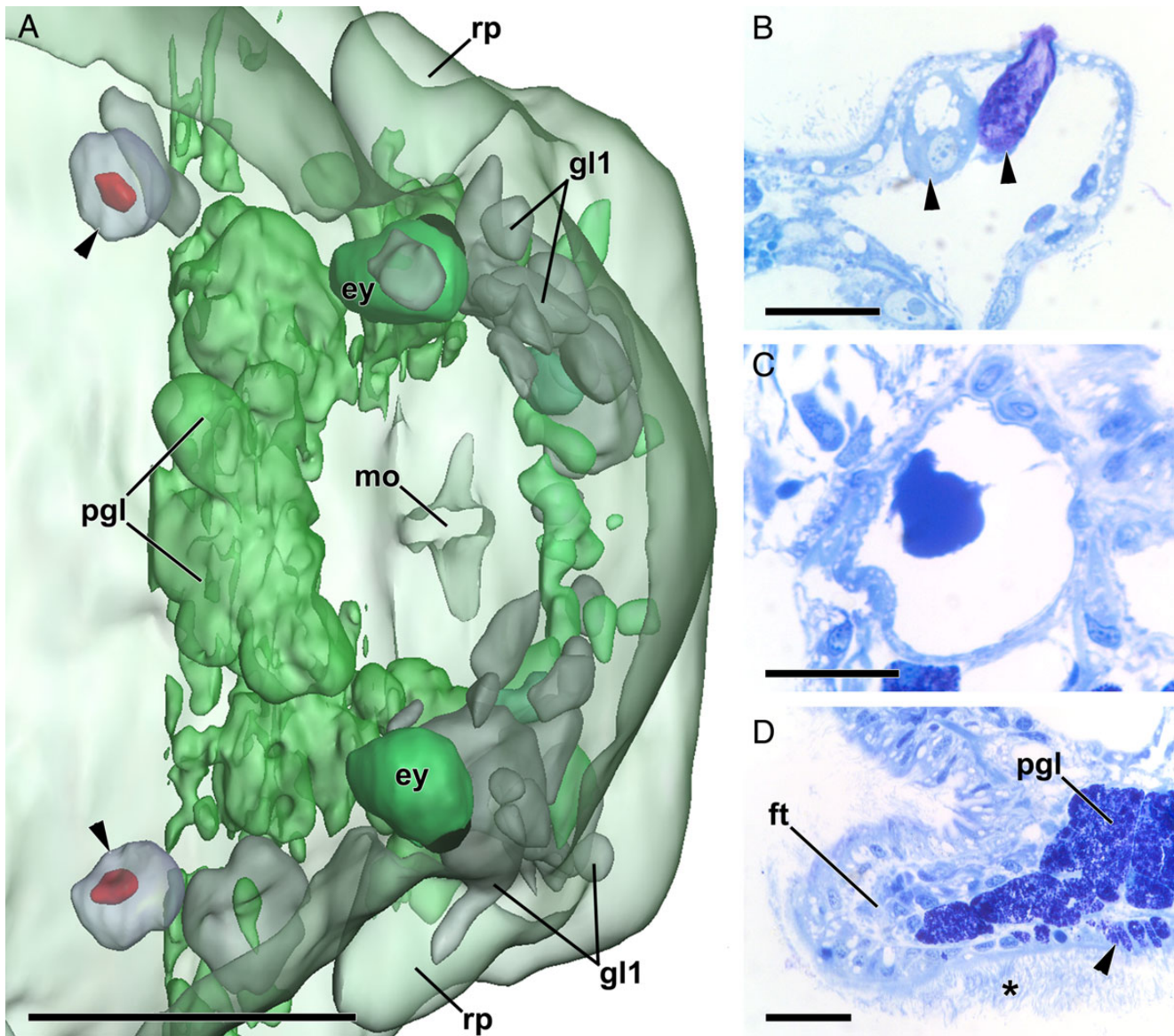


Figure 13. 3D-reconstruction and semithin cross-sections of glandular structures and enigmatic organ in *Ercolania halophilae* n. sp. **A.** Reconstruction of glandular structures in the anterior body, dorsal view. Arrowheads indicate enigmatic organ. **B.** Cross-section of cerata glands (arrowheads). **C.** Cross-section of the enigmatic organ. **D.** Cross-section of anterior right foot margin, arrowhead indicates small ductules of the pedal glands. Note dense ciliation of the foot sole (asterisk). Abbreviations: ey, eye; ft, foot; gl1, subepidermal gland 1; mo, mouth opening; pgl, pedal glands; rp, rhinophore. Scale bars: **A** = 100 μm ; **B–D** = 25 μm .

2007). The similarity of the teeth of *E. halophilae* and *A. nigra* has been pointed out above.

The size of the voluminous salivary glands in relation to the body size is remarkable and, to our knowledge, unique in sacoglossans. Also, salivary glands of sacoglossans normally appear to have granular content in histological cross-section (Grzybowski, Stemmer & Wägele, 2007; Kohnert *et al.*, 2013). Two pairs of differently staining oral glands were also found in *Gascoignella aprica* Jensen, 1985 (Kohnert *et al.*, 2013), and this may be the usual arrangement in sacoglossans, though no information is available for other species.

Penial armature in the Sacoglossa has been used in classification (Jensen, 1996) and a classification of the different types of armature has also been attempted (Gascoigne, 1993). The penial stylet of *E. halophilae* is thin, smooth and curved with a slightly flared tip, as in many species of *Ercolania*, *Limapontia* and

Placida (type A of Gascoigne, 1993). Other species of *Ercolania* have very short stylets (type B of Gascoigne, 1993), pointed in *E. coerulea* and with a widely flared tip in *E. margaritae* (see Jensen, 1985; Gascoigne, 1993), whereas that of *A. modesta* is nearly straight and has a scaly surface (Bleakney, 1988). There is no vaginal opening in *E. halophilae*, so the stylet is of the penetrant kind (Gascoigne, 1976).

Ercolania halophilae is androdiaulic, meaning there is an internal vas deferens branching from the postampullar hermaphroditic duct, and an oviduct containing the fertilization area and mucus gland (Ghiselin, 1966). Vaginal openings have been described in a few species of *Ercolania*, e.g. *E. coerulea* and *E. margaritae* (see Gascoigne, 1976; Schmekel & Portmann, 1982). However, the vaginal opening usually connects directly to the seminal receptacle, which is not triaulic (having three separate gonoducts) in the original sense (Ghiselin, 1966). Other species of *Ercolania* do

Table 1. Comparison of limapontiid genera.

Genus	Rhinophores	Cerata	Pericardium	Anus	Radular teeth	Penial stylet	Digestive gland branching	Albumen gland in cerata	Vaginal opening
<i>Placida</i>	Rolled, grooved, simple	Many; fusiform	Round; anterior (absent)	Papilla at anterior pericardium	Blade-shaped; smooth	Slightly curved	In rhinophores, head, cerata and foot	Present	Absent
<i>Stiliger</i>	Grooved, simple	Many; fusiform	Indistinct	Papilla at anterior pericardium	Blade-shaped; smooth or denticulate	Absent or unknown	In rhinophores, head and cerata	Present or absent	Present
<i>Calliopaea</i>	Simple	Few; inflated	Elongate; middorsal	Indistinct; anterior right of pericardium	Chisel-shaped	Long, thin	In cerata	Absent	Present
<i>Olea</i>	Short, triangular	Few; fusiform	Indistinct; middorsal	Indistinct; anterior right of pericardium	Reduced	Curved	In cerata	Absent	? Absent
<i>Ercolania</i>	Simple or basally flattened	Many; fusiform or inflated	Elongate or indistinct; anterior or middorsal	Papilla or no papilla; at anterior pericardium or at right side of this	Sabot-shaped; smooth	Curved	In cerata; present or absent in rhinophores and head	Present or absent	Present or absent
<i>Limapontia</i>	Absent (short and simple)	Absent	Indistinct	At right side or posterior end of pericardium	Sabot-shaped	Curved	Main tubules with short lobules	N.a.	? Present or absent
<i>Alderia</i>	Absent	Many; fusiform	Absent	Posterior papilla	Sabot-shaped	Straight, scaly	In cerata ? and foot	Unknown	Absent
<i>Alderopsis</i>	Short, triangular	One row; fusiform	Indistinct	Papilla to the right of pericardium	Sabot-shaped	Curved	In cerata	Absent	Groove

Data from literature (see text).

not have a vaginal opening. Instead the seminal receptacle is located immediately underneath the epidermis below the cerata on the right side, and the penial stylet penetrates directly into the seminal receptacle. This arrangement has been called pseudodialytic (Gascoigne, 1976) or heterotrialytic (Sanders-Esser, 1984). This is in fact a variety of the androdialytic system and is the arrangement seen in the present species. The number of gonad follicles is very low, corresponding to the small size of the animal. Histologically, the albumen gland looks almost identical to the salivary gland. However, ductules could be traced in sections to the salivary reservoirs from the latter.

Comparison with other seagrass-feeding *Sacoglossa*

Two other sacoglossan species feed on seagrasses, *Elysia catulus* and *E. serca* (= *E. clena* Marcus & Marcus, 1970), both from the east coast of North and South America (Clark, 1975; Jensen, 1982). *Elysia serca* prefers feeding on *H. engelmanni* and is able to penetrate both upper and lower epidermal cell layers of the leaves. *Ercolania halophilae* apparently penetrates only one epidermal cell layer during feeding (this study). The cusps of the radular teeth of *E. serca* are somewhat longer (56–72 µm) than those of *E. halophilae* (Jensen, 1983a; present study). The shape of the radular teeth of the two species of *Elysia* is very different from those of *E. halophilae*, having very tall bases and blade-shaped cusps, which may be denticulate or smooth (Jensen, 1982). The pharynx of *E. serca* is tall and has a very thick ventral ascus muscle. The same is seen in *E. halophilae*. Both species of *Elysia* are larger than the present species: *E. catulus* = 2–6 mm and *E. serca* = 2–5 mm (Jensen, 1982), but rather small compared with other species of *Elysia*.

The small size of *E. halophilae* may be an adaptation for a short life restricted to the leaves of *H. ovalis*. This seagrass produces a pair of small oval leaves on short petioles arising from each node of the rhizome. It is very fast-growing, with short-lived leaves having an estimated average longevity of 12.4 d (Duarte, 1991). Furthermore, the leaves are very thin, being only two cell layers thick over most of the leaf blade (Tomlinson, 1980). Most specimens of *E. halophilae* occurred alone on a leaf, or at the most two specimens on the same leaf, but in May 2011, when most of the egg masses were found, more than two specimens were occasionally found on one leaf. Most likely the lifespan of *E. halophilae* is very short and they reach sexual maturity, copulate and spawn on the same or neighbouring leaf pairs. *Halophila ovalis* is probably the most abundant and widespread seagrass in the Indo-Pacific region, but for a small sacoglossan that feeds exclusively by sucking out cell sap from the chloroplast containing cells of the leaves, the short life of individual leaves (or leaf pairs) may represent a limitation best overcome by fast growth and reproduction, followed by quick dispersal of larvae. Unfortunately we did not have the opportunity to study development time for the egg masses, but in other tropical sacoglossans with planktotrophic development it is usually a week or less (Jensen, 2001).

Miniaturization

Extremely small body sizes in marine invertebrates have often been associated with an interstitial mode of life (Worsaae *et al.*, 2012; Brenzinger, Haszprunar & Schrödl, 2013, Jörger *et al.*, 2014). So far only one sacoglossan has been described as truly interstitial, *Platyhedyle denudata*, and its body is worm-like and elongated, rather than particularly small (Rückert *et al.*, 2008). Most sacoglossans, including the smallest known species such as *E. halophilae*, live on (or in) their food plants, without the obvious selective pressure for extreme miniaturization of interstitial animals. However, a short-lived substrate may induce proge-netic shortening of life cycles in kelp-dwelling corambid

nudibranchs (Martynov & Schrödl 2011) and the same may be hypothesized for *E. halophilae*.

Small body size combined with short life cycle in *E. halophilae* thus could be suggestive of progenetic development. If so, sexually mature *E. halophilae* should display some simple, juvenile or larva-like features, as demonstrated in rhodopemorph heterobranchs, interstitial or not (Brenzinger, Wilson & Schrödl, 2011, Brenzinger *et al.*, 2013). Rhodopemorphs lack any tentacles or body appendages. Similarly, *E. halophilae* has very short rhinophores and also very few cerata, as seen in juvenile limapontiids (Rao, 1937; Jensen, 1986, 1997). Rhodopemorphs lack a heart and have a reduced pericardium, which develop late during molluscan ontogeny. A heart is also absent in fully mature *E. halophilae*, indicating progenesis. A few other sacoglossans also lack a heart, i.e. *A. modesta*, *A. willowi*, *P. viridis* and *P. denudata* (Evans, 1953; Gascoigne & Sordi, 1980; Krug *et al.*, 2007; Rückert *et al.*, 2008); because of larger body sizes and unclear phylogeny and ontogeny this potential paedomorphosis may refer to progenetic or neotenic processes.

As a heart and pericardium are absent, ultrafiltration is either also absent or takes place somewhere else. In *E. halophilae*, loosely connected groups of rhogocyte-like cells in the haemocoel are found adhering to digestive gland tubules and to the kidney. Similar aggregations of cells with large nuclei and vacuoles, but interpreted as glandular, have also been described in *E. kencolesi* Grzybowski *et al.*, 2007, which is endophytrophic, and *E. annyleorum*, which feeds on the filamentous green alga *Boodlea* (Wägele *et al.*, 2010). In those species they were located in the posterior half of the body directly under the epidermis. Rhogocytes are also found in *A. modesta* (see Fahrner & Haszprunar, 2001) and are responsible for ultrafiltration (Haszprunar, 1996), but not necessarily for excretion. Aggregations of rhogocytes found in *Ercolania* species do not show any lumen opening to the exterior. Instead, they resemble the blood gland of, e.g. dorid nudibranchs (Schrödl & Wägele, 2001), which show rhogocytes embedded in connective tissue (Fahrner & Haszprunar, 2002). The presence of the blood gland may have a wider distribution among euthyneurans (Wägele, 1997) and even among gastropods (Koller, Brenzinger & Schrödl, 2014), and the homology and function of rhogocyte-containing organs needs to be further investigated including ultrastructural analysis. The paired structure (Fig. 13A, C) found in *E. halophilae* (ZSM Mol 20310524) is enigmatic. Its position suggests it could correspond to the kidney in heterobranch veliger larva (Schmekel & Portmann, 1982: 43, fig. 5.1) and thus it could be another paedomorphic character.

While assuming progenetic development in *E. halophilae*, no unambiguous signatures were detectable in the quite complex androdiaulic reproductive or nervous systems. Only three hermaphroditic follicles were found in all mature specimens. Cerebral and pleural ganglia in *E. halophilae* are combined (i.e. fused or not yet separated) and the visceral loop is short, showing a (fused or not yet separated) suboesophageal/abdominal ganglion and a supraoesophageal ganglion. This is the usual condition in adult Sacoglossa. Ontogenetic data on sacoglossan nervous development have only been studied in the bivalved *Berthelinia limax* (Kawaguti & Baba, 1959). In that species, late veligers have cerebropleural ganglia, pedal ganglia and only one unpaired visceral ganglion. As the visceral loop elongates in juveniles, the sub- and supraoesophageal ganglia appear (Kawaguti & Yamasu, 1966). To evaluate whether or not cerebropleural ganglia of *E. halophilae* and other sacoglossans show paedomorphoses, more detailed data are needed, especially on early ontogeny. Five separate ganglia on the visceral loop, as expected under a Pentaganglionata hypothesis (Haszprunar, 1988) and found in progenetic rhodopemorphs (Brenzinger *et al.*, 2011, 2013), have not yet been observed in any sacoglossan. Regarding the three (at least) pairs of anterior cerebral nerves, *E. halophilae*

possesses the typical euthyneuran condition, rather than having a putatively progenetic single tentacular nerve as present in Thecosomata (Kubilius *et al.*, 2014). Like other limapontiids and plakobranchiids (Huber, 1993; Kohnert *et al.*, 2013), *E. halophilae* has paired rhinophoral ganglia, each with double roots. This feature is uncommon among opisthobranchs, but was hypothesized as an apomorphy of Panpulmonata (Brenzinger *et al.*, 2013), to which Sacoglossa are currently assigned (Jörger *et al.*, 2010; Wägele *et al.* 2014).

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REFERENCES

- ALDER, J. & HANCOCK, A. 1843. Notice of a British species of *Calliopa* d'Orbigny, and of four new species of *Eolis*, with observations on the development and structure of the nudibranchiate Mollusca. *Annals and Magazine of Natural History*, **12**: 233–238.
- BABA, K. 1937. A new noteworthy species of the sacoglossan genus *Alderia*, from Amakusa, Japan. *Zoological Magazine (Japan)*, **49**: 249–251.
- BABA, K. 1938. Opisthobranchia of Kii, Middle Japan. *Journal of the Department of Agriculture, Kyushu Imperial University*, **6**: 1–19.
- BABA, K. 1959. The family Stiligeridae from Japan (Opisthobranchia—Sacoglossa). *Publications of the Seto Marine Biological Laboratory*, **7**: 327–334.
- BABA, K. 1968. A revised description of *Alderia nigra* Baba, 1937, type species of *Alderopsis* n.g. from Japan (Opisthobranchia—Sacoglossa). *Bijdragen tot de Dierkunde*, **38**: 5–11.
- BABA, K. & HAMATANI, I. 1970a. Occurrence of specimens presumably identifiable with *Stiliger ornatus* Ehrenberg, 1831, at Seto, Kii, Middle Japan (Opisthobranchia: Sacoglossa). *Publications of the Seto Marine Biological Laboratory*, **18**: 199–206.
- BABA, K. & HAMATANI, I. 1970b. The anatomy of *Ercolania boodlea* (Baba, 1938) from Seto, Kii, middle Japan (Opisthobranchia: Sacoglossa). *Publications of the Seto Marine Biological Laboratory*, **18**: 215–222.
- BLEAKNEY, J.S. 1988. The radula and penial style of *Alderia modesta* (Lovén, 1844) (Opisthobranchia: Ascoglossa/Sacoglossa) from populations in North America and Europe. *Veliger*, **31**: 226–235.
- BREZINGER, B., HASZPRUNAR, G. & SCHRÖDL, M. 2013. At the limits of a successful body plan—3D microanatomy, histology and evolution of *Helminthope* (Mollusca: Heterobranchia: Rhodopemorpha), the most worm-like gastropod. *Frontiers in Zoology*, **10**: 37.
- BREZINGER, B., WILSON, N.G. & SCHRÖDL, M. 2011. 3D microanatomy of a gastropod 'worm', *Rhodope rousei* n. sp. (Heterobranchia) from southern Australia. *Journal of Molluscan Studies*, **77**: 375–387.
- BURN, R. 1974. Notes on some benthonic opisthobranchs from Port Phillip Bay, Victoria. *Journal of the Malacological Society of Australia*, **3**: 43–57.
- CABALLER, M., ORTEA, J. & ESPINOSA, J. 2006. Descripción de una nueva especie de *Alderopsis* Baba, 1968. *Avicennia*, **18**: 57–59.
- CLARK, K.B. 1975. Nudibranch life cycles in the northwest Atlantic and their relationship to the ecology of fouling communities. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, **27**: 28–69.

- COSTA, A. 1866. Saggio sui molluschi eolodidei del Golfo di Napoli. *Annuario del Museo Zoologico della R. Università di Napoli*, **3**: 59–90.
- CRANE, S. 1971. The feeding and reproductive behavior of the sacoglossan gastropod *Olea hansineensis* Agersborg, 1923. *Veliger*, **14**: 57–59.
- DACOSTA, S., CUNHA, C.M., SIMONE, L.R.L. & SCHRÖDL, M. 2007. Computer-based 3-dimensional reconstruction of major organ systems of a new aeolid nudibranch subspecies, *Flabellina engeli lucianae*, from Brazil (Gastropoda: Opisthobranchia). *Journal of Molluscan Studies*, **73**: 339–353.
- DUARTE, C.M. 1991. Allometric scaling of seagrass form and productivity. *Marine Ecology Progress Series*, **77**: 289–300.
- EHRENBERG, G.G. 1831. *Symbolae physicae seu icones et descriptiones animalium evertibratorum sepositis insectis quae ex itinere per Africam Borealem et Asiam Occidentalem*. Decas I Mollusca. Academie, Berlin.
- ELIOT, C. 1904. On some nudibranchs from East Africa and Zanzibar. Part VI. *Proceedings of the Zoological Society of London*, **1904**: 268–298.
- EVANS, T.J. 1953. The alimentary and vascular systems of *Alderia modesta* (Lovén) in relation to its ecology. *Proceedings of the Malacological Society of London*, **29**: 249–258.
- FAHRNER, A. & HASZPRUNAR, G. 2001. Anatomy and ultrastructure of the excretory system of a heart-bearing and a heart-less sacoglossan gastropod (Opisthobranchia, Sacoglossa). *Zoomorphology*, **121**: 85–93.
- FAHRNER, A. & HASZPRUNAR, G. 2002. Microanatomy, ultrastructure and systematic significance of the excretory system and mantle cavity of an acochlidian gastropod (Opisthobranchia). *Journal of Molluscan Studies*, **68**: 87–94.
- GASCOIGNE, T. 1976. The reproductive systems and classification of the Stiligeridae (Opisthobranchia: Sacoglossa). *Journal of the Malacological Society of Australia*, **3**: 157–172.
- GASCOIGNE, T. 1977. Sacoglossan teeth. *Malacologia*, **16**: 101–105.
- GASCOIGNE, T. 1978. The internal anatomy of *Stiliger fuscatus* (A. Gould, 1870) (Opisthobranchia: Sacoglossa). *Zoological Journal of the Linnean Society*, **63**: 265–274.
- GASCOIGNE, T. 1993. Stylets, styles, and other cuticular extensions of the male duct in the order Sacoglossa (Gastropoda). *Bollettino Malacologico*, **29**: 139–146.
- GASCOIGNE, T. & SORDI, M. 1980. A redescription of *Placida viridis* Trinchese, 1873 (Gastropoda: Ascoglossa). *Journal of Conchology*, **30**: 167–179.
- GHISELIN, M.T. 1966. Reproductive function and the phylogeny of opisthobranch gastropods. *Malacologia*, **3**: 327–378.
- GOULD, A.A. 1870. *Report on the invertebrates of Massachusetts*. Edn 2, Mollusca (W.G. Binney, ed.). Wright and Potter State Printers, Boston, MA.
- GRZYMBOWSKI, Y., STEMMER, K. & WÄGELE, H. 2007. On a new *Ercolania* Trinchese, 1872 (Opisthobranchia, Sacoglossa, Limapontiidae) living within *Boergesenia* Feldmann, 1950 (Cladophorales), with notes on anatomy, histology and biology. *Zootaxa*, **1577**: 3–16.
- HASZPRUNAR, G. 1988. On the origin and evolution of major gastropod groups, with special reference to the Streptoneura. *Journal of Molluscan Studies*, **54**: 367–441.
- HASZPRUNAR, G. 1996. The molluscan rhogocyte (pore-cell, Blaszencelle, cellule nucleale), and its significance for ideas on nephredial evolution. *Journal of Molluscan Studies*, **62**: 185–211.
- HUBER, G. 1993. On the cerebral nervous system of marine Heterobranchia (Gastropoda). *Journal of Molluscan Studies*, **59**: 381–420.
- IREDALE, T. & O'DONOGHUE, C. 1923. List of British nudibranchiate Mollusca. *Proceedings of the Malacological Society of London*, **15**: 195–233.
- JENSEN, K.R. 1980. A review of sacoglossan diets, with comparative notes on radular and buccal anatomy. *Malacological Review*, **13**: 55–77.
- JENSEN, K.R. 1981. Observations on feeding methods in some Florida ascoglossans. *Journal of Molluscan Studies*, **47**: 190–199.
- JENSEN, K.R. 1982. Occurrence of *Elysia serca* Marcus in Florida, with notes on the synonymy of the species. *Journal of Conchology*, **31**: 87–94.
- JENSEN, K.R. 1983a. Factors affecting feeding selectivity in herbivorous Ascoglossa (Mollusca: Opisthobranchia). *Journal of Experimental Marine Biology and Ecology*, **66**: 135–148.
- JENSEN, K.R. 1983b. Further notes on the ecology and systematics of *Elysia serca* Marcus (Opisthobranchia, Ascoglossa). *Journal of Molluscan Studies, Supplement*, **12A**: 69–72.
- JENSEN, K.R. 1985. Annotated checklist of Hong Kong Ascoglossa (Mollusca: Opisthobranchia), with descriptions of four new species. In: *Proceedings of the Second International Workshop on the Malacofauna Hong Kong and Southern China, Hong Kong 1983*, Vol. 1 (B. Morton & D. Dudgeon, eds), pp. 77–107. Hong Kong University Press, Hong Kong.
- JENSEN, K.R. 1986. Observations on feeding, copulation, and spawning in *Calliopa oophaga* Lemche (Opisthobranchia, Ascoglossa). *Ophelia*, **25**: 97–106.
- JENSEN, K.R. 1993a. Morphological adaptations and plasticity of radular teeth of the Sacoglossa (=Ascoglossa) (Mollusca: Opisthobranchia) in relation to their food plants. *Biological Journal of the Linnean Society*, **48**: 135–155.
- JENSEN, K.R. 1993b. Sacoglossa (Mollusca, Opisthobranchia) from Rottnest Island and central Western Australia. In: *Proceedings of the Fifth International Marine Biological Workshop: The Marine Flora and Fauna of Rottnest Island, Western Australia* (F.E. Wells, D.I. Walker, H. Kirkman & R. Lethbridge, eds), pp. 207–253. Western Australian Museum, Perth.
- JENSEN, K.R. 1993c. Evolution of buccal apparatus and diet radiation in the Sacoglossa (Opisthobranchia). *Bollettino Malacologico*, **29**: 147–172.
- JENSEN, K.R. 1996. Phylogenetic systematics and classification of the Sacoglossa (Mollusca, Gastropoda, Opisthobranchia). *Philosophical Transactions of the Royal Society B*, **351**: 91–122.
- JENSEN, K.R. 1997. Sacoglossa (Mollusca, Opisthobranchia) from the Darwin Harbour area, Northern Territory, Australia. In: *Proceedings of the Sixth International Marine Biological Workshop: The Marine Flora and Fauna of Darwin, Northern Territory, Australia* (J.R. Hanley, G. Caswell, D. Megirian & H.K. Larson, eds), pp. 163–186. Museums and Art Galleries of the Northern Territory and the Australian Marine Sciences Association, Darwin.
- JENSEN, K.R. 1999. A new species of Sacoglossa (Mollusca, Opisthobranchia) from Rottnest Island, Western Australia. In: *The seagrass flora and fauna of Rottnest Island, Western Australia* (D.I. Walker & F.E. Wells, eds), pp. 377–383. Western Australian Museum, Perth.
- JENSEN, K.R. 2001. Review of reproduction in the Sacoglossa (Mollusca, Opisthobranchia). *Bollettino Malacologico*, **37**: 81–98.
- JENSEN, K.R. & CLARK, K.B. 1983. Annotated checklist of Florida ascoglossan Opisthobranchia. *Nautilus*, **97**: 1–13.
- JÖRGER, K.M., STÖGER, I., KANO, Y., FUKUDA, H., KNEBELSBERGER, T. & SCHRÖDL, M. 2010. On the origin of Acochlidia and other enigmatic euthyneuran gastropods and implications for the systematics of Heterobranchia. *BMC Evolutionary Biology*, **10**: 323.
- JÖRGER, K.M., STOSCHEK, T., MIGOTTO, A.E., HASZPRUNAR, G. & NEUSSER, T.P. 2014. 3D-microanatomy of the mesopsammic *Pseudovermis salamandrops* Marcus, 1953 from Brazil (Nudibranchia, Gastropoda). *Marine Biodiversity*, doi 10.1007/s12526-014-0224-5.
- KAWAGUTI, S. & BABA, K. 1959. A preliminary note on a two-valved sacoglossan gastropod, *Tamanoalva limax* n.gen., n.sp., from Tamano, Japan. *Biological Journal of Okayama University*, **5**: 177–184.
- KAWAGUTI, S. & YAMASU, T. 1966. Development of the nervous system in a bivalved gastropod. *Biological Journal of Okayama University*, **12**: 61–67.
- KOHNERT, P., BRENZINGER, B., JENSEN, K.R. & SCHRÖDL, M. 2013. 3D- microanatomy of the semiterrestrial slug *Gascoignella aprica* Jensen, 1985—a basal plakobranchacean sacoglossan (Gastropoda, Panpulmonata). *Organisms, Diversity & Evolution*, **13**: 583–603.

- KOLLER, K., BRENZINGER, B. & SHRÖDL, M. 2014. A caenogastropod in 3D: microanatomy of the Munich endemic springsnail *Sadleriana bavarica* Boeters, 1989 (Hydrobiidae). *Spixiana*, **37**: 1–19.
- KRUG, P.J., ELLINGSON, R.A., BURTON, R. & VALDÉS, A. 2007. A new poecilogonous species of sea slug (Opisthobranchia: Sacoglossa) from California: comparison with the planktotrophic congener *Alderia modesta* (Lovén, 1844). *Journal of Molluscan Studies*, **73**: 29–38.
- KUBILIUS, R.A., KOHNERT, P., BRENZINGER, B. & SHRÖDL, M. 2014. 3D-microanatomy of the straight-shelled pteropod *Creseis clava* (Gastropoda, Heterobranchia, Euthecosomata). *Journal of Molluscan Studies*, **80**: 585–603.
- LANCE, J.R. 1962. A new *Stiliger* and a new *Corambella* (Mollusca: Opisthobranchia) from the Northwestern Pacific. *Veliger*, **5**: 33–38.
- LEMICHE, H. 1935. On some nudibranchiate gastropods from the Northern Atlantic. *Videnskabelige Meddelelser fra dansk Naturhistorisk Forening*, **99**: 131–148.
- LEMICHE, H. 1974. On *Calliopaea oophaga* n. sp., a new sacoglossan. *Proceedings of the Malacological Society of London*, **41**: 124–126.
- LOVÉN, S. 1844. Om nordiska hafs-mollusker. *Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar*, **1844**: 48–53.
- LUFT, J.H. 1961. Improvements in epoxy resin embedding methods. *Journal of Biophysical and Biochemical Cytology*, **9**: 409–414.
- MARCUS, E.D.B.-R. 1982. Systematics of the genera of the order Ascoglossa (Gastropoda). *Journal of Molluscan Studies, Supplement*, **10**: 1–31.
- MARCUS, E.R. 1955. Opisthobranchia from Brazil. *Boletins da Faculdade de Filosofia da Universidade de Sao Paulo, Zoologia*, **20**: 89–262, plates 1–30.
- MARCUS, E.R. 1959. Lamellariacea und Opisthobranchia. Reports of the Lund University Chile Expedition 1948–49. *Lunds Universitets Årsskrift*, **55**: 1–133.
- MARCUS, E.V. & MARCUS, E.R. 1956. On two sacoglossan slugs from Brazil. *American Museum Novitates*, **1796**: 1–21.
- MARCUS, E.V. & MARCUS, E.R. 1960. Opisthobranchs from American Atlantic warm waters. *Bulletin of Marine Science*, **10**: 129–203.
- MARCUS, E.V. & MARCUS, E.R. 1970. Opisthobranchs from Curaçao and faunistically related regions. *Studies on the Fauna of Curaçao and Other Caribbean Islands*, **33**: 1–129.
- MARTYNOV, A. & SHRÖDL, M. 2011. Phylogeny and evolution of corambid nudibranchs (Mollusca: Gastropoda). *Zoological Journal of the Linnean Society*, **163**: 585–604.
- MILLER, M.C. 1961. Distribution and food of the nudibranchiate Mollusca of the south of the Isle of Man. *Journal of Animal Ecology*, **30**: 95–116.
- NEUSSER, T.P., HEB, M., HASZPRUNAR, G. & SHRÖDL, M. 2006. Computer-based three-dimensional reconstruction of the anatomy of *Microhedyle remanei* (Marcus, 1953), an interstitial acoelid gastropod from Bermuda. *Journal of Morphology*, **267**: 231–247.
- RAO, K.V. 1937. Structure, habits and early development of a new species of *Stiliger* Ehrenberg. *Records of the Indian Museum*, **39**: 435–464, plates 7–9.
- RICHARDSON, K.C., JARETT, L. & FINKE, E.H. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technology*, **35**: 313–323.
- ROMEIS, B. 1989. *Mikroskopische Technik*. Urban & Schwarzenberg, München.
- RÜCKERT, I.-M., ALTNÖDER, A. & SHRÖDL, M. 2008. Computer-based 3D anatomical reconstruction and systematic placement of the mesopsammic gastropod *Platyhedyle denudata* Salvini-Plawen, 1973 (Opisthobranchia, Sacoglossa). *Organisms, Diversity & Evolution*, **8**: 358–367.
- RUTHENSTEINER, B. 2008. Soft Part 3D visualization by serial sectioning and computer reconstruction. *Zoosymposia*, **1**: 63–100.
- SALVINI-PLAWEN, L. VON. 1973. Zur Kenntnis der Philinoglossacea und der Acoelidiacea mit Platyhedylidae fam. nov. (Gastropoda, Cephalaspidea). *Zeitschrift für zoologische Systematik und Evolutionsforschung*, **11**: 110–133.
- SANDERS-ESSER, B. 1984. Vergleichende Untersuchungen zur Anatomie und Histologie der vorderen Genitalorgane der Ascoglossa (Gastropoda, Euthyneura). *Zoologische Jahrbücher, Anatomie*, **111**: 195–243.
- SCHMEKEL, L. & PORTMANN, A. 1982. *Opisthobranchia des Mittelmeeres. Nudibranchia und Sacoglossa*. Springer, Berlin & New York.
- SCHRÖDL, M. & WÄGELE, H. 2001. Anatomy and histology of *Corambe* Marcus, 1959 (Gastropoda, Nudibranchia, Doridoidea), with a discussion of the systematic position of Corambidae. *Organisms Diversity & Evolution*, **1**: 3–16.
- THOMPSON, T.E. 1973. Sacoglossan gastropod molluscs from eastern Australia. *Proceedings of the Malacological Society of London*, **40**: 239–251.
- TOMLINSON, P.B. 1980. Leaf morphology and anatomy in seagrasses. In: *Handbook of seagrass biology: an ecosystem perspective* (R.C. Phillips & C.P. McRoy, eds), pp. 7–28. Garland STPM Press, New York.
- TRINCHESE, S. 1872. Un nuovo genere della famiglia degli Eolididei. *Annali del Museo civico di storia naturale di Genova*, **2**: 86–132.
- TRINCHESE, S. 1873. Descrizione di alcuni nuovi eolididei del porto di Genova. *Memorie della Accademia delle Scienze dell'Istituto di Bologna*, **4**: 197–201.
- TRINCHESE, S. 1892. Nuovi ascoglossi del Golfo di Napoli. *Rendiconto dell'Accademia delle Scienze Fisiche e Matematiche, Napoli*, **33**: 154–155.
- TROWBRIDGE, C.D., HIRANO, Y.M. & HIRANO, Y.J. 2009. Sacoglossan opisthobranchs on northwestern Pacific shores: *Stiliger berghi* Baba, 1937 and *Elysia* sp. on filamentous red algae. *Veliger*, **51**: 43–62.
- VOGEL, R.M. 1971. The biology and a redescription of the opisthobranch mollusk *Hermaea cruciata* Gould, from Chesapeake Bay (Maryland). *Veliger*, **14**: 155–157.
- WÄGELE, H. 1997. Histological investigation of some organs and specialized cellular structures in Opisthobranchia (Gastropoda) with the potential to yield phylogenetically significant characters. *Zoologischer Anzeiger*, **236**: 119–131.
- WÄGELE, H., KLUSSMANN-KOLB, A., VERBEEK, E. & SHRÖDL, M. 2014. Flashback and foreshadowing—a review of the taxon Opisthobranchia. *Organisms Diversity & Evolution*, **14**: 133–149.
- WÄGELE, H., STEMMER, K., BURGHARDT, I. & HÄNDELER, K. 2010. Two new sacoglossan sea slug species (Opisthobranchia, Gastropoda): *Ercolania annelyleorum* sp. nov. (Limapontiidea) and *Elysia asbecki* sp. nov. (Plakobranchoidea), with notes on anatomy, histology and biology. *Zootaxa*, **2676**: 1–28.
- WÄGELE, H. & WILLAN, R.C. 2000. Phylogeny of the Nudibranchia. *Zoological Journal of the Linnean Society*, **130**: 83–181.
- WILLIAMS, J.C. & GOSLINER, T.M. 1973. Range extensions for four sacoglossan opisthobranchs of California and the Gulf of California. *Veliger*, **16**: 112–116.
- WORSAAE, K., STERRER, W., KAUL-STREHLOW, S., HAY-SCHMIDT, A. & GIRIBET, G. 2012. An anatomical description of a miniaturized acorn worm (Hemichordata, Enteropneusta) with asexual reproduction by paratomy. *PLoS ONE*, **7**: e48529.