



On a new *Ercolania* Trinchese, 1872 (Opisthobranchia, Sacoglossa, Limapontiidae) living within *Boergesenia* Feldmann, 1950 (Cladophorales), with notes on anatomy, histology and biology

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Abstract

A new sacoglossan species, belonging to the genus *Ercolania* Trinchese, 1872 is described. *E. kencelesi* sp. nov. has a similar biology as *E. endophytophaga* Jensen, 1999. Both are living within syncytial algal tubes of members of the Siphonocladales, *E. kencelesi* sp. nov. in *Boergesenia forbesii* and *E. endophytophaga* in *Struvea plumosa*. Assignment to the genus *Ercolania* is possible on the base of the cerata, which are round in cross-section, the digitiform rhinophores, sabot-shaped teeth and the presence of a penial stylet. The new species can be easily distinguished from *E. endophytophaga*, by the teeth with a prominent cusp, as well as other *Ercolania* species, by its unique colouration and its peculiar association to the food alga *Boergesenia*. Feeding, copulation, egg masses and development are noted. Differences in development further strengthen the distinction of *E. kencelesi* to *E. endophytophaga*. Measurement of photosynthetic activity of incorporated chloroplasts during several days clearly shows that chloroplasts are digested.

Key words: *Ercolania kencelesi* sp. nov., Australia, histology, feeding, photosynthesis, PAM, reproduction, development

Introduction

The opisthobranchiate taxon Sacoglossa is characterized by its unique feeding strategy. The slugs pierce algal cells with their single row of teeth and suck out the cell contents. This suctorial feeding mode is reflected in morphology; sacoglossans have a uniseriate radula with sickle-shaped teeth apt for piercing cell walls. Sometimes, the chloroplasts of the food algae are incorporated into the digestive gland and retain their ability to perform photosynthesis for a period of time ranging from hours to months (e.g., Rumpho *et al.* 2001; Wägele & Johnsen 2001; Evertsen *et al.* 2007). Usually sacoglossans have a close affinity to their food algae, which mainly belong to the Caulerpales and Bryopsidales (e.g., Jensen 1993a, 1997; Williams & Walker 1999).

According to Jensen (1985), the distinction between *Ercolania* Trinchese, 1872, *Stiliger* Ehrenberg, 1831 and *Placida* Trinchese, 1876 is difficult due to the scanty original descriptions. Du Bois-Reymond Marcus (1982) even synonymised *Ercolania* with *Stiliger* since she could not find characters delineating described species to one of these genera. Jensen (1985) diagnosed the genus *Ercolania* by the following characters: the presence of cerata, which are round in cross-section; digitiform rhinophores; presence of sabot-shaped teeth, presence of a curved penial stylet. Up to now, nearly 20 species have been described and probably meet this generic diagnosis. Atlantic species are *Ercolania cricetus* Marcus & Marcus, 1970, *E. fuscata* (Gould, 1870), *E. nigra* (Lemche, 1935), *E. talis* (Marcus, 1956), *E. vanellus* (Marcus, 1957) and *E. viridis* (A. Costa, 1866).

Mediterranean species are *Ercolania lozanoi* Ortea, 1981 and *E. trinchesei* (Pruvot-Fol, 1951). *Ercolania viridis* is reported from the Mediterranean Sea as well as from the Caribbean Sea. Following species are recorded from the Pacific: *Ercolania boodlea* (Baba, 1938), *E. emarginata* Jensen, 1985, *E. endophytophaga* Jensen, 1999, *E. evelinae* (Marcus, 1959), *E. felina* (Hutton, 1882), *E. fuscovittata* (Lance, 1962), *E. gopalai* (Rao, 1937), *E. margaritae* Burn, 1974, *E. subviridis* (Baba, 1959), *E. tentaculata* (Eliot, 1917), *E. translucens* Jensen, 1993. *Ercolania coerulea* Trinchese, 1892 is the only species, which is recorded from the Caribbean, Mediterranean and Pacific.

In 1999, Jensen described a species with a very peculiar biology, *Ercolania endophytophaga*. This sacoglossan is closely associated with *Struvea plumosa*, an alga of the taxon complex Cladophorales/Siphonocladales (taxonomic problems of this group were addressed by Bakker *et al.* 1994). The slug species was described to intrude into the algal cell, which it sucks out completely. It also lays its egg masses into the emptied algal cells.

During a study on Lizard Island, a hitherto undescribed species of *Ercolania* was detected, which has a similar biology as *E. endophytophaga*. It lives within the tubular green alga *Boergesenia forbesii* (Cladophorales). Six individuals were collected within several days and investigated.

Material and methods

Six specimens were collected by hand at Casuarina Beach, Lizard Island, Australia in July 2006 while snorkeling in front of the Lizard Island Research station along the seawater pipeline in the intertidal flat. All specimens were situated within the green alga *Boergesenia forbesii*. Four specimens were kept in small aquaria or cultivation bowls (200 ml) for a few days (see Table 1) in order to observe biology and to measure possible photosynthetic activity caused by incorporated chloroplasts. Measurements were taken with the help of a Pulse Amplitude Modulated Fluorometer (Diving PAM, Waltz, Germany). With this method, fluorescence emitted by photosystem II in chlorophyll a of chloroplasts is measured. F_0 values (in mV) have been obtained from dark-acclimated cells when the maximum of reaction centers of the photosystem are open. Yield values are relative values indicating the maximum quantum yield (Φ_{IIc}) of fluorescence for photosystem II in the organism investigated. The maximum quantum yield of fluorescence for PSII ($\Phi_{IIc-max}$) is defined as: $\Phi_{IIc-max} = (F_m - F_0)/F_m$. Details are given in Wägele and Johnsen (2001) and Burghardt and Wägele (2004). During these studies one specimen crawled out of the cultivation bowl and therefore was lost. Four animals were preserved in 96% EtOH and one animal in formaldehyde/seawater. This last animal was embedded in hydroxyethyl-methacrylate for serial sectioning and sections (2.5 μ m) were stained with toluidine blue. All histological investigations are based on this one animal. The radula was extracted from the paratype and investigated under the light microscope. For details of investigated specimens and type material see Table 1. This species was only observed in July. Investigation of *Boergesenia* in August did not reveal further specimens.

***Ercolania* Trinchese, 1872** (type species: *Ercolania siotti* Trinchese, 1872)

Diagnosis of the genus

According to Baba and Hamatani (1970a, b), Schmekel and Portmann (1982), and Jensen (1985), *Ercolania* is a distinct genus that is characterized by the following features: presence of cerata, which are round in cross-section; digitiform rhinophores; elongate or inconspicuous reno-pericardial prominence, presence of sabot-shaped teeth, presence of a curved penial stylet, probable absence of albumen gland in the cerata.

TABLE 1: Details of the six specimens of *Ercolania kencolesi* sp. nov. collected at Casuarina Beach, Lizard Island, North Queensland, Australia.

Date of collection	Date and kind of preservation	Used for photosynthetic measurements	Type of investigation
30.06.2006	2 July 06, Formaldehyde/seawater	2 days	Histology (serial cross sections)
02.07.2006	7 July 06, had crawled out of bowl	5 days	Lost for further studies
02.07.2006	30 July 06, 96% ethanol	27 days	Molecular studies
02.07.2006	2 July 06, 96% ethanol	Only once	Paratype , radula extracted and deposited together with rest of animal in Australian Museum Sydney C.209736
02.07.2006	5 August 06, 96% ethanol	39 days; starving interrupted by feeding periods	Holotype Australian Museum Sydney C.209735
07.07.2006	7 July 06, 96% ethanol	Only once	Molecular studies

Ercolania kencolesi sp. nov.

Repository

Australian Museum, Sydney: Type material: Casuarina Beach, Lizard Island, North Queensland, Australia; shallow water up to 1 m. 2nd July 2006. Holotype C.209735, length of preserved specimen 2.3 mm; 1 paratype C.209736, length of preserved specimen 2.1 mm;

Further material: Casuarina Beach, Lizard Island, North Queensland, Australia; shallow water up to 1 m depth. 30th June 2006 (1 specimen used for histology), 2nd July 2006 (1 specimen used for molecular studies), 7th June 2006 (1 specimen used for molecular studies).

Localities

Up to now, this species has been recorded from Casuarina Beach, in front of the Lizard Island Research Station (material studied here), from Hope Island, North Queensland (Loch 1989 and pers. comm., material deposited in AMS under the number C.153783) and from Guam (Carlson & Hoff 2003). A photo record of the Guam species by Carlson and Hoff is available in Rudman (2000b).

Etymology

This species is dedicated to Ken Coles, a great sponsor of the Lizard Island Research Station (LIRS). With his donations, he supports many scientists doing research in the well-equipped laboratories.

Description

External morphology and colour of living specimens based on five animals (Figs. 1A–E): Size from 4 mm up to 6 mm. Body elongate; foot tapering posteriorly; anterior foot without a notch and any propodial tentacles, but slightly extended to lateral sides; rhinophores long, solid and digitiform; eyes lying behind rhinophores on lateral sides; renopericardial prominence inconspicuous; cerata club-shaped to sausage-shaped, in one to two rows, with smaller ones on outer side; cerata not standing very close, those of similar size in opposite position; central notum free of any cerata (Fig. 1A, 1B).

Overall colour of body green; under higher magnification, green disintegrating to green dots representing terminal parts of numerous tiny digestive glandular branches. Stripes never present, not even in starving ani-

mals (Fig. 1B). Rhinophores green with white tips; in median part with a white blotch nearly circling rhinophore. Head completely green, eyes hardly visible. Anterior margin of foot light green to whitish (Figs. 1C, 1E). Cerata darker green, darkest at apical end; subapical white blotches present, forming an incomplete ring; more patches especially in dorsal areas of cerata. After starving for two to three days, animals showing a more brownish colour and eyes becoming more visible (Fig. 1B).

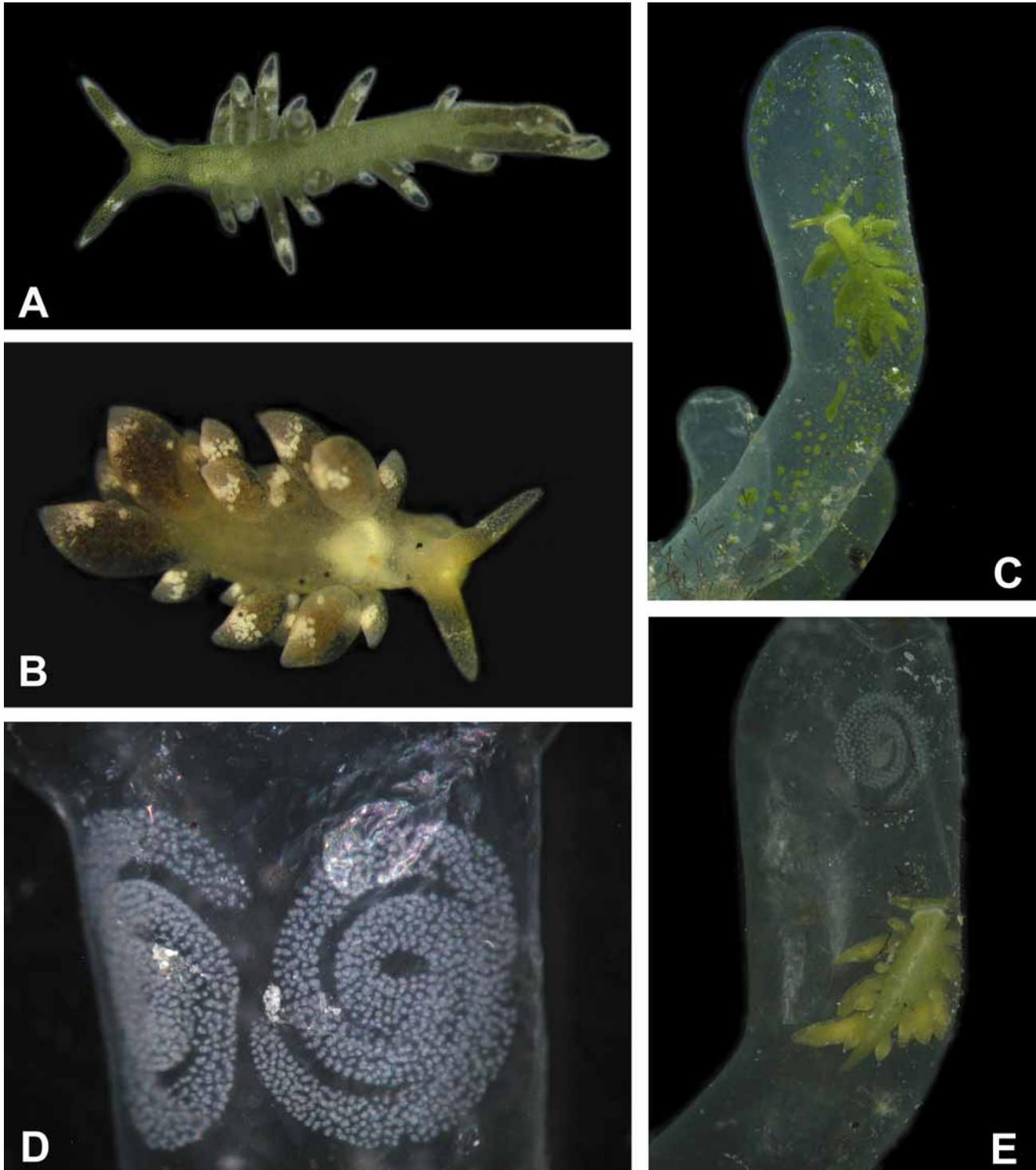


FIGURE 1: *Ercolania kencolesi* sp. nov.: Living animals from Lizard Island; A: Animal freshly taken out of algal tube; length about 5 mm. B: Animal starved for 4 days; length about 3 mm. C: Animal within algal tube of *Boergesenia* after two hours; D: Two egg masses laid after 3 days since intrusion of animal into algal tube. E: Animal and egg mass within algal tube after two days.

Description of anatomy and histology of preserved specimen (Figs. 2A–F).

Digestive tract: Oral tube with ciliated cells, many subepithelial glandular follicles with red-stained mucus surrounding and entering the oral tube. Radula formula 0.1.0. Radula of paratype with 2 teeth in the ascending limb, 7 teeth in the descending limb and 2 preradular teeth (Fig. 3C). Teeth sabot-shaped, with smooth edges.

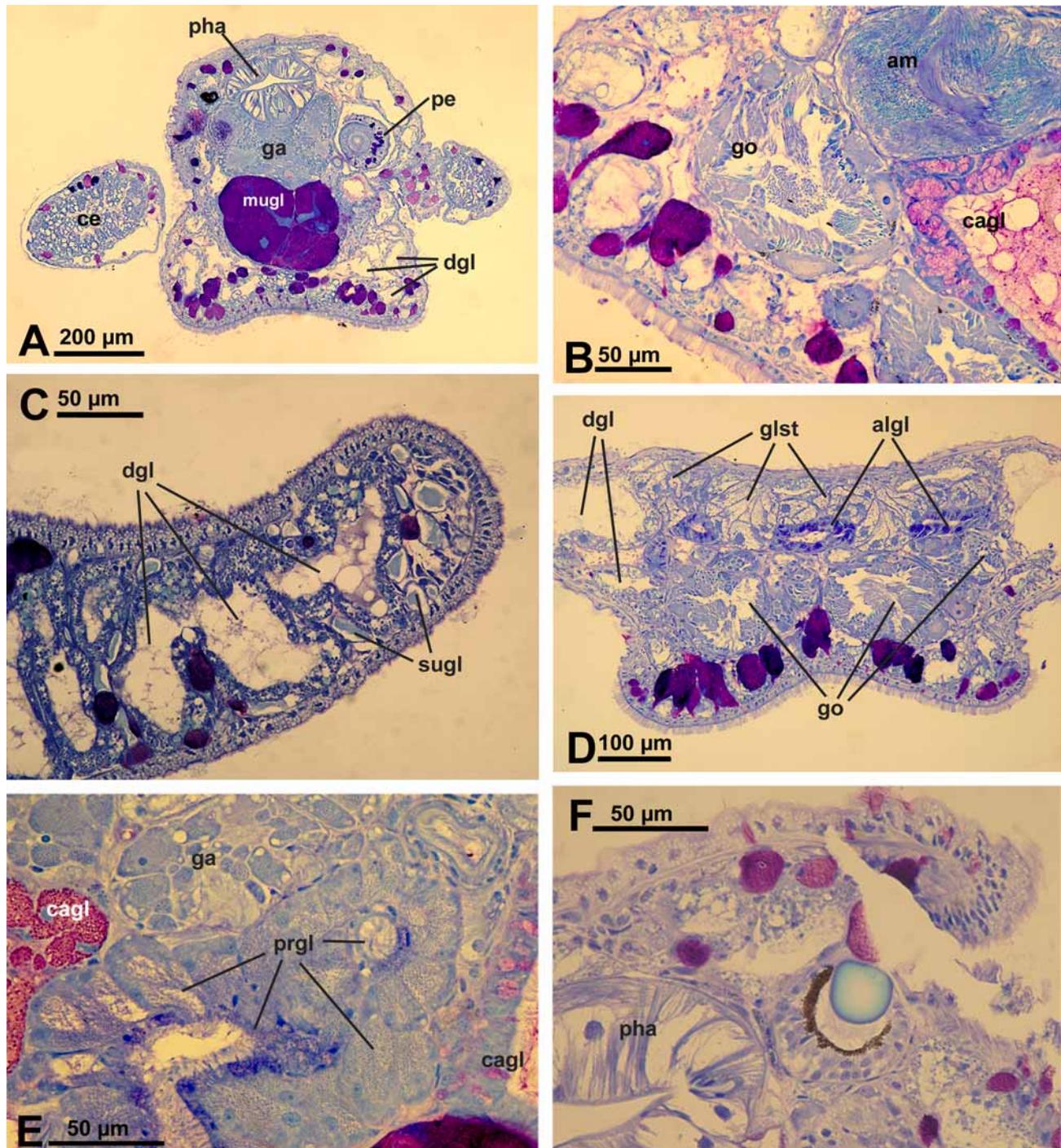


FIGURE 2: *Ercolania kencolesi* sp. nov.: Histology of organ systems. A: Cross section in the anterior third of body. B: Cross section in anterior half of body with genital system. C: Longitudinal section of rhinophore. Note that most of the algal chloroplasts are already consumed. D: Cross section in posterior third of body. E: Prostate gland. F: Cross section of eye. Abbreviations: algl albumen gland, am ampulla, cagl capsule gland, ce ceras, dgl digestive gland, ga ganglia, glst glandular structure, go gonad, mugl mucous gland, pe penis, pha pharynx, prgl prostate gland, sugl subepithelial glands.

Pharynx lined with a cuticle. Short, tube-like salivary glands present; glandular cells with tiny dark bluish stained granules. Oesophagus and stomach lined by a columnar, ciliated epithelium. Digestive gland highly tubulose with many branches running especially beneath epidermis and filling cerata nearly completely (Fig. 2A); these diverticula and branches reaching also into rhinophores (Fig. 2C) and foot. Intestine with a typhlosolis; epithelium with ciliated cuboidal to columnar cells; distal part of intestine a simple ciliated duct; no glands observable. Anus opening on top of pericardium, middorsally.

Genital system: Gonad lying partly ventral, consisting of follicles with oogonia lying peripheral and spermatogonia in median part (Fig. 2B). Ampulla large, with extremely flat epithelium (Fig. 2B); sperm in ampulla lying in groups; sperm heads very long, up to 20 μm . Nidamental glands comprising three distinct areas: albumen, capsule and mucus gland. Albumen gland forming several small tubes and running into posterior part of body between gonad and an unidentified gland (Fig. 2D). Cells rather broad, apical part filled with violet-staining granules. Capsule gland tubular and mainly situated dorsally to mucus gland. Cells of an intermediate size, i.e., capsule gland cells smaller than mucus cells and larger than albumen gland cells. Capsule gland cells filled with smaller vacuoles staining bright red (Fig. 2B). Mucus gland forming a thick tubular structure running ventrally of the pharynx (Fig. 2A) and bending back in head area. Cells very large with a red and greasy staining appearance. Presence or absence of receptaculum seminis could not be verified satisfactorily since some slides of that area were lost during preparation. A small bursa copulatrix with rather flat epithelium and very few disintegrating sperm present. Prostate gland composed of large glandular cells with tiny granular appearance and staining light bluish (Fig. 2E), forming a rather compact mass behind pharynx. Vas deferens composed of ciliated cells. Penis rather thick, lying within a sheath (Fig. 2A). Vas deferens inside of penis with ciliated epithelium, surrounded by musculature. Penis tissue with few subepithelial glandular cells, staining dark violet, in a similar way as observed in body tissue and especially in foot (see below). Distal part of penis with one hollow and curved stylet. Penis opening not observed.

Excretory system: Kidney saclike, situated dorsally above digestive system; epithelium highly folded, with larger cells containing several non-staining vacuoles. Ureter forming a simple ciliated duct. No syrinx present.

Sensory organs: Eyes with pigment cup and round lens. This lens staining homogeneously light blue (Fig. 2F). Statocyst large, with one otolith.

Epithelia and glandular structures: Epidermal cells with many vacuoles and very long and densely set cilia especially in anterior part of body and rhinophores (Fig. 2C). Tip of rhinophores with larger subepithelial glandular cells having homogeneously light bluish stained contents (Fig. 2C).

Subepithelial glandular follicles beneath epidermis of body composed of several large cells. Contents of these cells staining violet, indicating mucus. Cerata epithelium with very flat cells. Few glandular cells staining more homogeneously dark violet present subepithelially (Fig. 2A). Anterior pedal glands present as subepithelial glandular follicles staining violet to red. Number of these pedal glandular follicles high throughout foot sole.

A special glandular structure starting in middle of body and stretching along whole posterior body part on top of gonad and albumen gland (Fig. 2D). Gland composed of follicles with round cells characterized by a large nucleus and large, non- or slightly bluish-staining vacuoles. Neither ducts nor outside opening observed.

Biological notes

Feeding: To observe feeding, two slugs were chosen, which had been kept without algae for four days. Several fresh algal tubes were collected the day before starting the experiment. When these algae were offered, *Ercolania kencolesi* sp. nov. crawled on top of the first third of one algal tube, sat there for about three to four minutes with the ventral part of the head firmly attached to the alga. In these minutes, the slug pierced the algal cell wall (a process that could not be directly observed) and then started to push the head into the alga. After seven minutes the slug had penetrated completely into the tube. Within the algal tube, it moved

immediately to the proximal part of the alga, next to the attachment to the coral rubble. A broad transparent creeping track could be observed, where the chloroplasts were missing. The slug then turned round and crawled to the tip of the algal sac, slurping the chloroplast layer at the inner wall of the alga and leaving a second transparent track. It crawled up and down while slurping the cell sap. The mouth opened regularly for this action. After 45 minutes, 2/3 of the whole sac was sucked out by the slug. After 70 minutes most of the chloroplast layer was consumed. Even after that time the slug still crawled from the bottom to the top, searching for untouched areas within the algal sac. After 80 minutes it stopped searching and was crawling rather lazily. We observed sucking movements now and then, but the slug did not take up any of the small patches of chloroplasts which were still recognizable (Fig. 1C). After 18 hours, even those patches were gone (Fig. 1E). After consumption of the cell contents, the slug remained in the alga, which did not collapse. Two days after the intrusion, an egg clutch attached to the algal cell wall was observed (Fig. 1E). In another experiment, two egg clutches were laid after two and a half days (Fig. 1D). In both experiments, the slugs left the algal sac and intruded into another algal tube. These were sucked out immediately in the same way as described above.

Reproduction: For copulation experiments, two animals of similar size (about 5 mm) were chosen. One was taken out of an algal sac; another was sitting outside of *Boergesenia*. Copulation started with a head to head contact. The rhinophores were touching each other and a few seconds later the animals came to lie side by side with their heads. The penis of one animal was inserted into the body of the other animal. It was not possible to observe whether copulation was reciprocal. This process was observed several times while these animals were kept together for several hours.

Egg lying was observed within the algal sac several times, but also occurred free in the aquaria when no algae were available. The egg mass is a cylindrical tube (up to 1 mm in diameter) coiled anticlockwise into planar spirals of two and a half whorls (Figs. 1D, 1E). It is attached to the inner wall of *Boergesenia forbesii*. Usually one animal laid two egg masses inside the same algal sac. Egg masses measure 4–5 mm in diameter and contain around 500 egg capsules, each with one white egg (size about 100 μm) inside. Capsules are rather spherical and measure around 215 μm (measurements taken from 5 eggs).

Development (Figs. 3A–B): Spawning took place in July under laboratory conditions. 14 egg masses were observed until their free-swimming larval stage. Egg masses were kept at room temperature (around 20°C) in small vials. Water was changed every two days. Cleavage of eggs happened within several hours and first preveliger were observed after one day. Veliger stage was observed on the 3rd day after spawning; eyes developed one day later. Shell of preveliger and veliger measured around 130 to 140 μm (Figs. 3A, 3B). After five days, egg masses started to disintegrate and free-swimming larvae were observed within the algal sac. The algal sac disintegrates at the same time; therefore free-swimming larvae escape soon from the alga and tend to swim towards the light. Unfortunately, these larvae were trapped at the water surface and died soon after. Settlement of larvae could not be observed, even in bowls, where *Boergesenia forbesii* as the food of the adults was offered.

Measurements of the photosynthetic activity (Figs. 4A–B): Figure 4A shows the yield values of *Ercolania kencolesi* sp. nov. (Holotype) plotted versus the number of cultivation days under starving conditions in the aquarium. The yield values of all investigated specimens are on a very low level around 0.1. The ground fluorescence values (F_0 [mV]) plotted against the number of cultivation days under starving conditions in the aquarium decrease quickly from an average of about 150 mV in the beginning to approximately 20 mV after 10 days (Fig. 4B).

Starved and pale animals of *Ercolania kencolesi* sp. nov. with photosynthetic activity approaching zero were allowed to feed again on *Boergesenia forbesii* for four days. Their green colouration returned and values of their photosynthetic activity (yield) (Fig. 4A), as well as ground fluorescence (Fig. 4B), increased, but after one to two days of starvation the activity declined to zero again.

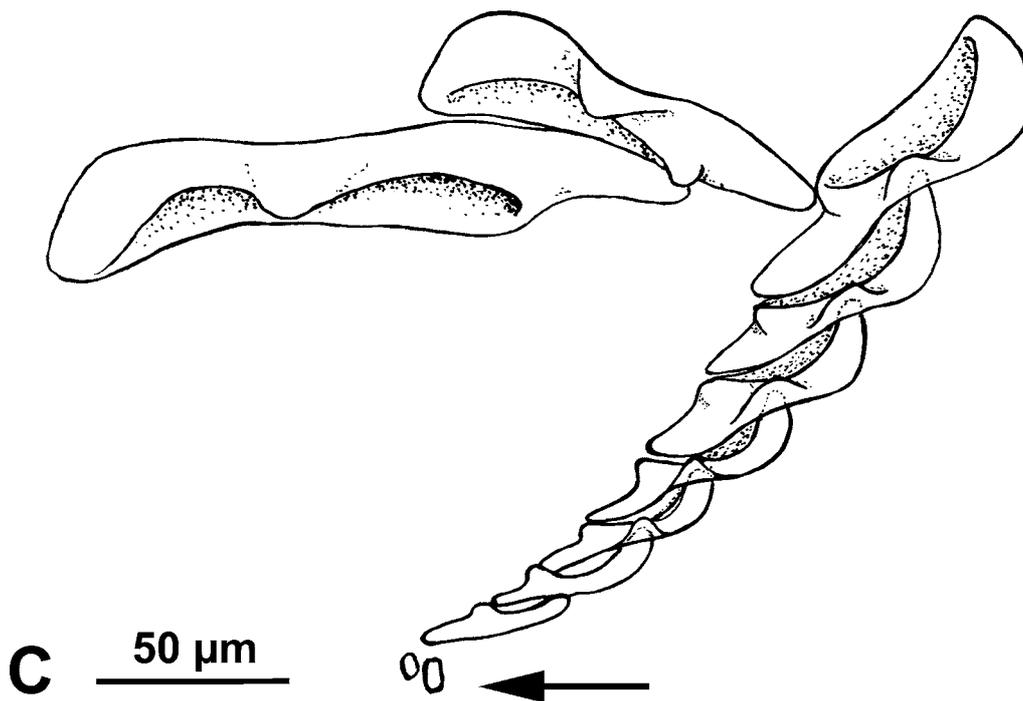
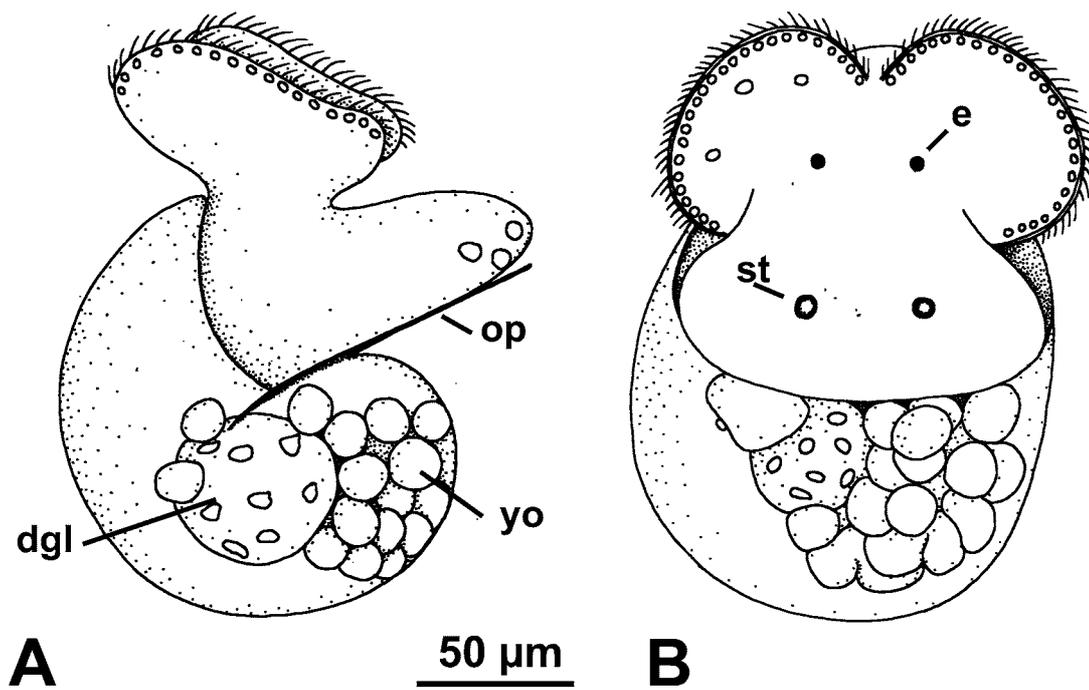


FIGURE 3: *Ercolania kencolesi* sp. nov.: larval development and adult radula. A: Veliger larva (day 5), lateral view after hatching from egg mass. B: Frontal view of similar developmental stage. C: Radula of paratype. Note the two preradular teeth (arrow). Abbreviations: dgl digestive gland, e eye, op operculum, st statocyst, yo yolk.

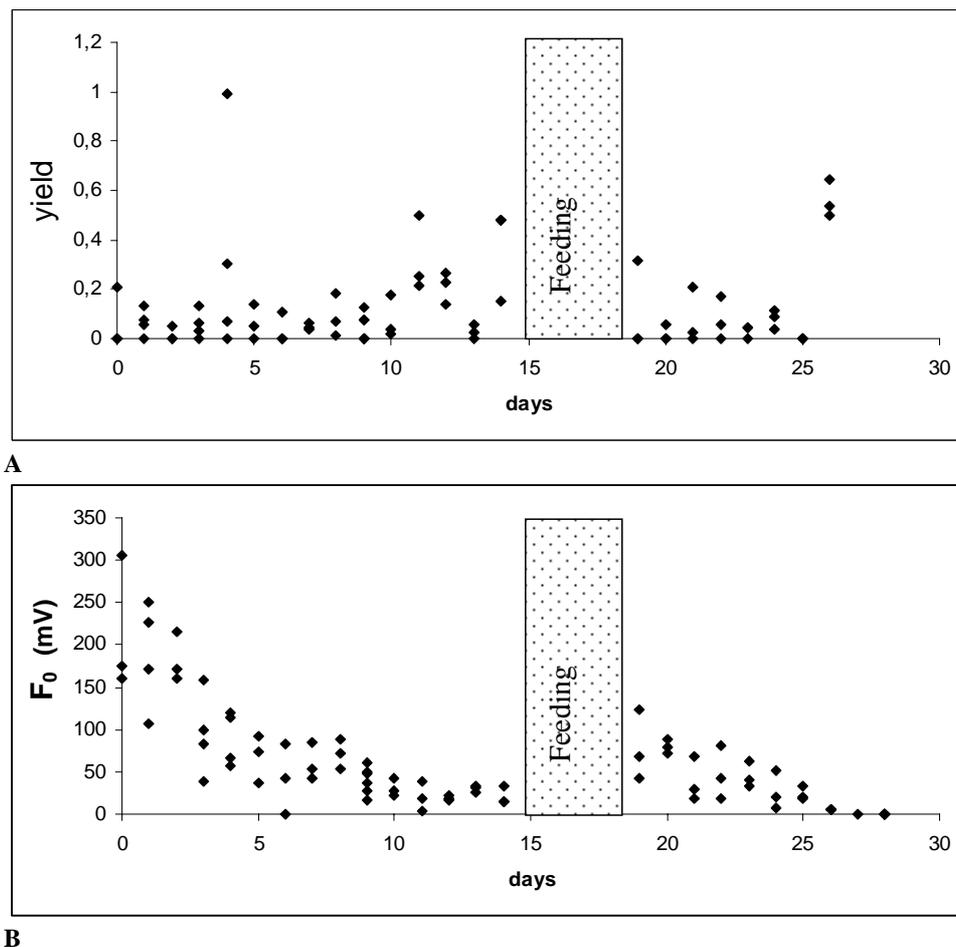


FIGURE 4: *Ercolania kencolesi* sp. nov.: Measurements of photosynthetic activity during a period of starvation interrupted with a period of four days, where feeding on *Boergesenia* was allowed. A: Yield values of photosynthesis measured over time. B: Ground fluorescence of photosynthesis (F₀ [mV]) measured over time. Decline of values indicates the decrease of numbers of chloroplasts. Note the increase of ground fluorescence after feeding.

Discussion

Taxonomy: No thorough phylogenetic analyses of the Limapontioidea exist, thus the validity (i.e., monophyly) of the different genera of the Limapontiidae (*Limapontia*, *Alderia*, *Stiliger*, *Placida* and *Ercolania*) is unclear. Whereas Baba and Hamatani (1970a, b) mentioned distinct characters for the genus *Ercolania*, du Bois-Reymond Marcus (1982) rejected this, on the basis that several characters discussed as genus-specific for *Stiliger* or *Ercolania* show a high degree of variation in other genera, e.g., tooth shape. Here, we follow Schmekel and Portmann (1982) and Jensen (1985) and preliminarily assign the new species to the genus *Ercolania* due to the presence of digitiform rhinophores, the presence of a penial stylet, the presence of sabot-shaped teeth and the absence of the albumen gland in the cerata. The very similar *Placida* and *Stiliger* differ especially by the auriculate rhinophores and the blade-shaped teeth. *Placida* has an oesophageal crop, which could not be found in the specimen investigated and *Stiliger* lacks a penial stylet.

About 20 valid species of *Ercolania* are recognized. Several of them have been described from the Mediterranean and Atlantic Sea. These Atlantic and Mediterranean species can be separated from the new species described here not only by the different geographic distribution but also by the different colouration. Only one

species, *Ercolania coerulea*, has been recorded from both the Atlantic and Pacific. Due to the inflated numerous cerata, this species differs considerably from the new species described here.

Several of the Pacific species can be distinguished from the presently described one by their colour. *Ercolania boodleae* is dark green, nearly blackish, with distinct white stripes along the head. The eyes are situated within these stripes (Sanders-Esser 1984). Very similar to *Ercolania boodleae* is *E. emarginata* with very dark green cerata and also very distinct white stripes along the head surrounding the eyes. Additionally, this species has a white dorsal notum (Jensen 1985, 1993a) and the teeth have a distinct denticle on the ventral margin. Jensen (2005) considered *Ercolania emarginata* as a synonym to *E. boodleae*. A similar denticle as is described for *Ercolania emarginata* is also present in *E. evelinae* from Chile (Marcus 1959). *Ercolania endophytophaga* also resembles *E. boodleae* and *E. emarginata* in the very dark colour, but whereas this colour comes from the darker pigments of the algae in *E. endophytophaga*, the dark pigment in *E. boodleae* and *E. marginata* is epidermally. Furthermore, in *Ercolania endophytophaga* the head and rhinophores are white, with a dark green stripe coming from the lateral cerata and running on top of the head along the dorsal sides of the rhinophores. In this species, there is a white triangular patch on the pericardium (Jensen 1999) similar as in *Ercolania emarginata*. *Ercolania felina* is also a very dark species, but has only two white patches located in the head area, and the eyes are situated in these patches (Jensen 1993b, Rudman 2000c). Our species differs from these mentioned ones, since it has no white stripes or patches in the head area, and has no white areas at all in the dorsal notum.

Some of the Pacific species of *Ercolania* have many more cerata attached not only along the lateral sides as is typical for *E. kencolesi* sp. nov., but also covering the dorsal notum. This is the case for *Ercolania boodleae*, *E. coerulea*, *E. felina* and *E. margaritae* (see Burn 1974; Jensen 1985; Rudman 2000a, b). A similar cerata distribution with similar colours as in *Ercolania kencolesi* sp. nov. can be observed in *E. subviridis* and *E. translucens*. *Ercolania subviridis* is characterized by distinct green stripes in the cerata, which are caused by the digestive glandular branches (Rudman 2005). Our species never showed these stripes but always exhibited a granular appearance, independent of being well fed or starved. The starved animal exhibited also a granular appearance but in these animals the colour faded to light brown. *Ercolania translucens* was described as being translucent with reddish brown pigment stripes along the sides of the body, on the dorsal surface and on the head. The green tubules of the digestive gland are visible through the epidermis. "In some specimens, a reddish sphere is visible through the epidermis in the neck region." (Jensen 1993b: 242). This species does not only differ from *Ercolania kencolesi* sp. nov. by this overall colouration, but also by the white tips of the cerata. These are dark in fed animals or translucent in starved animals of *Ercolania kencolesi* sp. nov.. Only the presence of white subapically lying dots in the cerata of *Ercolania translucens* resembles our specimens, but in *E. kencolesi* sp. nov., these white areas are patch-like and not single dots (Jensen 1996, 1999).

Rudman (2000b, d; 2002a, b; 2006) listed several undescribed *Ercolania* species (*Ercolania* sp. 1 up to sp. 5). We compared information from his website (www.seaslugforum.net) with our analysis and could exclude sp. 2 to sp. 5, since these animals clearly differ in colour and shape. The colour slide of *Ercolania* sp. 1 provided by Carlson and Hoff from Guam definitely shows the same species as *E. kencolesi* sp. nov.. It is the merit of Clay Carlson and Patty Jo Hoff to have observed this peculiar species in its home alga for the first time in 1970. In 1989, Loch described the detection of an unknown sacoglossan in *Boergesenia forbesii* at East Hope Island, North Queensland. According to his biological notes (same alga, similar coiled egg mass within the empty cell) and some slides he provided for identification (Loch, pers. comm.), these specimens can be assigned to our new species described here.

Considering literature data on food organisms and feeding strategies, only one species can be compared with our new species here, *Ercolania endophytophaga* described by Jensen (1999). *Ercolania endophytophaga* feeds on *Struvea plumosa* and in aquaria also *Valonia* sp. (Jensen 1999). These algal species, as well as *Boergesenia*—the food of our new species—belong to the Siphonocladaceae, Cladophorales. Although the teeth of *Ercolania kencolesi* sp. nov. have the typical sabot-shaped form of the genus, the teeth differ consid-

erably from those of *E. endophytophaga*. Whereas the teeth of *Ercolania kencolesi* sp. nov. clearly exhibit a pointed apex, the teeth of *E. endophytophaga* have a very big, rounded tip (Jensen 1999).

Comparing literature data on general anatomy of *Ercolania*, there are some details that can be mentioned here. A separate vaginal opening is described for few species (*Ercolania coerulea*, see Jensen 1993b, and *E. margaritae*, see Burn 1974) but this is probably absent in *E. kencolesi* sp. nov.. Unfortunately, sections of that region were partly broken; therefore this feature should be re-investigated in better-preserved material. The presence of the albumen gland within the cerata is also a rare feature in *Ercolania* and is described only for *E. boodlea* and *E. margaritae* by Sanders-Esser (1984). *Ercolania kencolesi* sp. nov. does not exhibit this character. The albumen gland lies as a tubular structure in the visceral cavity. The presence of a bursa copulatrix seems likely, since a structure could be identified, which fulfils the requirements as described by Sanders-Esser (1984) for sacoglossans; i.e., flat epithelium without a thick underlying muscular layer and no orientation of sperm heads towards the wall. But the absence or presence of a receptaculum seminis has to be clarified yet. Peculiar is the presence of a separate glandular structure in the posterior part of the body. This prominent gland has not been described hitherto from any *Ercolania* species. But since only few histological investigations exist for members of the Sacoglossa, its significance is unknown. The non- or lightly bluish stained large cells arranged in follicles have a similar appearance as some gill glands in Doridoidea (Wägele 1997). But it has to be emphasized that description of anatomy is based here on the histological analysis of one animal and future investigations are needed to complete description and to analyse intraspecific variability.

Biological notes

Feeding: Penetration of the algal cell wall by *Ercolania kencolesi* sp. nov. is very similar as described for *E. endophytophaga* by Jensen (1999). The algal cells of *Boergesenia* seem to be much bigger than *Struvea*, because *Ercolania kencolesi* sp. nov. intrudes completely into the algal cell and moves like a vacuum cleaner. Pulsation of the cerata, as was described for *Ercolania endophytophaga*, was not observed for *E. kencolesi* sp. nov..

Reproduction: Copulation and egg laying of *Ercolania kencolesi* sp. nov. is very similar to that described by Jensen (1999) for *E. endophytophaga*, but the shape of the egg masses differ. Whereas *Ercolania endophytophaga* lies sausage-shaped egg masses (Jensen 1999), the spawn of *E. kencolesi* sp. nov. is spirally coiled (see also Loch 1989). Jensen (1996) considered the sausage-shaped egg mass as a generic character for *Ercolania*, although she described a spirally coiled eggmass for *E. coerulea* (see Jensen 1985). The spirally coiled egg mass of *Ercolania kencolesi* sp. nov. therefore represents a further exception for the genus *Ercolania*.

The number of eggs per egg clutch is considerably higher in the new species than in *Ercolania endophytophaga* (about 500 in the former, up to 117 in the latter). Size of egg capsules and egg diameter (215 µm and around 100 µm in *Ercolania kencolesi* sp. nov.; around 140 µm and 77 µm in *E. endophytophaga*) differs also considerably between these two species. Jensen (2001) listed and discussed a number of sacoglossan species with egg and capsule diameter. Compared with these data on about 10 species, *Ercolania kencolesi* sp. nov. is the species with the largest eggs and egg capsules.

Development: The ontogeny of *Ercolania kencolesi* sp. nov. is very similar to that of *E. endophytophaga*, as described by Jensen (1999). In *Ercolania kencolesi* sp. nov., first veliger larvae have been observed after three days, in *E. endophytophaga* already after two. The bigger eggs in the former, which might prolong development, can explain this. One day after having developed into the veliger state, eyes were visible in both species. Hatching was not observed in *Ercolania endophytophaga*, but Jensen (1999), following Thompson (1976), discussed the eyes developing before hatching as an evidence for lecithotrophic development. But see also Goddard (2001), who discussed this phenomenon in more detail. All other *Ercolania* species known from the Indo-Pacific have planktotrophic development (Jensen 1985, 1996, 1999, 2001).

Photosynthetic activity: Yield values of the alga *Boergesenia forbesii* lie between 0.6 and 0.8 indicating a healthy photosynthetic activity. *Ercolania kencolesi* sp. nov. (Figs. 4A, 4B) shows much lower values already within a few hours after capture and after feeding/starvation experiments. The decrease of the ground fluorescence values (F_0) over time as well as loss of green colour strongly indicates that the number of chloroplasts declines. Feeding experiments after starving periods and *vice versa* clearly show that digestion of chloroplasts occur within a few hours up to three days. According to these results, it can be concluded that chloroplasts are not retained as discussed in some other sacoglossans (Rumpho *et al.* 2001, Wägele & Johnsen 2001, Evertsen *et al.* 2007).

Short-term, non-functional retention is also reported for another *Ercolania* species by Clark *et al.* (1990). They reported that freshly collected animals of *Ercolania coerulea* retain plastids in digestive gland diverticula for at least two hours of starvation, but no photosynthate was detectable by isotope tracer techniques.

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