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SACOGLOSSA (MOLLUSCA: GASTROPODA: OPISTHOBRANCHIA) FROM SINGAPORE

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***ABSTRACT.* –** Eleven species of sacoglossan opisthobranchs (Mollusca: Gastropoda: Opisthobranchia: Sacoglossa) are described from Singaporean waters. Internal anatomy is described for the first time for three species of *Costasiella* originally described from Okinawa, Japan. Spawn and fecundity for *Placida daguilarensis* Jensen, 1990, is also described for the first time. The synonymy of *Elysia ornata* (Swainson, 1840) and *Elysia grandifolia* Kelaart, 1857, is discussed.

***KEY WORDS*. –** Sacoglossa, Opisthobranchia, systematics, anatomy.

INTRODUCTION

The opisthobranch fauna of Singapore has been described in several publications (Lim & Chou, 1970a, b; Sigurdsson, 1991; Sachidhanandam et al., 2000). However, the Sacoglossa have been largely ignored in these publications. During the "14th International Marine Biology Workshop" held at the Tropical Institute of Marine Science, National University of Singapore in April 2006, eleven species of Sacoglossa were collected. Additional material has been collected by the present author in 1995 and by Prof. J. B. Sigurdsson in 1985.

Most shallow water habitats in Singapore are heavily modified. Many islets have been developed as recreation facilities, where beaches are protected by seawalls and fine white sand present within imported from elsewhere. After completion of this development, new growth of reef-forming corals and macro-algae has appeared close together in shallow water (Huang et al., 2006). Whereas tourist activities may be harmful for the corals, they may well be beneficial for macro-algae, at least at the present level of tourism. Macro-algae in coral reef environments are usually restricted by lack of nutrients, but human activities invariably cause addition of nutrients. The research activities carried out at the Tropical Marine Science Institute at St John's Island should be able to monitor ecological changes so that human activities can be regulated accordingly.

MATERIAL AND METHODS

Algae were collected from various localities in the Singaporean archipelago. Most were collected intertidally during low tide, but also snorkeling in shallow subtidal waters. A few samples were collected by SCUBA by Dr. R. C. Willan. Algae were brought to the laboratory at St John's Island and carefully examined in shallow trays with seawater. Sacoglossans were picked out and kept in separate containers with seawater and food algae. After several days in the laboratory, animals were relaxed in 7% $MgCl_2$, fixed in 4% seawater-buffered formalin, and preserved in 80% EtOH. Radulae were extracted by placing the pharynx in NaOH or in household bleach to dissolve the tissue. Radulae were then rinsed in demineralized water and two rinses of 96% EtOH, dried and transferred to SEM-stubs. Penises with stylets, where present, were dissected out, dehydrated in a series of EtOH and acetone, dried and placed on SEM-stubs. Where nothing else is mentioned, algae and sacoglossans were collected by the author.

SYSTEMATICS

Volvatella vigourouxi (Montrouzier, in Souverbie, 1861)

(Figs. 1A–C, 2A, 3)

Synonyms. – *V. kawamurai* Habe, 1946? (fide Baba, 1966).

Material examined. – 14 specimens on *Caulerpa sertularioides?* and *C. ramosa* collected intertidally at Pulau Tekukor, on 11 Apr.2006. Three specimens (3–4 mm shell length) were dissected.

Two specimens from New Caledonia collected by W. B. Rudman (AM C200637) were used for comparison of Singaporean specimens with specimens from the original locality.

***Description.* – Colour alive.** White and orange glands are distributed on the mantle fold; orange spots are especially dense in a band behind the gill (hypobranchial gland), but also along the anterior mantle margin. A dark green spot is seen anteriorly on the dorsal side (to the left of the heart) and ventrally in front of the gill; also a broad dark band is seen under the hypobranchial gland (visible both anterior and posterior of this gland). Head and foot are white; a few orange dots are located on and between the cephalic tentacles of the largest specimen. In preserved specimens the orange colour has disappeared.

External features. The largest specimen had a shell length of 10 mm, the second largest of 6 mm, and the remaining specimens had shell lengths of 2–4 mm. The shell is very thin and fragile, with numerous fractures in the preserved specimens. It is covered by a transparent, overhanging periostracum. The posterior shell "spout" (extension of aperture) is moderately long and skewed towards the right side. The posterior cephalic lobes are large, forming tentacles (Fig. 1A). The anterior tentacles are broad and directed outwards. The foot is short and broad, anteriorly widened to form rounded lobes with a slight median notch; posteriorly it is rounded. The gill filaments form a wide band extending from the right mantle edge to the ventral attachment of the mantle fold.. The hypobranchial gland is behind the gill (the narrow orange band in Fig. 1A–C).

Internal anatomy. The pharynx is small and has a large muscular pouch posteriorly (Fig. 3A, B). The radular teeth have triangular, denticulate cusps (Fig. 2A). The conical, muscular penis is unarmed (Fig. 3C).

Observations on live specimens. As other species of *Volvatella*, this species exudes a lot of milky fluid when disturbed - both through the spout and through wide part of the shell aperture. One egg mass was discovered in the container holding *V. vigourouxi* on 15 April; the embryos were large veligers, so it must have been deposited several days before. It contained 294 eggs and formed a flat disc on a frond of *C. sertularioides*. The egg capsules measured 113 ± 5.4 × 100.5 ± 5.4 µm (n=10) and the veliger shell had a diameter of 84 ± 3.9 µm.

***Remarks.* –** *Volvatella vigourouxi* was originally described from one preserved specimen from New Caledonia (Montrouzier in Souverbie, 1861). As usual with old descriptions, only the shell was described, and no type material is available. The colour plate indicates yellowish coloration of the periostracum. In 1966 Baba described specimens collected in Japan, which were tentatively identified with this species. More recently it was described from Taiwan (Huang et al., 2007). In that paper also feeding and copulation were described. The specimens from New Caledonia (AM C200637) were much larger than the specimens from Singapore. But apart from size, they appeared to be identical. Three other species of *Volvatella* have been described as being orange or even red: *Volvatella pyriformis* Pease, 1868, described from Huaheine, *V. cincta* Nevill & Nevill, 1869, from Sri Lanka, and *V. evansi* (Kay, 1961) (as *Arthessa evansi*) from Hawaii. The latter species has been synonymized with *V. pyriformis* (Kay, 1979). *V. vigourouxi* externally approaches *V. pyriformis*, but even to this day the anatomy of the latter species remains unknown. *Volvatella vigourouxi* has a distribution ranging from New Caledonia to Japan, and has also been found in Guam (Montrouzier, 1861; Baba, 1966; Carlson & Hoff, 2003). If it turns out to be synonymous with the other two orange species, as suggested by Rudman (2002), the distribution will be broad Indo-Pacific (Sri Lanka to Hawaii). Another species that has a similar shell, but for which colouration is unknown, is *V. elioti* (Evans, 1950), from Zanzibar in the western Indian Ocean.

Volvatella sp.

(Figs. 1D–E, 3E)

***Material examined.* –** 1 specimen collected at same locality and date as previous species.

***Description.* – Colour alive.** White with transparent spots of different sizes in mantle.

External features. The anterior cephalic lobes are much wider than posterior ones. The gill is seen as a band of narrow filaments encircling the body. No hypobranchial gland is visible. The foot is short and broad, posteriorly rounded, anteriorly broader than body. The foot corners are rounded. There is no median notch in the anterior foot margin.

***Remarks.* –** With only one specimen available internal anatomy has not been examined. Morphologically the specimen is similar to *Volvatella angeliniana* Ichikawa, 1993. However, no orange or yellow coloration was seen in the present specimen, and also, the colour pattern seems to be a "negative" of *V. angeliniana*, which has white spots in an otherwise unpigmented mantle (Ichikawa, 1993). None of the previously described species of *Volvatella* has this color pattern, but there are a number of species for which only the shell has been described.

Placida daguilarensis Jensen, 1990

(Figs. 1F–G, 2B, 4A)

Material examined. – Many specimens on *Bryopsis* spp. collected at 14 m at Darat patch reef near Pulau Hantu by R. C. Willan on 5 Apr.2006; many specimens on *Bryopsis* spp. collected at 0.5 m at Kusu Island by R. C. Willan on 6 Apr.2006; 3 specimens on *Bryopsis* sp. at Pulau Tekukor on 11 Apr.2006.

***Description.* – Colour alive.** Animals appear bright green due to the contents of the digestive gland. There are also some dark brown pigment spots, which in some specimens form longitudinal stripes along the sides of the body below the cerata and extending anteriorly to the ventral surface of

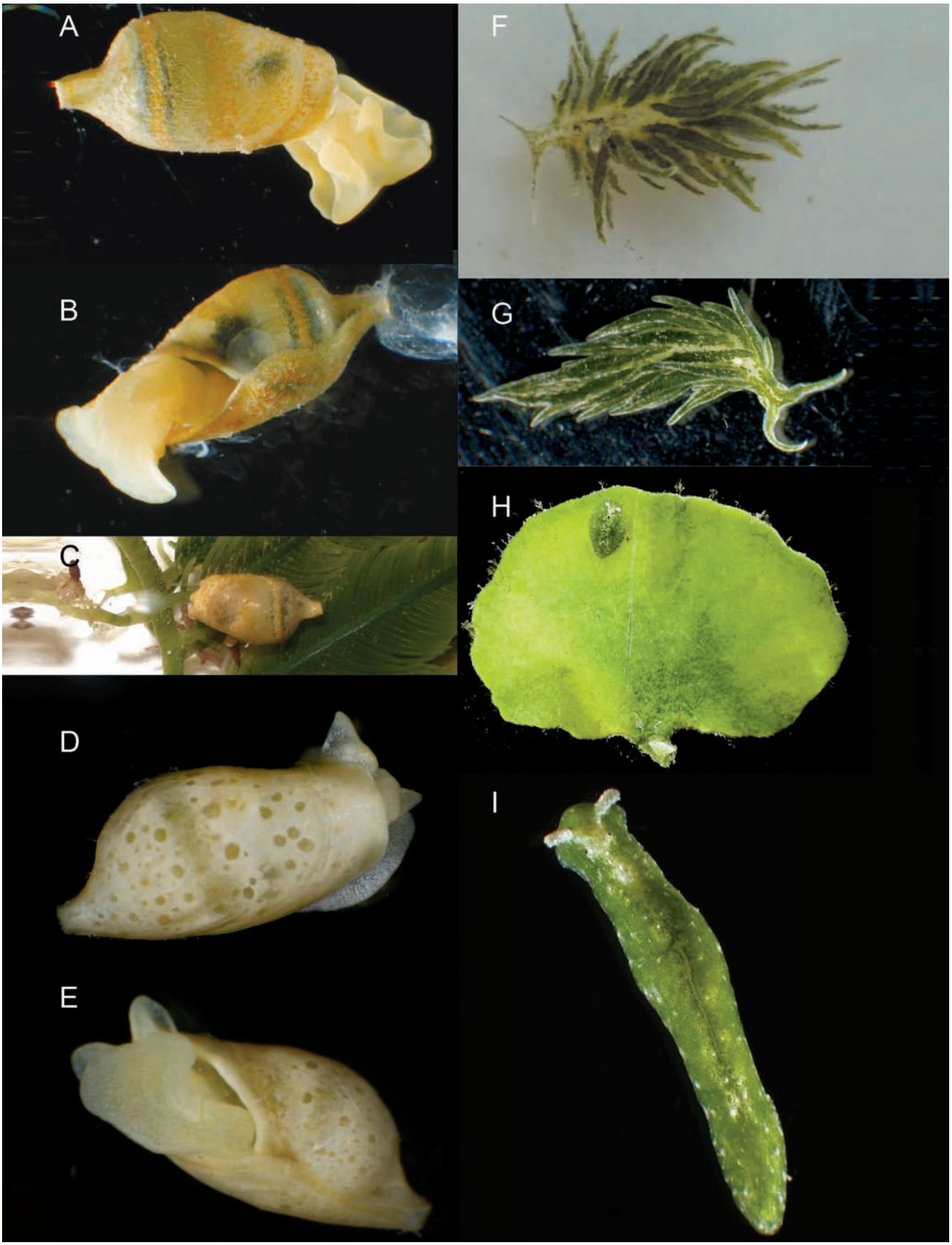

Fig. 1. Color photographs of Sacoglossa from Singapore. **A**, *Volvatella vigourouxi* dorsal view; **B**, same, ventral view; **C**, same on *Caulerpa serularioides*; **D**, *Volvatella* sp. dorsal view; **E**, same, ventral view; **F**, *Placida daguilarensis* specimen with dark pigment dorsally; **G**, another specimen with very broad rhinophores; **H**, *Elysiella pusilla* on segment of *Halimeda* sp.; **I**, same, crawling in petri-dish. Photos **A, B, D, E, G, H, I**: Michelle Lee; **C** and **F**: K.R. Jensen.

the oral lobes. Brown dots are also scattered on the cerata. The renopericardial ridge is white in some specimens.

External features. The smallest specimens were only slightly over 1 mm long and had at least 3 cerata that were as long as the body, plus several smaller ones. The larger specimens were about 8 mm long when collected, but grew larger while in the laboratory (see below). The head is concave anteriorly. Some specimens had extremely long, thin rhinophores, but most had rhinophores that were flattened at the bases. The digestive gland extends into the rhinophores, at least at the proximal part. The degree of branching of the digestive gland is highly variable between specimens. Some have an apparently unbranched tubule in each ceras; others have highly branched tubules of the digestive gland in cerata as well as rhinophores. In some specimens the central tubule has numerous thin, short, branching tubules extending at right angles so that the terminal branches are very close to the surface. The anus is located on a papilla anteriorly on the pericardial swelling. There is a long vaginal ridge on the right side. The anterior foot corners are rounded and slightly wider than the foot sole; there is no median notch.

Internal anatomy. This has been described previously (Jensen, 1990). The radula of a 3 mm (preserved length) had 5 teeth in the ascending limb, 10 in the descending one, plus 3 pre-radular teeth. A second specimen contained 6 teeth in the ascending limb, 12 teeth plus 3 pre-radular teeth in the descending limb, which formed a beginning spiral. In a third specimen the radula had 8 teeth in the ascending limb and 13 teeth plus 3 pre-radular ones in the descending limb, also forming a beginning spiral. The size of the teeth decrease rapidly in the descending limb. The teeth are blade-shaped with a characteristically curved blade and smooth cutting edges (Fig. 2B). The penis is armed with a short, curved stylet (Fig. 4A). There is an apparently unbranched tubule

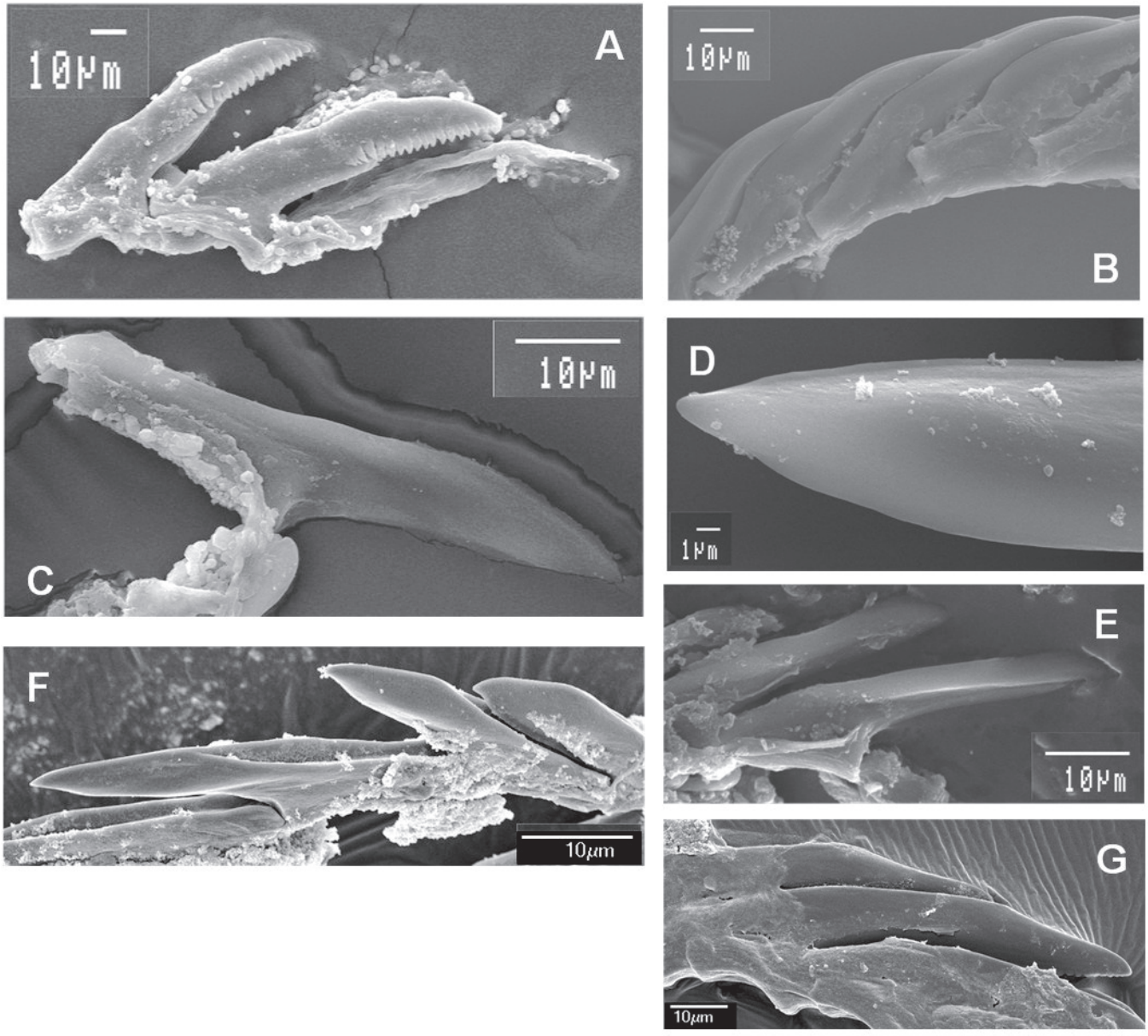

Fig. 2. SEM photos of sacoglossan radular teeth. **A**, *Volvatella vigourouxi*; **B,** *Placida daguilarensis*; **C**, *Costasiella paweli*; **D**, *C. paweli*, tip of tooth; **E**, *Costasiella usagi*; **F**, *Costasiella kuroshimae*; **G**, *Elysia* cf. *verrucosa*, small specimen.

Table 1. Fecundity in *Placida daguilarensis* from Singapore. Animals were kept separately after copulation.

Length at day 1	Length at day 5	# egg masses	Total # eggs	Mean (±s.d.) # eggs per day
8mm	7mm	8	10196	1949±424
7mm	6mm	3	2132	*
8mm	6.5mm	5	8897	1779±544

* Eggs were only produced at days 2 and 3.

of the albumen gland in the cerata, winding around the digestive gland tubule.

Spawning. The animals began spawning shortly after collection. The egg mass is shaped like an irregularly coiled sausage. It is very sticky and it is almost impossible to dislodge it from the substrate without breaking it. The egg capsules are arranged in a slightly irregular spiral inside the egg mass. The capsules contain foamy "albumen", which is still visible at the gastrula stage, though the "granules" appear smaller than immediately after spawning. Egg capsules were measured in three egg masses: 128.3 ± 7.2 × 98.3 ± 2.5 µm (n=12); 113.8 ± 10.0 × 103.0 ± 7.3 µm (n = 30); 133.3 ± 6.5 × 114.2 ± 7.9 µm (n=12). In the first egg mass all eggs were in the gastrula stage. In the second egg mass most eggs were in the 2- or 4-cell stage, but some were uncleaved. In the third egg mass all eggs were uncleaved. Egg diameter was: 59.0 ± 2.1 µm (n=10) and 60.0 ± 0.0 µm (n=12). Development at room temperature took 5–6 days. The shell of the veliger larvae just before hatching was 99.2 ± 5.1 µm long (n = 12). The larvae had distinct statocysts and a black spot (larval renal organ). Capsule size as well as development time indicates planktotrophic development for this species. A few specimens were separated and the number of egg masses produced as well as the number of eggs per egg mass was counted (Table 1).

***Remarks.* –** This species was originally described from Hong Kong (Jensen, 1990). Recently it has also been described from Japan (Hirano et al., 2006). The present material agrees with previous descriptions, though there seems to be greater variability in branching pattern of digestive gland, length of cerata and amount of brown epidermal pigment. The present collection increases the distribution to at least the northwestern Pacific rim, from Japan to Singapore.

Costasiella paweli Ichikawa, 1993
(Figs. 2C–D, 4C, 5A, 6, 9B)

***Material examined.* –** 3 specimens collected from *Avrainvillea* at Pulau Subar Laut on 4 Apr.2006; more than 10 specimens collected on *Avrainvillea* at Pulau Hantu on 10 Apr.2006 (4 dissected); several specimens collected on *Avrainvillea* at Pulau Hantu on 26 Apr.1995 (2 dissected); serial sections of one specimen collected by Prof. J. B. Sigurdsson, 1985.

***Description.* – Colour alive.** The rhinophores have a broad black band towards the outer half; in some specimens the outer half was black. There is a spot of rust-coloured and black dots behind the eyes, extending up the front of the pericardium. Also, there are some scattered rust-colored dots on the anterior surface of the head. The cerata appear dark green from dense digestive gland tubules; the tips have subterminal bands of white and orange or pale red and the narrow tips are transparent. Black dots are scattered on the green part of the cerata as are tiny fine asterisk-shaped white dots. The anterior margin of the head has a thin row of rust-colored spots. The tip of the tail is transparent, like the tips of the cerata. There is a row of dark dots along the foot margin and in one specimen also along the anterior margin of the head.

External features. The rhinophores are simple; in some specimens the bases appear flattened. The pericardium is about half as long as the body (excluding head and tail); it is distinctly longer than wide (Fig. 6A). The anus is on a distinct papilla adjacent to the right side of the pericardium. The anterior foot corners are drawn out to form spatulate tentacles (Fig. 9B). The oviducal opening is seen under the anteriormost cerata. The cerata are inflated with thin tips. They are arranged in 3 to 5 rows with the largest dorsally. One specimen had 26 cerata on either side. The tail is shaped like a ceras and is separated from the rest of the foot sole by a distinct groove (Fig. 9B).

Internal anatomy. The pharynx has a large muscular pouch posteriorly (Fig. 6F). The radular teeth are blade-shaped with a smooth cutting edge (Fig. 2C, D). One radula contained 6 teeth in the ascending limb and 6 teeth in the descending limb. Another radula had 8 teeth in the ascending limb and 5 teeth in the descending limb. In both specimens the ascus was lost during preparation. In the sections the ascus was seen squeezed in between the pharyngeal pouch and the ventral ascus-muscle, which - besides the small size of the teeth - explains why it was lost when dissolving the musculature. Behind the ganglionic ring there is an esophageal pouch with a muscular wall and a large lumen. The stomach is short, and the intestine ascends from the anterior end. Three branches of digestive gland leave the stomach on either side. The penis is long and muscular, but without a stylet (Figs. 4C, 6G). The prostate gland is composed of several lobes. The albumen gland sends one or two narrow branches into the larger cerata (Fig. 6D, E). The follicles of the ovotestis fill out most of the space in the body cavity.

***Remarks.* –** The present specimens differ somewhat in

coloration from the original description. The specimens from Okinawa had all black rhinophores, and no red or orange spots or rings subterminally on the cerata were mentioned (Ichikawa, 1993). However, the spatulate foot tentacles, the dark pigment along head and foot margins, and the black dots and white tips of the cerata indicate that they are the same species. *C. paweli* was originally described from Okinawa, Japan, but the description did not include anatomical characters, which are described here for the first time. Ichikawa's holotype is supposedly deposited as serial sections in the Natural History Museum of Vienna (Austria). Paratypes were said to be deposited at the Seto Marine Biological Laboratory (Ichikawa, 1993), but this material is either lost or was never deposited (Trowbridge, pers. comm.). Hence voucher specimens have been deposited at the Zoological Museum in Copenhagen (Denmark). Several specimens of *Costasiella* with a similar color pattern have been published on the Sea Slug Forum. Unfortunately it is not possible to see the anterior foot in these pictures and hence their identity remains uncertain. Some of them have been identified as *C. kuroshimae*, but contrary to the specimens here identified as that species (see below), they have orange pigment on the cerata.

Costasiella usagi Ichikawa, 1993
(Figs. 2E, 4B, 5B–C, 7, 9A)

Material examined. – 3 specimens, on *Avrainvillea*, intertidal sandflat, Pulau Subar Laut, 4 Apr.2006 (1 dissected); 2 specimens, on *Avrainvillea*, intertidal sandflat, Pulau Hantu, 10 Apr.2006 (1 dissected); 2 specimens, on *Avrainvillea*, intertidal coral rubble/sand, Pulau Tekukor, 11 Apr.2006; 3 specimens (2 dissected) and serial sections of one specimen collected by Prof. J. B. Sigurdsson in 1985 (no locality or date given).

Description. – **Colour alive.** The general body colour appears dark green from the contents of the digestive gland. The cerata have some thin longitudinal silvery lines, which make the medial (largest, oldest) cerata look grayish. Some specimens have a lot of black pigment on the cerata; other specimens just have black tips. Some brown pigment is found on the pericardium, in some specimens continuing in a line running down both sides of the body as a kind of "necklace". The rhinophores have longitudinal stripes of dark pigment anteriorly and posteriorly, sometimes almost the entire length of rhinophores, giving a resemblance of a lateral groove (the unpigmented part). Some specimens have a dark rust-colored spot in front of the eyes as well as some rust-colored or orange pigment on the frontal part of the head. The epidermis covering the eyes is transparent. There are some white dots along the margin of the foot and an opaque white stripe in the posterior part of the foot sole.

External features. The pericardium is short and tall and the anus is on a short papilla at the right anterior corner (Fig. 7A). The rhinophores are thick and simple, possibly slightly flattened at the base. The large eyes appear to be located in a small protuberance between the rhinophores (Fig. 7B). The cerata are fusiform. There are about 30 cerata on either side arranged in 3–5 rows. The anterior corners of the foot form long, thin tentacles. The tail is not shaped as a ceras (Fig. 9A). The penial opening is located below the base of the right rhinophore.

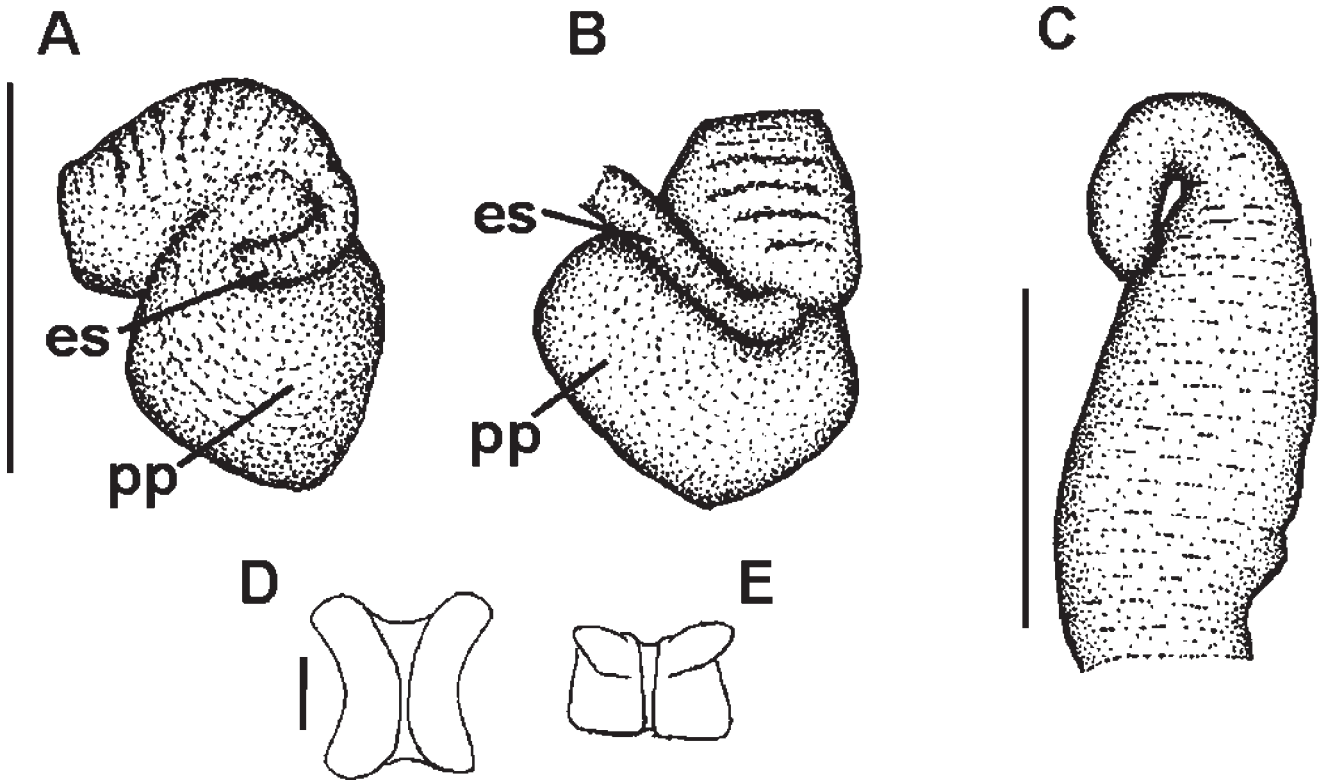

Fig. 3. *Volvatella* spp. **A**, *V. vigourouxi* pharynx, lateral view; **B**, same, dorsal view; **C**, *V. vigourouxi* penis; **D**, *V. vigourouxi* frontal view of head; **E**, *Volvatella* sp. frontal view of head. Scale bars: **A–C**: 0.5 mm; **D–E**: 1 mm.

Internal anatomy. The pharynx is small and has a small pharyngeal pouch (Fig. 7E). The radula is very difficult to extract; it appeared to dissolve when the pharynx musculature was dissolved in either NaOH or dilute commercial bleach, so probably the teeth are not mineralized. The radula contained 7 teeth in the ascending limb and 8 teeth in the descending limb; no ascus was seen. The teeth are blade-shaped and smooth (Fig. 2E). The most distinguishing feature of this species is the long, spirally coiled penial stylet (Fig. 4B, 7F). Apparently there is no albumen gland in the cerata (Fig. 7C). The digestive gland in the cerata forms thin, cup-like layers partly covering large spheres of vacuolized tissue (Fig. 7D). This tissue is not seen in the smallest cerata and may be degenerate digestive gland cells after complete digestion of chloroplasts. There appears to be a long convoluted vaginal duct under the cerata at the same level as the anal papilla. An opening into the seminal receptacle was not seen, but there was a distinct patch of tall epithelial cells (scar tissue from penetration of stylet?) of the seminal receptacle adjacent to this duct. The seminal receptacle contains sperm with the heads attached to the receptacle wall.

***Remarks.* –** Swennen (2007) summarized the characters of all species of this genus. *Costasiella usagi* is the only species of the ones described by Ichikawa (1993) that is easily recognizable. The longitudinal striping of the cerata and the thin pedal tentacles are not found in any other species of *Costasiella*. Also the long, coiled penial style is a unique character, though no anatomical features were described by Ichikawa (1993). The undivided foot sole is also rare in the genus. The species was originally described from Okinawa, Japan and has subsequently been recorded from Guam and Pohnpei, Northern Marianas (Carlson & Hoff, 2003) and from Lizard Island, Australia (Wägele et al., 2006). The present study is the first record from Singapore and the first description of the anatomy.

Costasiella kuroshimae Ichikawa, 1993
(Figs. 2F, 4D, 5D, 8, 9C)

***Material examined.* –** Two specimens from *Avrainvillea* collected at Pulau Subar Laut, 4 Apr.2006; 6 specimens from *Avrainvillea* collected at Pulau Hantu, 10 Apr.2006 (3 dissected).

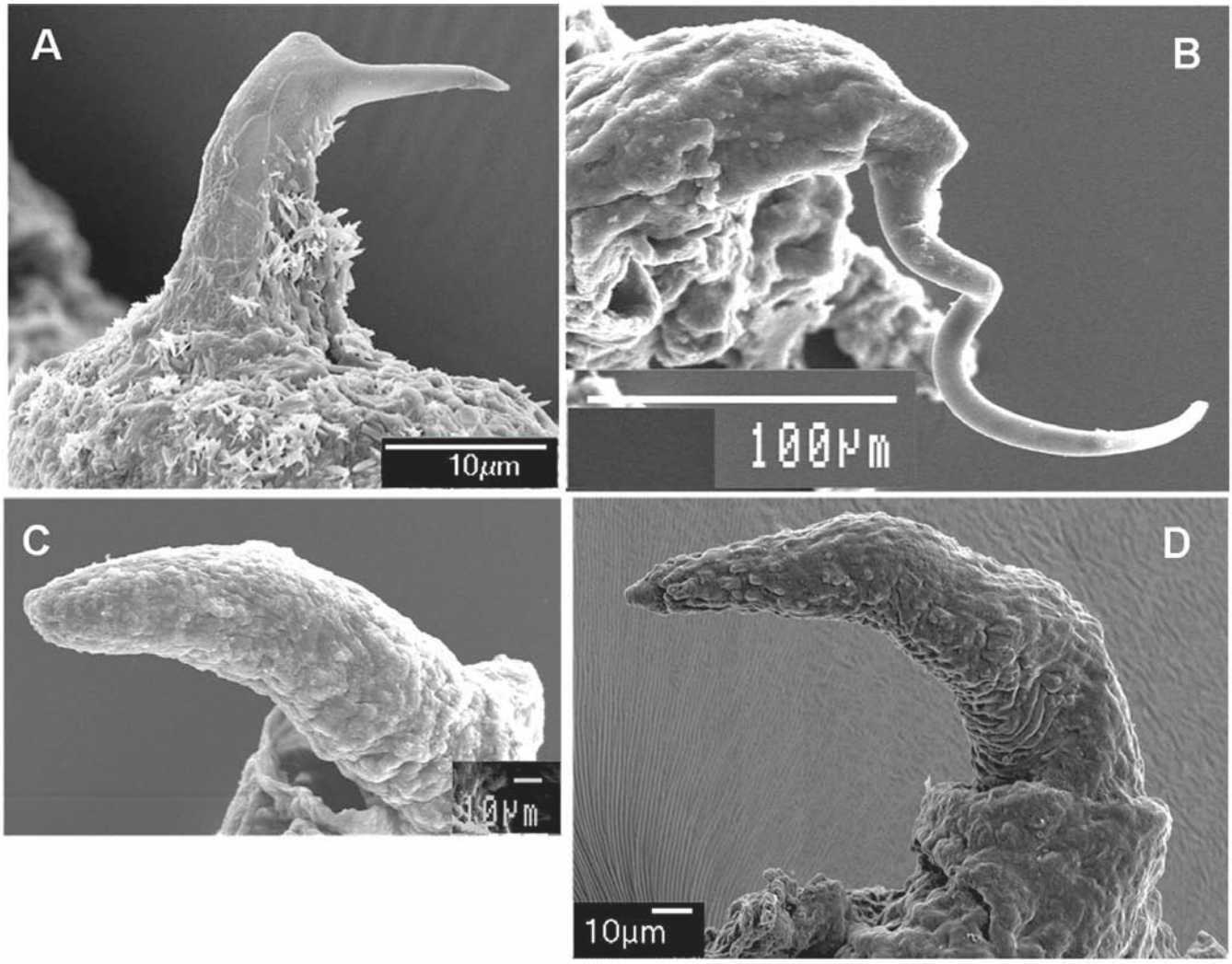

Fig. 4. SEM photos of sacoglossan penises. **A**, *Placida daguilarensis*; **B**, *Costasiella usagi*; **C**, *Costasiella paweli*; **D**, *Costasiella kuroshimae*.

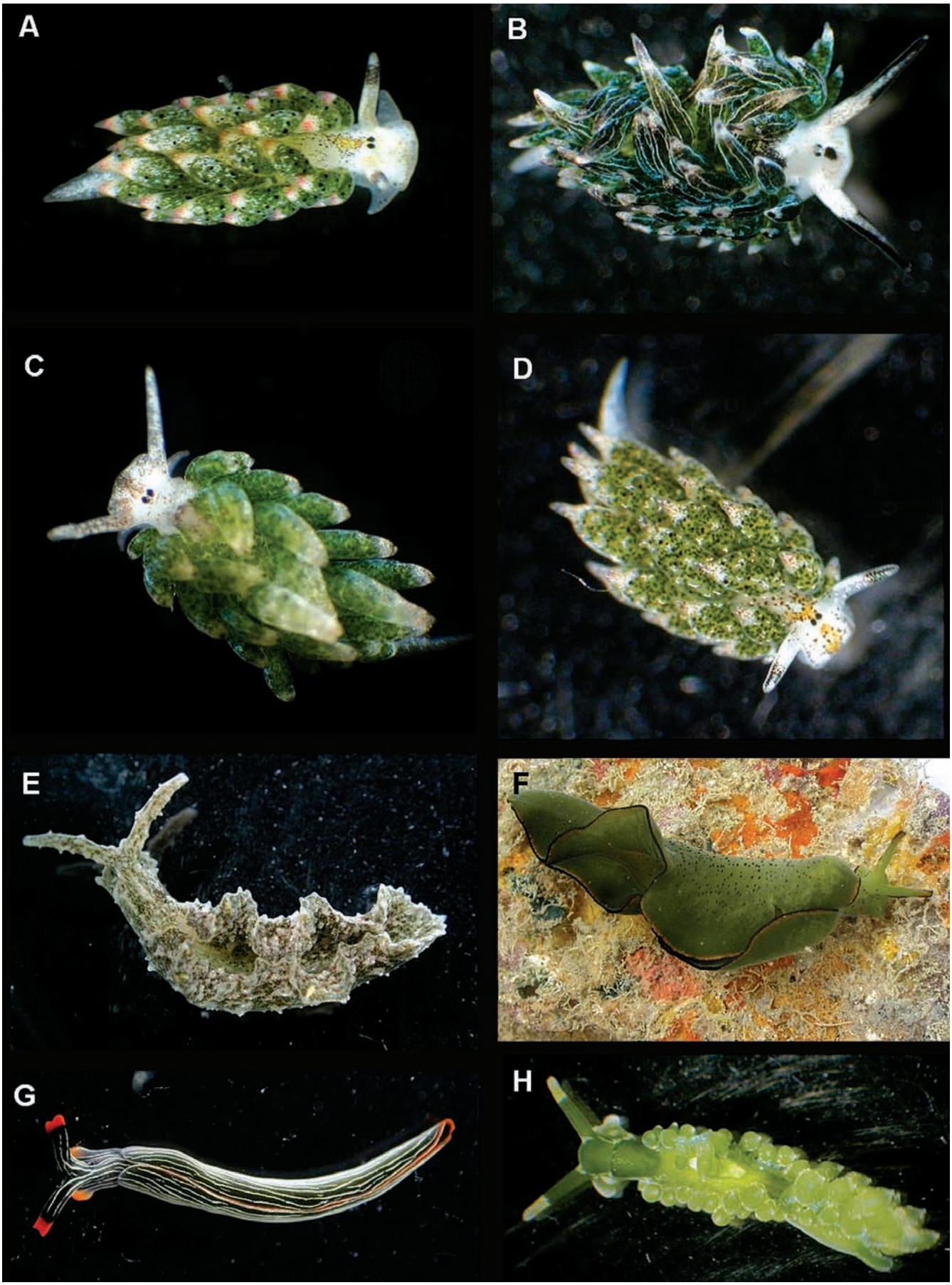

Fig. 5. Color photographs of Sacoglossa from Singapore. **A**, *Costasiella paweli*; **B**, *Costasiella usagi*, dark specimen; **C**, *C. usagi*, bright green specimen; **D**, *Costasiella kuroshimae*; **E**, *Elysia* cf. *verrusosa*; **F**, *Elysia ornata*; **G**, *Thuridilla ratna*; **H**, *Stiliger smaragdinus*. Photos: Michelle Lee.

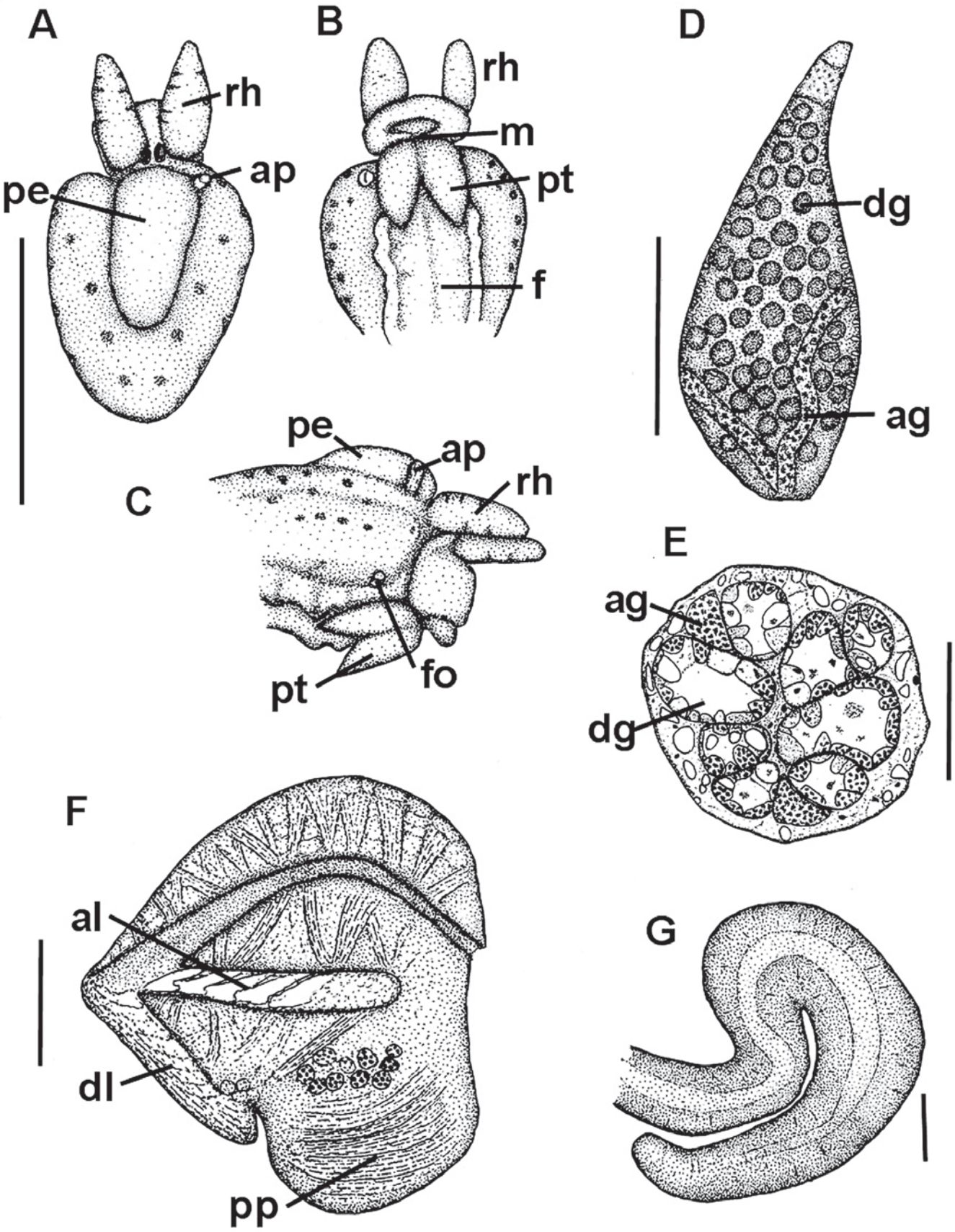

Fig. 6. *Costasiella paweli*. **A**, dorsal view of body after removal of cerata; **B**, ventral view of body after removal of cerata; **C**, lateral view of body after removal of cerata; **D**, ceras; **E**, cross-section of ceras; **F**, pharynx; **G**, penis. Scale bars: **A–C**: 1mm; **D**: 0.5 mm; **E–G**: 0.1 mm. Legend: **ag** - albumen gland; **al** - ascending limb of radula; **ap** - anal papilla; **dg** - digestive gland; **dl** - descending limb of radula; **e** - eye; **f** - foot; **fo** - female genital opening; **m** - mouth; **p** - penis; **pe** - pericardium; **pp** - pharyngeal pouch; **pt** - pedal tentacles; **rh** - rhinophore.

***Description.* – Colour alive.** There is a pigmented stripe, orange or rust-coloured, down the anterior surface of the head. This is visible through the epidermis from the ventral side. Some specimens have black tips, or a narrow submarginal black band on the rhinophores, but most specimens do not have any black pigment on the rhinophores. Rust-coloured and white dots are scattered on the rhinophores. In the largest specimens the rust-coloured dots almost form a line on the posterior surface of the rhinophores. There is an iridescent blue spot in the neck in front of the pericardium; this is not found in the smallest specimens. Iridescent and white dots are also found on the cerata; in some specimens there is only one large iridescent spot on the medial side of the largest cerata. Small rust-coloured dots are scattered all over the body. The digestive gland in the cerata is only visible as small green dots, whereas the opaque white albumen gland takes up a lot of the space in the cerata. The pericardium has rust-coloured dots on anterior surface. Tiny white dots (glands) dispersed along the foot margin. There are 2 tiny black dots flanking the mouth.

External features. The rhinophores are simple. The large eyes take up almost all space between the rhinophores. The anterior margin of the rhinophores continues into the edge of the front of the head (Fig. 8C). The anterior foot corners are rounded, not forming tentacular processes and the tail is shaped as a ceras, separated from the rest of the foot by a groove (Fig. 9C). The pericardium is tall and only slightly longer than wide; the width is about the same as the diameter of the largest cerata. The anus is on a papilla to the right of the anterior corner of the pericardium (Fig. 8A). There appears to be a vessel entering the pericardium posteriorly. This vessel branches shortly behind the pericardium. The cerata are in 4 or 5 rows with the largest dorsally. A 1.5 mm specimen had 17 cerata on the right side and 16 on the left side.

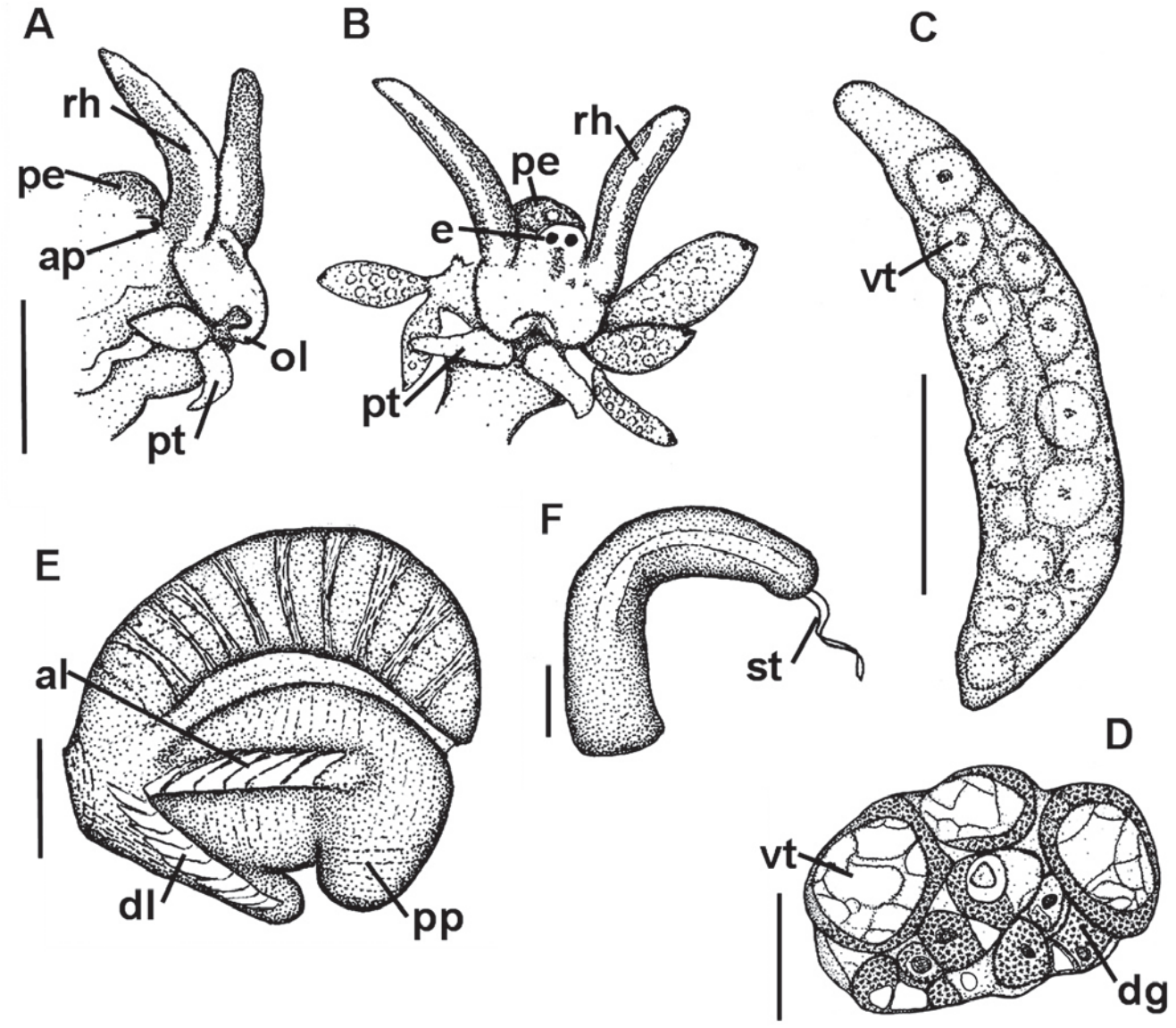

Fig. 7. *Costasiella usagi*. **A**, lateral view of anterior body; **B**, frontal view; **C**, ceras; **D**, cross-section of ceras; **E**, pharynx; **F**, penis. Scale bars: **A–C**: 0.5 mm; **D–F**: 0.1 mm. Legend: **al** - ascending limb of radula; **ap** - anal papilla; **dg** - digestive gland; **dl** - descending limb of radula; **e** - eye; **ol** - oral lobes; **pe** - pericardium; **pp** - pharyngeal pouch; **pt** - pedal tentacles; **rh** - rhinophore; **st** - penial stylet; **vg** - vacuolized tissue.

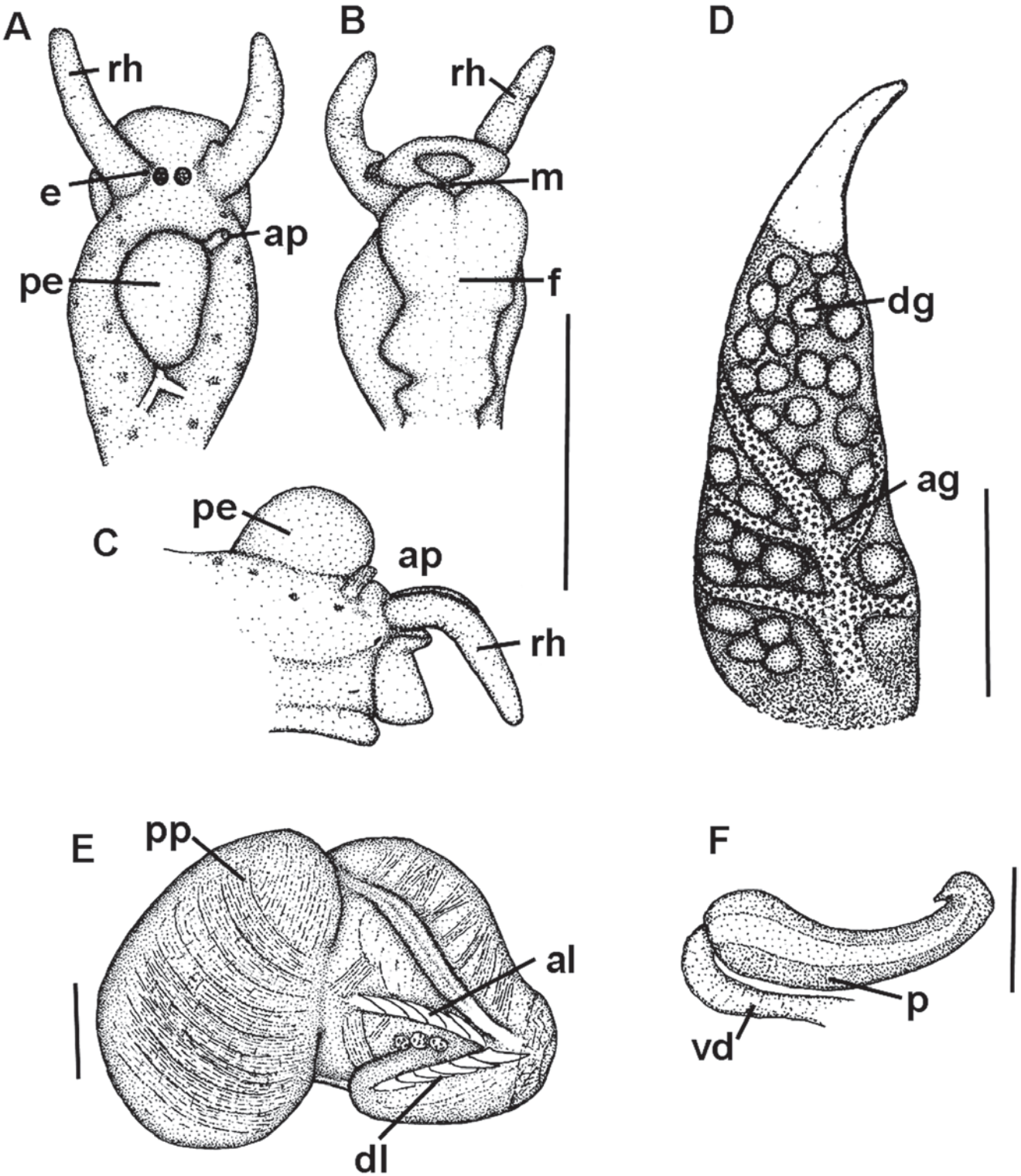

Fig. 8. *Costasiella kuroshimae*. **A**, dorsal view of body after removal of cerata; **B**, ventral view of body after removal of cerata; **C**, lateral view of body after removal of cerata; **D**, ceras; **E**, pharynx; **F**, penis. Scale bars: **A–C**: 1 mm; **D**: 0.5 mm; **E–F**: 0.1 mm. Legend: **ag** - albumen gland; **al** - ascending limb of radula; **ap** - anal papilla; **dg** - digestive gland; **dl** - descending limb of radula; **e** - eye; **f** - foot; **m** - mouth; **p** - penis; **pe** - pericardium; **pp** - pharyngeal pouch; **rh** - rhinophore; **vd** - vas deferens.

Internal anatomy. The pharynx has a large muscular pouch (Fig. 8D). A 3 mm long specimen (preserved length) had a radula with 7 teeth in the ascending limb and 6 teeth in the descending limb. The ascus was lost The teeth are blade-shaped and smooth (Fig. 2F). The penis is relatively short, conical and unarmed (Figs. 4D, 8F).

Remarks. **–** The colouration of the present specimens is most similar to that described for *Costasiella kuroshimae* from Okinawa (Ichikawa, 1993). Unfortunately the original description was not very detailed and no anatomical data were given, not even radular teeth. To allow future comparisons with other species, voucher specimens have been deposited at the Zoological Museum in Copenhagen (ZMUC). *C. kuroshimae* is separated from the above two species by having rounded foot corners and by having iridescent blue spots on the body and cerata. It is also very similar to *C. mandorahae* Jensen, 1997, but unlike this species and *C. paweli* it does not have a subterminal orange or pale red ring on the cerata. It is also separated from *C. mandorahae* by the absence of a fourth genital opening on the left side of the body (Jensen, 1997). Due to the poor original description the identity of several specimens published on the Sea Slug Forum (http://www.seaslugforum.net/) remains uncertain, as does the distribution of this species. Possibly the species identified as *Costasiella* cf. *ocellifera* from Lizard Island (Wägele et al., 2006) can be referred to *C. kuroshimae*. With the anatomical description of three of the six species described by Ichikawa (1993) it should be easier to identify species of *Costasiella* from the Indo-West Pacific region in the future. Characters that should invariably be examined are: (1) shape of anterior foot margin; (2) shape of foot sole; (3) size and shape of pericardium. If possible also position of anus and genital openings should be located, and of course, the colour pattern should be described. Radular teeth are of uniform shape within the genus, but shape and size of pharyngeal pouches and penis size and armature show distinct differences that can be used for species identification.

Stiliger smaragdinus **Baba, 1949**
(Figs. 5H, 10)

Material examined. **–** 1 specimen collected on *Caulerpa microphysa*?, Pulau Tekukor, 11 Apr.2006.

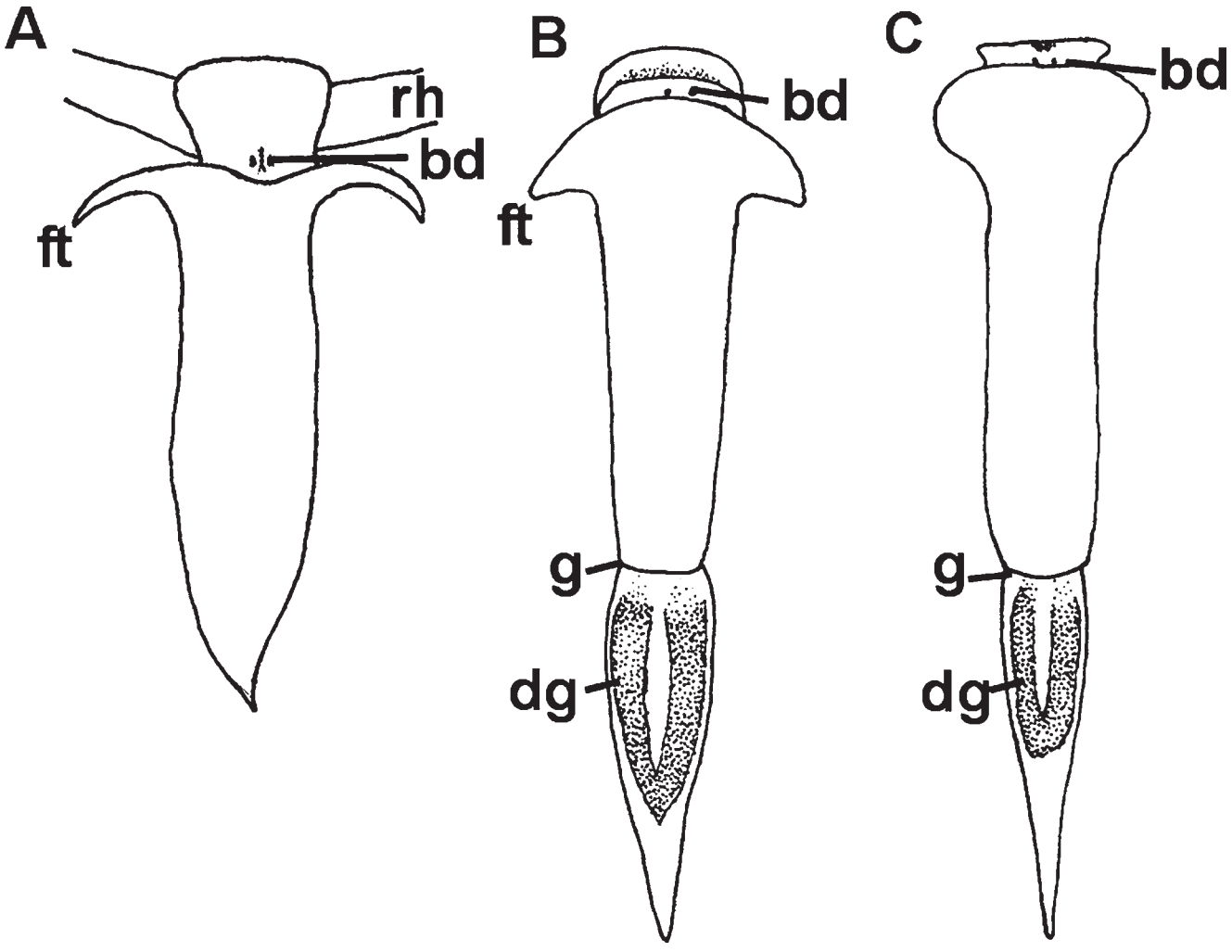

Fig. 9. Ventral view of foot sole of *Costasiella spp*. from Singapore. A, *C. usagi*; B, *C. paweli*; *C, C. kuroshimae*. Abbreviations used: bd, black dot; dg, digestive gland; ft, pedal tentacles; g, groove; rh, rhinophore

***Description.* – Colour alive.** This specimen had a white line along oral and pedal lobes. Also tips of anal papilla and rhinophores were white, and white lines extend from the eyes around the dorsal base of the rhinophores. Numerous iridescent blue dots are located around the bases of the cerata.

External features. The specimen was about 12–13 mm long alive. It lost some cerata when removed from the alga. The cerata had a ring of papillae (glandular?) around the widest part (Fig. 10). When the animal contracts the cerata, the papillae flatten out and form a "layer" on the back of the animal, resembling the utricles of *Caulerpa lentillifera*. In the present specimen cerata were placed in four rows with the largest towards the center of the dorsum.

***Remarks.* –** With only one specimen, internal anatomy has not been examined. This has been described previously (Jensen, 1993, 1997). *Stiliger smaragdinus* has a distribution from the eastern Indian Ocean in Western and Northern Australia and along the West Pacific rim from Japan through Guam and Singapore to eastern Australia, New Caledonia and New Zealand (Baba, 1949, Jensen, 1993, 1997; Carlson & Hoff, 2003; Morrison, 2005; Burn, 2006; present study). Comparing photographs on the Sea Slug Forum with the present specimen and specimens from Western Australia, it appears that the shape of the cerata varies with the location, and possibly the species of *Caulerpa* that the animals feed on. Whether one animal can actually change the shape of its cerata when it switches diet remains to be studied. The generic placement of this species has been discussed (Jensen, 1993, 1997). It has been placed in *Aplysiopsis (= Hermaeina)* because of its denticulate teeth (Baba & Hamatani, 1970) and in *Ercolania* without giving any explanation (Ichikawa, 1993). It has also been suggested that it should be placed in a separate, new genus (Jensen, 1997), but in phylogenetic terms it may be described as a *Stiliger* with many autapomorphies.

Elysia cf. *verrucosa* Jensen, 1985
(Figs. 2G, 5E, 11)

***Material examined.* –** 1 specimen collected from the drainage pool of the aquarium room of the TMSI, St John's Island, 5 Apr.2006; 1 specimen collected from *Chaetomorpha spiralis*? attached to stranded fishing net on Pulau Tekukor, 11 Apr.2006 (partly dissected).

***Description.* – Colour alive.** The general body colour is grayish green composed of dark green digestive gland tubules overlain by dense white dots (epidermal glands). There are also numerous orange dots, which appear to be surrounded by a dark green ring. However, this is digestive gland without any white dots. Also, there are some black ocellate spots. The parapodial margins are white. The large specimen had a white elongate spot in the neck region, in front of the pericardium. The pericardium was green anteriorly and white posteriorly.

External features. The body is covered by rounded white papillae, also found on the rhinophores. The pericardium has a slight constriction separating the green and white parts. There is one pair of distinct, branching renopericardial vessels extending from the posterior part of the pericardium. Another pair of less distinct and less branching vessels occurs more anteriorly. The parapodia usually form 3 waves ("funnels") (Fig. 5E).

Internal anatomy. The small specimen was partially dissected. The pharynx had a long, ventral ascus-muscle. Internally a pigmented line is seen along the edges of the pharyngeal cavity. Lightmicroscopic as well as SEM examination of the radular teeth showed a pointed tip and coarsely serrated blade (Fig. 2G). The penis was short and conical.

Observations on live specimens. When the animal moves it makes "jerking" movements, swiping the rhinophores backwards and then generating a "wave" through the parapodial lobes (Fig. 11). Copulation may be by hypodermic impregnation from the external side of the parapodia; the small specimen was observed while attempting copulation with the large one, which was not reciprocating.

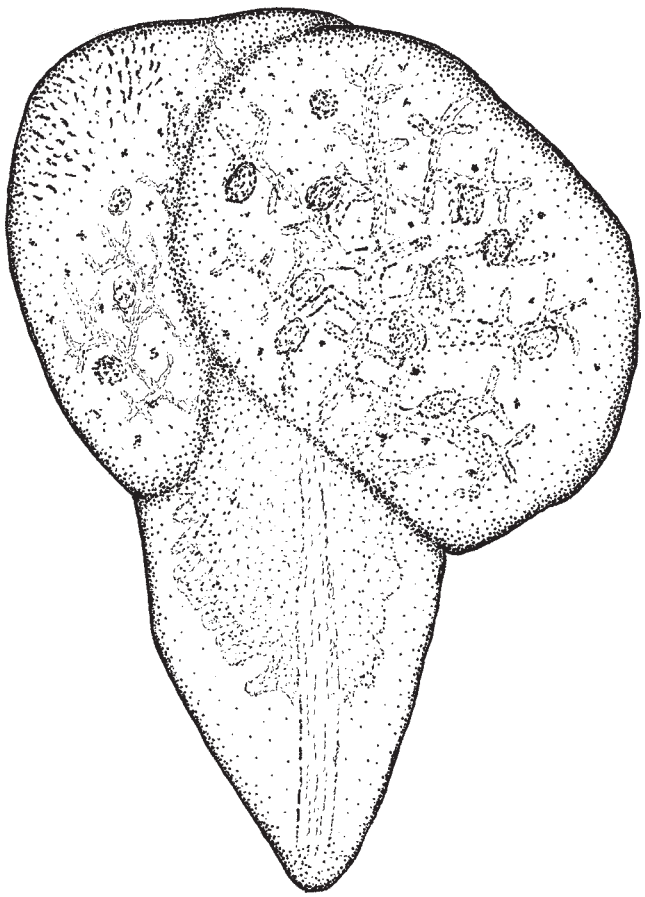

Fig. 10. *Stiliger smaragdinus*, ceras.

Remarks. **–** The present animals appeared externally identical to the original description of *Elysia verrucosa*, except that they had fewer black spots and lacked the submarginal brown line on the parapodia (Jensen, 1985). However, re-examination of the colour-slides of the type material showed that this line was not visible. Also, the present animals fed on *Chaetomorpha* in the laboratory, as did the Hong Kong specimens. Since copulation was observed, it was concluded that the two specimens were the same species, though copulation was not reciprocal. However, the shape of the radular teeth of the small specimen differed in important details from the blunt tipped, finely serrulate teeth of the Hong Kong animals. Hence the present animals have been referred to as *Elysia* cf. *verrucosa*. There is a slight possibility that radular preparations from the Hong Kong material have been switched with another species, but only re-examination of specimens from Hong Kong can clarify this.

Elysia ornata **(Swainson, 1840)**
(Fig. 5F)

Material examined. **–** One specimen collected at 2–5 m at North Point, St John's Island by B. W. Hoeksema on 29 Mar.2006.

Remarks. **–** The specimen grew from about 7 cm when collected to more than 12 cm in length during the workshop, and it was decided to let it live after the workshop to see how big it would eventually grow. Unfortunately the specimen died and disappeared before it could be preserved, so no anatomical details could be studied. Colouration agrees with that of *E. ornata* from elsewhere, with a black marginal and orange submarginal line along parapodial edges. This pattern is repeated on the tips of the rhinophores. Numerous black dots are scattered all over the body. The highly branched dorsal vessels are distinct, but the parapodia are kept folded on the back, so it was not possible to count the number

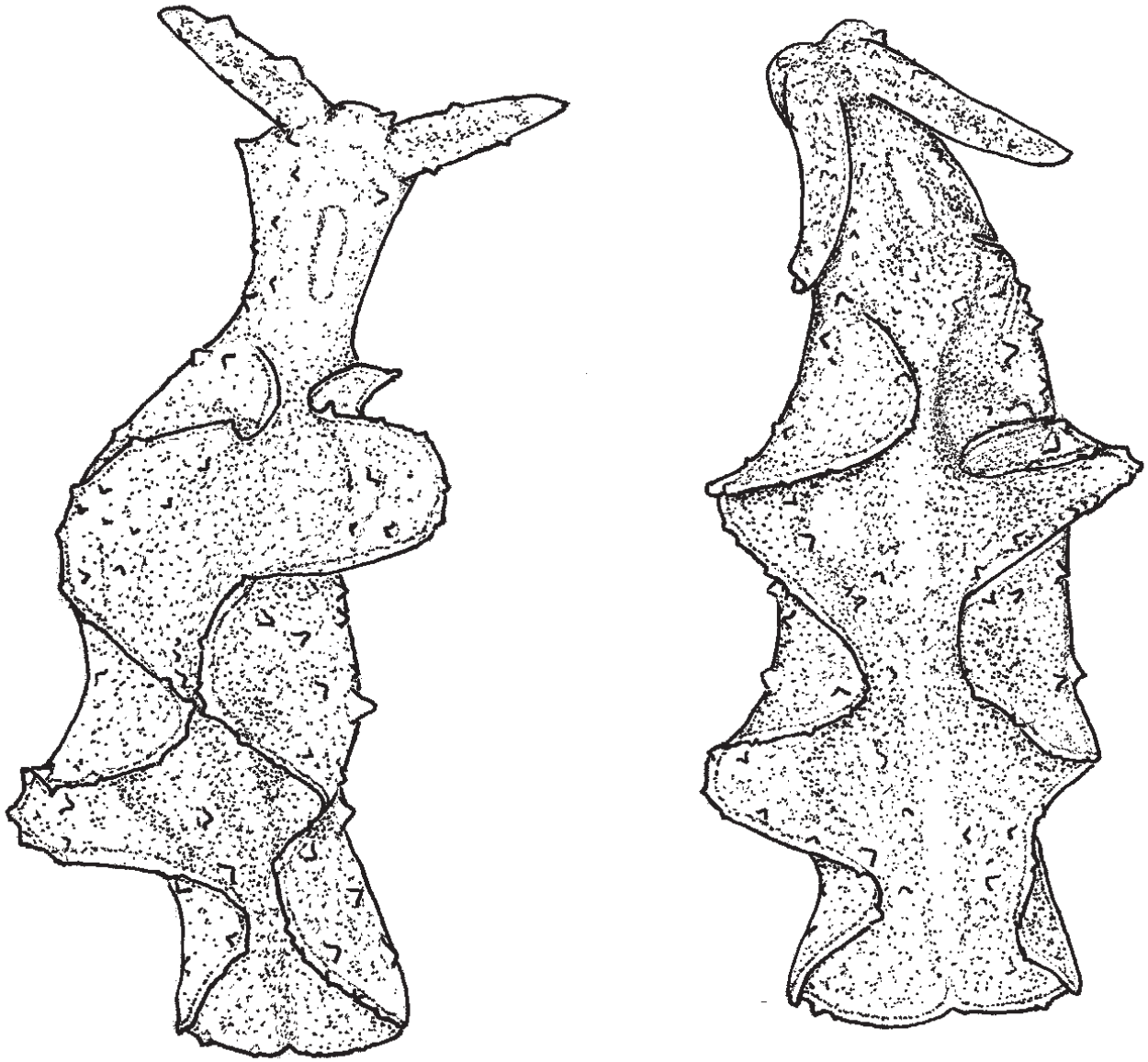

Fig. 11. *Elysia* cf. *verrucosa* drawn from photographs of large specimen by K.R. Jensen. The two pictures show the movement of rhinophores and parapodia during locomotion. Total length of animal approx. 30 mm.

of vessels. The animal was feeding on *Bryopsis* sp. in the large outdoor aquarium of TMSI, and it was the intention to preserve it after its natural death to study the radular teeth. *Elysia grandifolia* Kelaart, 1857, is very similar to *E. ornata*, but the radular teeth are finely serrated. This has been confirmed by the present author in specimens collected in southern India, which were externally identified as *E. ornata* (Jensen, 2001). It is possible that the fine serration depends on the species of *Bryopsis* eaten, or on the size of the animals, though the specimens from southern India were smaller than the present animal. However, it is also possible that there are two species, and unfortunately the opportunity to rule out the effect of size was lost when the present specimen disappeared before it could be preserved. In Guam both specimens with serrated teeth, identified as *E. grandifolia*, and specimens with smooth teeth, identified as *E. ornata* (as *E. marginata* (Pease, 1871)) have been found (Carlson & Hoff, 1978). The smallest specimen of *E. grandifolia* was the same size as the largest *E. ornata*, but still had serrated teeth, which supports the existence of two species. Hirose et al. (2003) described colour variation related to size and season in specimens identified as *E. ornata* from Japan. The specimens reached a maximum length of 15 cm, but unfortunately radular morphology was not examined. The confused state of *Elysia* species with black and orange marginal bands on the parapodia has been discussed previously (Carlson & Hoff, 1978; Heller & Thompson, 1983; Jensen, 1992). There are at least two species feeding on *Bryopsis*, namely *E. ornata* and *E. rufescens* (Pease, 1871), the latter having a brownish colouration with rather large white spots. There are also a couple of species with red parapodial margins that seem to feed on *Chlorodesmis*, namely *E. bennettae* Thompson, 1973 and possibly *E. yaeyamana* Baba, 1936. The latter two do not have black parapodial margins.

Elysiella pusilla Bergh, 1872
(Fig. 1H, I)

***Material examined.* –** 1 specimen from *Halimeda*, Pulau Tekukor, 11 Apr.2006; 2 specimens on *Halimeda*, Pulau Hantu, 26 Apr.1995.

***Description.* – Color alive.** White papillae are scattered on the parapodia, some of them along the margins (but not forming a regular papillose border). There is a dark green line along the parapodial margin, formed by densely branching digestive gland tubules. One specimen had a row of white spots along the parapodial margins. There was a white area in the neck, in front of the pericardium. A few brown dots were present on the rhinophores.

External features. The specimens were 5–7 mm long. The rhinophores were short and knobby. The eyes were small, comma-shaped and located behind the rhinophores. The parapodia do not extend to the tip of the tail. The tail is rounded behind the parapodia. The foot sole is smooth with the central part pulled up to create a vacuum to help the animal attach to the algal surface. There is a distinct groove across the foot sole at the anterior attachment of the parapodia.

***Remarks.* –** The anatomy has been described previously (Jensen & Wells, 1990; Jensen, 1992). The separation of the genus *Elysiella* has also been discussed previously (Jensen, 1992; Gosliner, 1995; Bass & Karl, 2006). Molecular studies have shown that this species nest within species of *Elysia* (Bass & Karl, 2006; Händeler & Wägele, 2007), but also indicate that the genus *Elysia* may need subdivision. The description of a second species, *Elysiella stylifera* from northern Australia (Jensen, 1997), supports this. Hence inclusion of *Elysiella pusilla* in *Elysia* seems to be premature. The distribution of *E. pusilla*, including its synonyms (see Jensen & Wells, 1990) is wide Indo-West Pacific with a possible extension to the East Pacific (Behrens & Hermosillo, 2005). The latter may be a human-mediated introduction (Jensen, 2007).

Thuridilla ratna (Marcus, 1965)
(Fig. 5G)

***Material examined.* –** 4 specimens, 3 Apr.2006, Terembu Pampang Tengah, patch reef SE of Pulau Hantu, collected by R. C. Willan.

Remarks. – Animals were kept in the laboratory for the duration of the workshop. Several species of algae were presented to the slugs, including *Chaetomorpha*, *Bryopsis*, *Avrainvillea*, *Caulerpa* and *Halimeda*, but none of the animals even attempted feeding on any of these, and the diet, therefore, remains unknown. This species has been synonymized with *Thuridilla bayeri* (Marcus, 1965) (Gosliner, 1995) as well as *T. gracilis* (Risbec, 1928) (Rudman, 2000). Recent molecular analysis (Händeler & Wägele, 2007) has shown that *T. gracilis* and *T. ratna* are almost identical whereas *T. bayeri* appeared as a separate species. Since only one gene was studied, it appears premature to dismiss Marcus' species. The original description of *T. gracilis* could fit either one, and it is not clear how the specimen identified as *T. gracilis* was identified (Händeler & Wägele, 2007). The present specimens agree with the *T. ratna* colour pattern with a broad orange band subterminally along the inner parapodial edges and oral and pedal lobes and no iridescent blue spots as seen in *T. bayeri*. Also, the longitudinal white stripes on the rhinophores do not extend into the terminal orange band. It probably has a wide Indo-West Pacific distribution (Jensen, 1992, 2007), but after Rudman's synonymization of all three species (Rudman, 2000), it has become difficult to tell which of the different color forms occur where.

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