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STYLETS, STYLES, AND OTHER CUTICULAR EXTENSIONS OF THE MALE DUCT IN THE ORDER SACOGLOSSA (GASTROPODA)

KEY WORDS: Sacoglossa, Opisthobranchia, copulation studies, hypodermic injection

Abstract

Sacoglossans form an interesting group of specialized sea slugs. They are vegetarians and feed by slitting open algal cells and sucking out the contents. The radula is the hall mark of the order. They are hermaphrodites. Fertilization is internal and usually reciprocal. There are two genital openings: a male opening and a female opening. When not in action, the penis is enclosed in a penial sheath that is adjacent and posterior to the male opening and along the inside from the penial sheath and is thrust outside the body. The female opening functions only as an outlet for the egg-string on during egg-laying. Apparently there is no vaginal opening in many sacoglossans. The transfer of sperm is effected by a cuticular extension of the male duct. Aided by considerable pressure of the body, a stylet, style or other extension pierces through the body wall of the other conjugant and so sperm is transferred. The process is called *hypodermic injection*. Next follows a section describing the details on the cuticular extensions which the author has dissected. Precise *hypodermic injection* is described. There follows the uses in classification in which the reproductive system is recommended as an important family character. One section especially emphases copulation studies and discusses in merit of other methods.

Riassunto

I Sacoglossi sono un ordine di opistobranchi ermafroditi, con fecondazione interna e generalmente reciproca. Essi presentano due aperture genitali: una maschile ed una femminile. Il pene, quando non è estroflesso, è contenuto in una guaina posta posteriormente all'apertura maschile. Attraverso l'apertura femminile avviene solo la deposizione del nastro ovigero e, apparentemente in molte specie manca una vera e propria apertura vaginale. Il passaggio degli spermi, infatti, avviene attraverso un'iniezione ipodermica del pene grazie anche alla presenza di stili, stiletti ed altri accessori, che vengono descritti in dettaglio. Viene inoltre discussa l'importanza del sistema riproduttore nella sistematica dei sacoglossi e le diverse tecniche necessarie per lo studio di questo ordine.

Introduction

This Introduction is intended to help the reader who may have little or no knowledge of sacoglossan sea slug. This was recommended at the 11° International Malacological Congress at Siena, Italy, in 1992.

The Sacoglossa is a small compact order of about 200 species. Most species are small and their lengths are measured in mm. Styles, styles and other extensions are measured in μm. They are vegetarians and feed on filamentous algae that grow, littorally, in rock pools. Three species have taken to feeding on the eggs of other opisthobranchs. Two species (*Limapontia depressa* and *Alderia modesta*) live on the damp mud of estuarine salt marshes. The order is essentially a tropical or subtropical group, but its range is extensive, from Norway to Australia. 10 species are found along the coast of Great Britain.

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Some brief remarks now follow for the species n° 1 to 10, depicted in Fig. 1.

1. Elysia viridis (Montagu, 1804)

This large species, 45 mm long, is characterized by a pair of parapodia with which it can balance and move about and even swim a little. Feeds on the seaweed *Codium*.

2. Limapontia capitata (O.F. Müller, 1773)

A very small sacoglossan of length 2 mm. Without rhinophores or cerata. Looks like a bit of dirt when placed in the hand. It will glisten with mucus and slowly crawl with its foot. Two black eyes in pale lemon patches will be seen. Feeds on filamentous seaweed of the genus *Cladophora*.

3. Limapontia cocksi (Alder & Hancock, 1848)

Similar in size and appearance to *L. capitata*, except that it has a pair of rhinophores which form a key character. It feeds on *Cladophora*. It lives in the same habitat as *L. capitata* but it is less common. It is more active than *L. capitata* that seldom strays from its tuft of *Cladophora*. Contrary other sacoglossans which have a veliger, *L. cooksi* shows a direct development.

4. Limapontia depressa Alder & Hancock, 1862

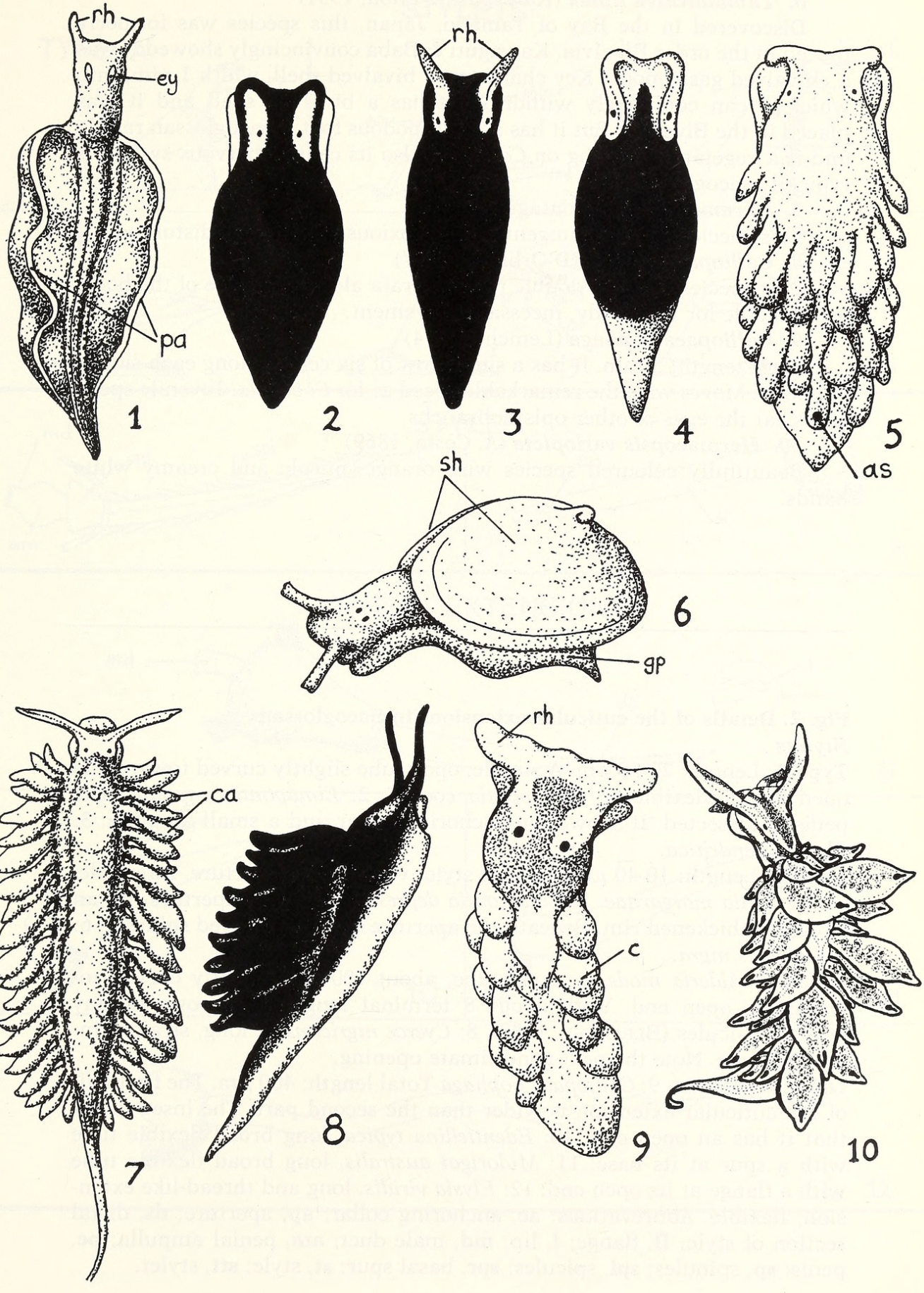
Limapontia depressa and *L. capitata* are alike in that they have no external appendages and are small, 2 mm long. *L. capitata* has a slightly raised crest-like head, whereas, the head of *L. depressa* is depressed. This last species lives on the damp mud of estuarine salt marshes whereas *L. capitata* lives in rocky shore pools. Dark forms of *L. depressa* have been mistaken for *L. capitata*, but their stylets are quite different (Figs. 2: 2-5). That of *L. depressa* is squat and has a wide aperture with a rim with 3 or 4 spinules underneath the aperture. The stylet of *L. capitata* is a simple open tube, slightly curved towards the tips. *L. depressa* feeds on *Vaucheria*.

5. Alderia modesta (Lovén, 1844)

Total length: 10 mm. A pair of short stubby ear-like parapodia. With a raised dorsal prominence, cerata are arranged round its posterior border. Key character, a terminal anal spout. Habitat: semi-terrestrial, lives on the dump mud of estuarine salt marshes as for *L. depressa*. Less common than *L. depressa*. It feeds on *Vaucheria*.

Fig. 1. Sacoglossan external features

1. *Elysia viridis*; 2. *Limapontia capitata*; 3. *Limapontia cocksi*; 4. *Limapontia depressa*; 5. *Alderia modesta*; 6. *Tamanovalva limax*; 7. *Hermaea bifida*; 8. *Calliopea bellula*; 9. *Calliopea oophaga*; 10. *Hermaeopsis variopicta*. Abbreviations: **rh**, rhinophores; **pa**, parapodia or sideflaps; **ey**, eye; **as**, anal spout; **sh**, bivalved shell (juvenile length: 1 mm): **gp**, gastropodous foot; **ca**, cerata (plural); **c**, ceras (singular).
(Based on a Figure by Gregory Brown in *Biology of Opisthobranchs*, vol 2 by T.E. Thompson and G. Brown).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6. Tamanovalva limax (Kawaguti & Baba, 1951)

Discovered in the Bay of Tamano, Japan, this species was formerly placed in the order Bivalvia. Kawaguti & Baba convincingly showed it was a «bivalved gastropod». Key character, a bivalved shell, width 1 mm, into which it can completely withdraw. It has a bivalved shell and it was placed in the Bivalvia. But it has a gastropodous foot, a sacoglossan radula and is a vegetarian feeding on *Caulerpa*. Also its central nervous system is typically sacoglossan.

7. Hermaea bifida (Montagu, 1815)

This species emits a pungent and obnoxious smell when disturbed.

8. Calliopaea bellula (D'Orbigny, 1837)

This species shows a double row of cerata along each side of the body. Remarkable for its speedy, incessant movement.

9. Calliopaea oophaga (Lemche, 1974)

Total length: 3 mm. It has a single row of six cerata along each side of the body. Moves with the remarkable speed as for *C. bellula*. Juvenile specimens eat the eggs of other opisthobranchs.

10. Hermaeopsis variopicta (A. Costa, 1869)

Beautifully coloured species with orange, purple and creamy white bands.

Fig. 2. Details of the cuticular extensions in Sacoglossans
Stylets

Type A. Length: 20-60 μm. A simple, open tube slightly curved towards its open end. Inflexible. 1: *Limapontia cocksii*. 2: *Limapontia capitata*. The penis is dissected. It shows the anchoring collar and a small ampulla. 3: *Placida dendritica*.

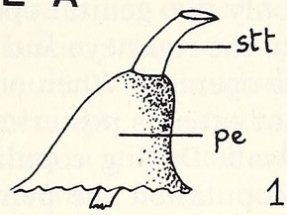
Type B. Length: 10-40 μm. A squat stylet with a wide aperture. Inflexible. 4: *Ercolania margaritae*. 5: *Limapontia depressa*. The wide aperture has an irregular thickened rim. Beneath the aperture are 3-4 recurved spinules. 6: *Alderiopsis nigra*.

Stiles. 7: *Alderia modesta*. Long tube, about 100 μm, slightly curved towards its open end. With about 8 terminal longitudinal rows of tiny pointed spicules (Bleakney, 1988). 8; *Cyerce nigricans*. A long, straight inflexible tube. Note the neat penultimate opening.

Other extensions. 9: *Calliopaea oophaga* Total length: 400 μm. The first half of the cuticular extension is wider than the second part. The inset shows that it has an open end. 10: *Edenttellina typica*. long broad flexible tube with a spur at its base. 11; *Midorigai australis*. long broad flexible tube with a flange at its open end. 12: *Elysia viridis*. long and thread-like extension; flexible. Abbrevations: **ac**, anchoring collar; **ap**, aperture; **ds**, distal section of style; **fl**, flange; **l**, lip; **md**, male duct; **am**, penial ampulla; **pe**, penis; **sp**, spinules; **spi**, spicules; **spr**, basal spur; **st**, style; **stt**, stylet.

STYLETS

TYPE A



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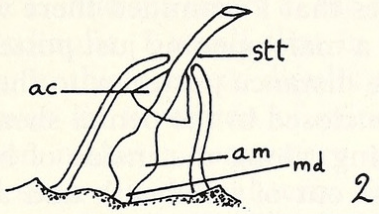

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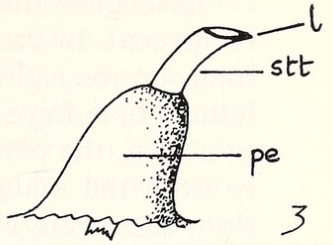

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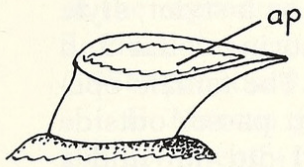
TYPE B



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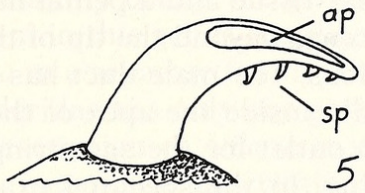

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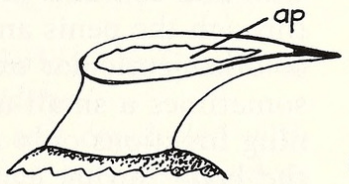

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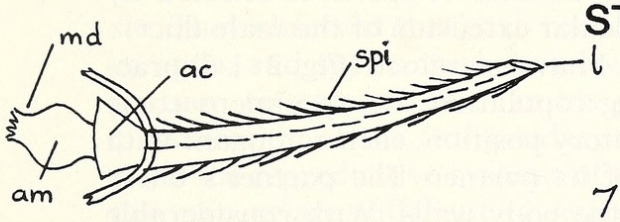
STYLES



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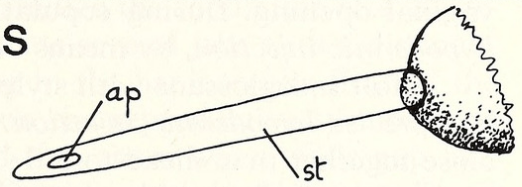

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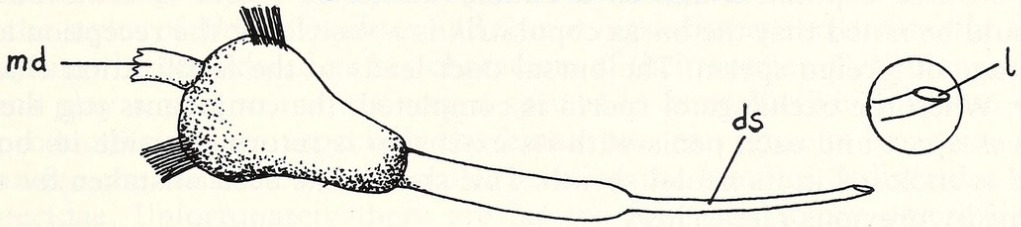
OTHER EXTENSIONS



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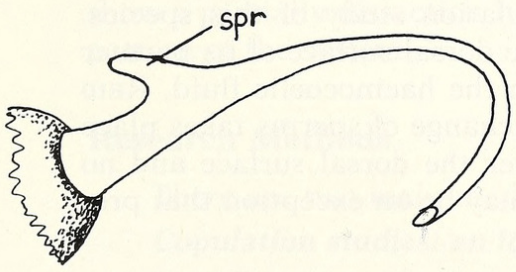

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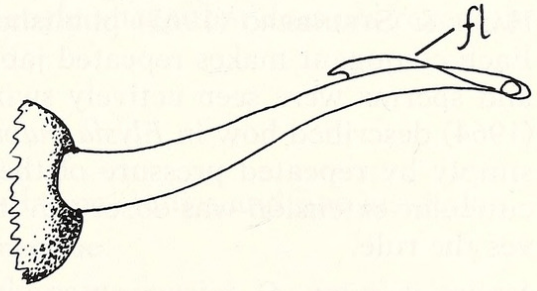

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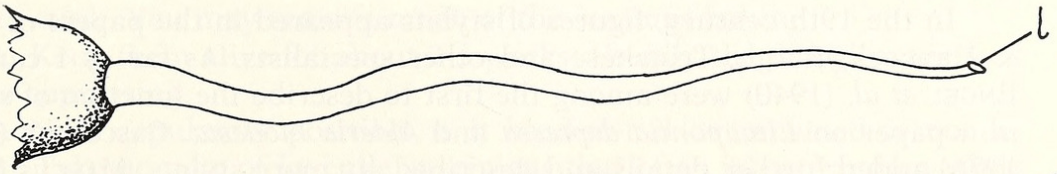

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Cuticular extensions of the male duct in Sacoglossans

Sacoglossans are hermaphrodites. Fertilization is internal and usually reciprocal. In the species that I examined there were only two genital openings on the right side: a male opening just posterior to the right eye and a female opening at some distance posterior to the male opening. When not in action, the penis is enclosed in the penial sheath that extends posteriorly from the male opening, along the inside of body wall. During copulation, the penis is thrust out of its sheath and after copulation the penis retracts inside its sheath. The penis is a conical outpushing of the body wall and contains muscular tissue and a penial nerve. The male duct runs through the penis and extends beyond the tip of the penis as a stylet, style or other cuticular extensions. The male duct has an anchoring collar and sometimes a small ampulla inside the apex of the penis. The female opening functions only as an outlet for the egg string when it passes outside the body during egg-laying. In the species I examined, I did not find a vaginal opening. During copulation, the transfer of sperm is effected by *hypodermic injection*, by means of a cuticular extension of the male duct.

Adult sacoglossans with stylets that I have examined (Fig. 2: 1-6) practice *precise hypodermic injection*. During copulation two specimens come close together in a «head-to-tail» copulatory position, each conjugant with its penis poised over the target area of its partner. The partner's bursa copulatrix is attached to the inside of the body walls. With considerable pressure from both conjugants, each stylet is thrust directly into its partner's bursa copulatrix and so a double tranfer of sperm is achieved. It should be noted that the bursa copulatrix is a vesicle for the reception and storage of foreign sperm. The bursal duct leads to the fertilization chamber. When the exchange of sperm is completed, the conjugants tug themselves apart and each penis with its extension is returned inside its body and is enclosed in its penial sheath. This sheath has been mistaken for the penis by previous researchers.

Alderia modesta exchanges sperm by *imprecise hypodermic injection*. Hand & Steinberg (1965) published a copulation study of this species. Each conjugant makes repeated jabs over the dorsal surface of its partner and sperms were seen actively swimming in the haemocoelic fluid. Reid (1964) described how in *Elysia maoria* the exchange of sperms takes place simply by repeated pressure of the body over the dorsal surface and no cuticular extension was observed. It so this may be an exception that proves the rule.

Uses in Classification

In the 19th century, figures of stylets appeared in the papers of Alder & Hancock, Bergh, Trinchese and other specialists. As far as I can tell, Engel *et al.* (1940) were among the first to describe the function of stylets in a paper on *Limapontia depressa* and *Alderia modesta*. Gascoigne (1956; 1976) added further details and described six more stylets. Marcus (1982) published a pamphlet on the systematics of the genera of the order Ascoglossa. It is a comprehensive pamphlet, including 71 figures and 156 references. A novel feature is a key to the genera. It will be a valuable reference work for ascoglossan specialists for many years to come. In the classifica-

tion, E. Marcus uses only stylets as generic characters; styles and other extensions are omitted. The stylets are not described. Among the 71 figures there is not one of a stylet. E. Marcus refers to the reproductive systems as diaulic, triaulic or pseudodiaulic. I recall that in my 1976 paper, I used diaulic and pseudodiaulic. In this context, diaulic means with two genital openings. Pseudodiaulic was an invention of mine. It refers to the time when a cuticular extension makes a temporary opening by hypodermic injection. I have not used such a technical term since 1976. I suggest two improvements to E. Marcus's classification of the Stiligeridae.

1. Remove the taxon subfamily before the genus *Limapontia*. The pattern of the reproductive system in this genus is stiligerid.
2. Place the genus *Alderia* in a separate family, the Alderidae, as GASCOIGNE (1976) proposed. The Alderidae has only one genus, *Alderia*.

E. Marcus gave a description of *Alderia modesta* and concluded with the comment «with this incomplete description it must be considered as *incertae sedis*». HAND & STEINBERG (1965) studied the copulation of *A. modesta*. They observed that each conjugant made several jabs with a distinctive style, and sperm were seen swimming in the haemocoelic fluid of the partner. GASCOIGNE (1976) in his paper on the reproductive systems and classification of the Stiligeridae, showed that there was a wide gap between the reproductive systems of the Stiligeridae and the Alderidae. These two papers are sufficient to remove the doubts expressed by E. Marcus. More could be added. If a sacoglossan possesses a stylet, it is and indication it belongs to the family Stiligeridae. A few cuticular extensions, such as the styles of *Alderia modesta* and *Cyerce nigricans*, are so distinctive that they may be considered as key characters of their species. Cuticular extensions play a part in maintaining a species in reproductive isolation. The reproductive system, especially the female one, can prove to be a wide gap between the families. For example, between the families Stiligeridae and Alderidae. Unfortunately there are far too few satisfactory descriptions, with figures, of the sacoglossan reproductive system. Most of the figures show a partially dissection lying in a tangled heap that does not reveal the pattern of the system.

Research Methods

There are two main methods: copulation studies and fine dissection.

Copulation studies: an ideal programme

1. Collect about six specimens of the same species. Do not over collect. Avoid collecting too much filamentous seaweed and leave it in the laboratory to stink. You may remove much of the seaslugs habitat.
2. Keep them alive for about 2-3 weeks. Usually the sacoglossans feed before copulating.
3. Note copulatory position: is it head-to-tail or entwined or nondescript? Take no note of juvenile specimens that often jab at random while the adult is in action.
4. Observe the injections: precise or imprecise?
5. Take a pair of copulants and slightly disturb them. This will reveal the exact point of injection.

6. Once a pair starts, the other adults will follow their example. This suggests that, during mating, an attractive chemical substance is secreted.

7. After copulation there is a period of apparent rest. The penis is withdrawn inside the body, into the penial sheath, until egg laying begins. The eggs are laid as an egg-string (flat spiral) or an egg-mass (compressed spiral). Within the envelope of an egg-string, the eggs are arranged on a single helix.

8. Estimate the number of eggs in the egg-string or egg-mass. There may be differences between families.

9. It may be that a species is not a good laboratory animal. If so, I suggest *Elysia viridis or Hermaea bifida*.

Fine dissection

The fine dissection is essential for displaying the pattern of the reproductive system. Do no represent the reproductive system by a confused figure of a partial dissected mass that does not show the pattern of the system. Serial sections can be essential for examining the follicles or acini of the hermaphrodite gland and cellular details. Not recommended for cuticular extensions or ducts which may be cut and displaced or lost in the elaborate process of making sections.

Whole mounts are suitable for cuticular extension of the male duct. Free the penis from the body and mount in Berlese's fluid is recommended. Also the smear technique is useful: from a freshly killed sacoglossan remove the vesicle, or part required and stain appropriately.

Finally electronic microscope methods (TEM and SEM) could be used if they make a cuticular extension clearer than before.

Acknowledgements

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