



Taxonomic and biological observations on the tiger flatworm, *Maritigrella crozieri* (Hyman, 1939), new combination (Platyhelminthes, Polycladida, Euryleptidae) from Florida waters

LESLIE J. NEWMAN^{†1}, JON L. NORENBURG[†] and SHERRY REED[‡]

[†]*Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560-0163, USA; e-mail: norenburg@oxyn.si.edu*

[‡]*Smithsonian Marine Station, Link Port, 5612 Old Dixie Highway, Fort Pierce, FL 34946, USA; e-mail: reed@sms.si.edu*

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The tiger flatworm, *Maritigrella crozieri* (Hyman, 1939) (Platyhelminthes, Polycladida, Cotylea) new combination, is redescribed from eastern Florida and the Florida Keys. This marine flatworm is one of the most common polyclads within warm temperate to tropical west Atlantic, yet it has been misidentified consistently as a pseudocerotid. Animals were kept alive in the laboratory for 3 weeks for biological observations. Findings indicated that these euryleptids employ hypodermic insemination, with multiple copulations occurring over several days. Sperm was transferred in sperm bundles bilaterally with little apparent damage to the epidermis of the copulating worms. Copulation sessions were variable and lasted an average of 15.4 min. *In situ* and laboratory observations indicated that *M. crozieri* fed exclusively on the mangrove ascidian *Ecteinascidia turbinata* Herdman, 1880, an individual consuming one prey zooid in an average of 17 min and an average of 19 zooids over 24 h.

KEYWORDS: Platyhelminthes, Polycladida, Euryleptidae, hypodermic insemination, feeding, Florida.

Introduction

Crozier (1917) first reported a striped polyclad flatworm from Bermuda, which Hyman (1939) later named in his honour as *Pseudoceros crozieri*. Hyman claimed this species lacks any cuticular elements, leading Faubel (1984) to place this species within his newly erected genus *Cryptoceros*, although he did not examine any specimens. The tiger flatworm is one of the most common and conspicuous polyclad flatworms from the Caribbean, Bermuda and the warm temperate to tropical west

¹Current address: Faculty of Science, University of the Sunshine Coast, Locked Bag No. 4, Maroochydone D.O., Qld 4558, Australia; e-mail: leslienewman@erols.com

Atlantic and is still incorrectly referred to as *Pseudoceros crozieri* (Ruppert and Fox, 1988; Humann, 1992; Pechenik, 1996).

Little is known about the biology of polyclad flatworms and only a few observations are available on the feeding biology of pseudocerotids (Newman and Cannon, 1994). *In situ* observations indicated that *Pseudoceros*, Lang, 1884 feed by extending a ruffled pharynx into the zooids of colonial and solitary ascidians and they exhibit no apparent prey specificity (Prudhoe, 1985; Newman and Cannon, 1994). However, these studies were limited to occasional *in situ* observations since these flatworms would not feed in captivity.

Even less is known about the reproductive biology of polyclad flatworms. Newman and Cannon (1994) first observed hypodermic insemination in pseudocerotids; this was later studied in detail by Michiels and Newman (1998). Nothing is known about reproduction in other cotyleans, including euryleptids.

The tiger flatworm is commonly found on its food, the mangrove ascidian *Ecteinascidia turbinata* Herdman, 1880, upon which it feeds by extruding its tubular pharynx into individual zooids (Ruppert and Barnes, 1994). Over 40 specimens were collected with which to redescribe *Maritigrella crozieri* (Hyman, 1939), new combination, and to make substantial observations on its biology. It is placed in the family Euryleptidae Lang, 1884 on the basis of its morphology: tubular pharynx, marginal tentacles and two elongate cerebral eyespots, as well as details of the male reproductive apparatus. Its gross morphology and lack of uterine vesicles place it within the genus *Maritigrella* Newman and Cannon, (2000). Serial sections of the holotype were also examined and compared to recently collected specimens from Florida. Copulatory and feeding behaviour were observed for animals retained in the laboratory for 3 weeks.

Material and methods

Specimens were hand-collected from submerged hanging lines at Little Jim's Marina, Fort Pierce, Florida (27°28.42' N, 80°18.4' W) and from prop roots of the red mangrove, *Rhizophora mangle* Linné, 1773, covered with the ascidian *E. turbinata* at about 3 m depth, Zane Grey Creek, Long Key, Florida Keys (24°50.20' N; 80°50.20' W). Live flatworms were photographed and collected in September and October 1997; and February, March and September 1998.

Some individuals were retained separately in small containers with unfiltered seawater, changed daily for 3 weeks. Ascidian prey were collected every week and kept separately in a flow-through sea-water system. An individual worm was measured, checked for maturity and randomly selected for observations of copulatory behaviour. Prior to feeding observations, the worms were starved for 48 h and the results are expressed as mean \pm standard deviation.

Specimens for taxonomic studies were fixed on frozen polyclad fixative (Newman and Cannon, 1995) and later preserved in 70% ethanol. Whole-mounts were stained with Mayer's haemalum, dehydrated in graded alcohols and then mounted in Canada balsam. Longitudinal serial sections of the reproductive region were obtained from specimens embedded in Paraplast (56°C), sectioned at 5–7 μ m, and stained with Mayer's haemalum and eosin Y solution.

Body measurements were taken from live animals in a quiescent state and are given as length \times width in mm. Measurements are used as a guide only, due to the 'plastic' nature of these animals. Drawings were made with the aid of a camera lucida (L.J.N.). All specimens were collected by L.J.N., S.R. and Andrew Flowers

unless otherwise stated. Voucher specimens are lodged at the National Museum of Natural History (USNM) as whole-mounts (WM), longitudinal serial sections (LS) and wet specimens (S). Colour transparencies are held by L.J.N. Type material is at the Peabody Museum of Natural History, Yale University (YPM).

Results

Systematics

Euryleptidae Lang, 1884

Maritigrella Newman and Cannon, 2000

Maritigrella crozieri (Hyman, 1939), new comb. (figures 1–10)

Pseudoceros unidentified sp. Crozier, 1917: 725–729, figures 1–7.

Pseudoceros crozieri Hyman, 1939: 17–18, figures 23–26; Marcus and Marcus, 1968: 73–75, figures 75–77; Prudhoe, 1985: 194.

Prostheceraeus zebra Hyman, 1955: 266–267, figures 7, 8.

Cryptoceros crozieri: Faubel, 1984: 207, 215.

Material examined

HOLOTYPE: YPM No. 201153, sagittal sections 1–7, Bermuda.

Other material

USNM181300, S, two specimens, 22 Sept. 1997, from old hanging ropes, Little Jim's Marina, Fort Pierce Inlet; USNM181301, S, same data; USNM181302, S, 25 Sept. 1997, in empty oyster shells on floating dock, Fort Pierce City Marina, M. Thiel; USNM181308, S, 2 Oct. 1997, on *Ecteinascidia turbinata* attached to mangrove roots, Zane Grey Creek, Long Key; USNM181303, LS, 3 Oct. 1997, same data; USNM181304, LS, same data; USNM181305, LS, same data; USNM181306, WM, same data; USNM181307, LS, same data; USNM181312, S, 21 Feb. 1998, Little Jim's Marina, Fort Pierce Inlet; USNM181309, two, 27 Feb. 1998, same data; USNM181310, S, 5 Mar. 1998, on *E. turbinata* attached to mangrove roots, Zane Grey Creek, Long Key; USNM181311, S, same data; USNM181313, S, 22 Sept. 1998, same data; USNM181314, S, two spec., same data.

Diagnosis

Dorsal colour pattern variable; background semi-transparent white-biege with fine transverse, wavy, black lines; lines often broken and branching laterally and may end in black blotch or orange irregular patch; opaque white spots either regularly or irregularly distributed over dorsal surface (figures 1, 5, 6). Margin with narrow irregular banding of opaque white and outer orange semi-transparent band at the rim, intensity of orange pigment variable. Marginal tentacles orange with anterior black band, white tips. Ventrally cream-white. Smaller individuals usually darker with few wide black lines.

Body oval, fragile, with deep marginal ruffles (figures 1, 5, 6). Marginal tentacles elongate and held erect. Cerebral eyes two elongate clusters, about 35 eyes each. Dorsal eyes between marginal tentacles, ventral eyes two elongate clusters; auricular groove extends along anterior rim. Pharynx anterior, small, oval and tubular (figure 2). Gonopores behind pharynx and anterior to mid-body line. Sucker mid-body and

prominent. Vas deferens branched. Male antrum narrow and deep. Seminal vesicle rounded oval (590 μm long); with thick muscular walls, ejaculatory duct long and winding (figure 3). Prostate rounded oval (360 μm long), with thick muscular walls and smooth lining, ejaculatory and prostatic ducts do not join in penis papilla but continue separately to stylet tip. Sclerotized stylet 130 μm long, 50 μm wide; ratio = 1:2.6. Female antrum deep with several folds at cement pouch. Uterine vesicles lacking.

Remarks

It is surprising that Hyman (1939, 1955) and Marcus and Marcus (1968) did not recognize that this animal belonged to the Euryleptidae since morphological characters, such as a small anterior tubular pharynx, elongate marginal tentacles and cerebral eyes in two elongate clusters, are diagnostic. Pseudocerotids, on the other hand, possess a large ruffled pharynx, pseudotentacles and one horseshoe-shaped cerebral eyespot (Newman and Cannon, 1994, 2000). Hyman (1939) did not mention or illustrate the pharynx in her specimens, but Marcus and Marcus (1968, figure 75) did draw a tubular pharynx. Hyman did note that the male system differed from *Pseudoceros* and stated that her specimens lacked 'any cuticular elements'. This led Faubel (1984) to place Hyman's species in a new genus, *Cryptoceros*, based solely on the absence of a penial stylet. However, our re-examination of the holotype clearly shows a sclerotized (cuticular) stylet measuring 130 μm long, 50 μm wide (figure 4), exactly as found in our Florida specimens (see Diagnosis). These characteristics define these animals as euryleptids. They are placed in the recently erected genus *Maritigrella* Newman and Cannon (2000), other members of which are generally cream with transverse black stripes and characteristically lack uterine vesicles (Newman and Cannon, 2000).

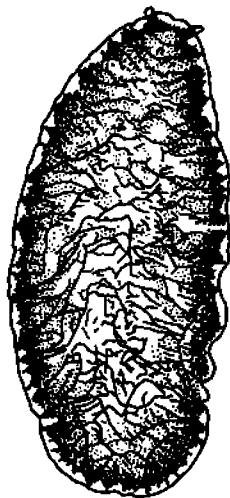
In 1955, Hyman also described a striped flatworm, *Prosthecleraeus zebra*, from sea grass habitats in Florida and Jamaica. She noted that *P. zebra* lacks uterine vesicles, although members of the genus characteristically have them. We believe that this species also is *M. crozieri*, due to its colour pattern and details of its reproductive anatomy.

Crozier (1917) noted that colour differences in this species were directly due to ingestion of pigments from their ascidian prey; animals fed on *E. turbinata* appeared orange compared to darker animals which fed on *Phallusia nigra* (Savigny, 1816). Among our specimens, only slight differences in colour pattern were found between animals from Long Key and those from Fort Pierce, probably because both populations were found feeding on *E. turbinata*. Animals from Long Key were cream with fewer stripes and irregular white opaque blotching, whereas Fort Pierce specimens were generally light orange with more numerous fine stripes and regular opaque white spots on the dorsal surface.

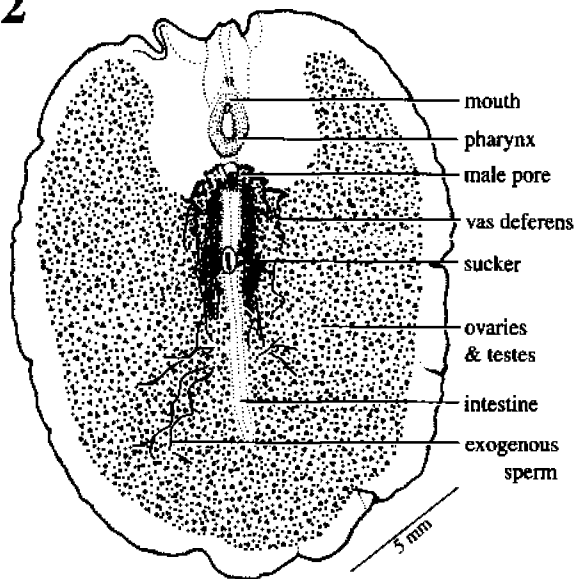
Distribution

Common from east-central Florida and the Florida Keys. Also reported from Bermuda (Crozier, 1917), Jamaica (Hyman, 1955) and South Carolina (Ruppert and Fox, 1988).

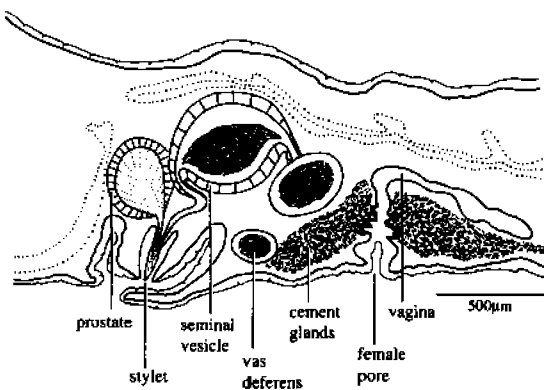
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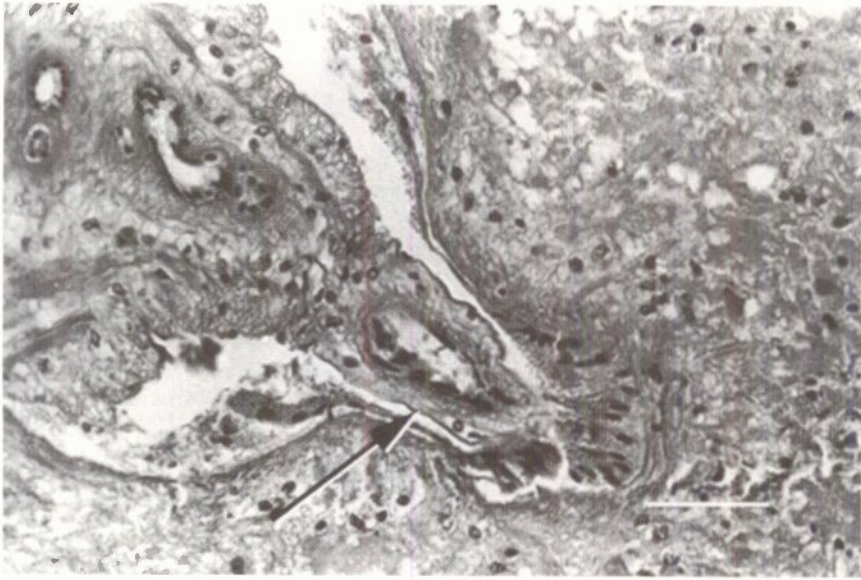


FIG. 4. Penial stylet of the holotype of *Maritigrella crozieri* nov. comb., from Bermuda. Bar = 50 μ m.

Biology

Feeding

Our animals were consistently found associated with their prey, *E. turbinata*, either crawling over the surface of the zooids or, at times, between zooids (figure 5). Most of the flatworms were collected in pairs, i.e. two to a colony, on *E. turbinata* colonies attached to mangrove roots. Animals were found on the benthic leaf litter only occasionally.

In the laboratory, flatworms did not feed until they had glided and left mucus trails over the entire surface of their container. Worms from Long Key were brought back to Fort Pierce and readily fed on locally collected *E. turbinata*. Once an animal found a colony of *E. turbinata*, it commenced feeding, whether day or night; it settled on the anterior end of an individual zooid, extended its sucker and then wrapped its lateral margins around both siphons as if to suffocate the prey (figure 7). After several minutes the worm slowly everted its pharynx into the zooid until it reached the entire length of the prey. Feeding rates per zooid ranged from 12 to 22 min (17 ± 4 , $n = 12$), often with 'rest' breaks.

After a few minutes of feeding, dark orange pigment from the ascidian could be seen in the flatworm's pharynx and intestine. After approximately 15 min of feeding, this pigment could be seen moving down the intestine, eventually disseminating throughout the intestinal branches and giving the animal a slight orange tinge. Animals regurgitated an orange pigment bolus several hours after ingestion. Twelve animals were kept in separate aquaria, each with a clump of 25 zooids, for 24 h. During this time they consumed from 14 to 24 zooids (19 ± 4).

In order to observe feeding selection, three different species of colonial ascidians were randomly placed at one end of a 5-litre aquarium. *M. crozieri* consistently selected *E. turbinata* within 10–15 min, even after not feeding for several days, and did not attempt to feed on other prey during a 12 h observation period.



Fig. 5



Fig. 6



Fig. 7



Fig. 8



Fig. 9



Fig. 10

FIGS 5–10. Living *Maritigrella crozieri* new comb.; (5) *in situ*, mangrove creek, Long Key, Florida; (6) colour pattern variation in specimens from Long Key; (7) feeding on *Ecteinascidia turbinata* Herdman, 1880 with its tubular pharynx fully extended; (8) bilateral hypodermic insemination in a copulating pair; (9) sperm bundle on the posterior dorsal surface, post-copulation; (10) egg-mass laid *in situ* on *E. turbinata*.

Reproductive behaviour

Observations on the copulatory behaviour of *M. crozieri* show that these flatworms employed hypodermic insemination, but behaviour differed from that described for *Pseudoceros bifurcus* (Michiels and Newman, 1998). Copulation was initiated when one animal approached another from behind. The approaching animal stopped, reared up, fully everted its penis papilla, fell forward, stabbed its partner posteriorly and commenced inseminating (figure 8). At this time, the partner usually

turned and attempted to stab the first worm, also in its posterior end, thus frequently resulting in bilateral (simultaneous) insemination. The animals stayed in a head-to-tail position while repeatedly stabbing and injecting each other in the same area. Ten animals were observed copulating and each event was highly variable, lasting from 2.9 to 46.8 min (15.7 ± 12.9). Sometimes they paused during copulation events. In three instances one partner appeared disinterested and did not reciprocate copulation. An animal would not necessarily stop feeding while it was being inseminated. *In situ*, animals were observed copulating during the day, in direct sunlight, either on *E. turbinata* colonies or on turtle weed. A copulation event ended when one worm retracted its penis papilla and moved away.

Sperm was transferred in white bundles that adhered to the posterior epidermis of the partner (figure 9). On the epidermis, sperm bundles appeared in strings of several packets of sperm; these were absorbed after approximately 12 h. In the laboratory, animals would alternately feed, copulate and rest.

Egg-laying was observed 48 h after the first copulation. Up to five cream-white egg-masses, per pair of animals, one layer thick were laid on the side of the containers. In the field, egg-masses were found on *E. turbinata* zooids (figure 10). Larvae hatched into Müller's larvae and survived for up to 2 weeks in the laboratory with constant sea-water changes, but they did not settle or metamorphose.

Discussion

Surprisingly little is known about the taxonomy and biology of polyclad flatworms, despite their importance as predators in tropical marine ecosystems. However, it is understandable that the taxonomy of pseudocerotids and euryleptids has been confusing, since they resemble each other superficially in size, colour pattern, movement and body ruffling. Upon closer examination of the living tiger flatworm, it is clearly a typical euryleptid, with a tubular pharynx, marginal tentacles and two cerebral eyespots. This places it with five other species in the recently erected genus *Maritigrella*, previously known only from tropical Australian coral reefs (Newman and Cannon, 2000). *Maritigrella crozieri* is strikingly similar to other members of the genus in its colour pattern (cream with transverse black stripes) and in the details of its copulatory anatomy. Newman and Cannon believed that this colour pattern is disruptive, enabling the Australian species to blend in with the colour patterns of their colonial ascidian prey. Crozier (1917) noted changes in the colour of tiger flatworms after consuming different ascidian prey. However, to human eyes *M. crozieri* appears to be conspicuously patterned. Possibly this is aposematic patterning, since they are easily seen underwater in mangrove creeks and are active during the day. Furthermore, they may contain the same cytotoxic substances known to occur within *E. turbinata*; especially the ecteinascidins, which are in pre-clinical trials as anticancer agents (Rinehardt *et al.*, 1990; Carté, 1996).

There are few reports of feeding and prey selection in cotyleans. Newman and Cannon (1994) found that *Pseudoceros bifurcus* Prudhoe, 1989 fed on a variety of ascidians and showed little prey specificity. Crozier (1917) found the tiger flatworm of Bermuda on three species of ascidian and some within the test of at least one species. He believed that once an individual fed on a particular ascidian it would always return to that species. If true, that might explain why the tiger flatworms in this study from the Florida Keys and Fort Pierce, which were only found on *E. turbinata*, did not choose other prey offered in the laboratory.

Prudhoe (1985) suggested that pseudocerotids produce proteolytic secretions to

macerate and soften zooid tissue prior to ingestion. Newman and Cannon (1994) disputed this and suggested that only *Pseudoceros* feed by this method, whereas equally diverse species of *Pseudobiceros* Faubel, 1984, engulf their prey whole. Our observations indicate that members of the euryleptid genus, *Maritigrella*, extend the entire pharynx into the zooid and, possibly facilitated by secretion of proteases into the zooid, suck the zooid test empty by peristaltic muscular action of the body. This takes about 17 min, and an individual can consume up to 19 zooids within 24 h. Because the animals were found on this prey they appear to have ready access to food. It is not known how long they feed on one colony or how readily they move to other colonies. It is also unknown what happens to the flatworms in the winter months, when colonies of *E. turbinata* die off.

Michiels and Newman (1998) described the complex behaviour of penis fencing and hypodermic insemination in *P. bifurcus* from the Great Barrier Reef, Australia as a contest to strike and inseminate first. This behaviour results in unilateral transfer of sperm, where the asymmetrical outcome favours worms that can inject first. This 'aggressive' behaviour was not observed for *M. crozieri*; copulation was more like a duet, with both worms inseminating each other simultaneously. This also resulted in less damage to the epidermis. At times, only one worm would be inseminating while the other was (1) busy feeding, (2) just 'not interested' or (3) already copulated and was ready for egg-laying.

To date, there are no published reports on the rearing of cotyleans from the Müller's larval stage. Although we have kept the larvae alive for 2 weeks in the laboratory, they did not settle. Scarpa *et al.* (1996) determined that Müller's larvae of *M. crozieri* feed on plankton. This provides evidence that they could live for extended periods of time in the plankton but the length of their larval life or cues for settlement have not been determined.

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References

- CARTÉ, B. K., 1996, Biomedical potential of marine natural products, *Bioscience*, **46**, 271–286.
- CROZIER, W. J., 1917, On the pigmentation of a polyclad, *Proceedings of the American Academy of Arts and Sciences*, **52**, 723–730.
- FAUBEL, A., 1984, The Polycladida, Turbellaria. Proposal and establishment of a new system. Part II. The Cotylea, *Mitteilungen aus dem hamburgischen zoologischen Museum und Institut*, **8**, 189–259.
- HUMANN, P., 1992, *Reef Creature Identification; Florida, Caribbean and Bahamas* (Jacksonville, Florida: New World Publications), p. 320.

- HYMAN, L. H., 1939, Acoel and polyclad turbellarians from Bermuda and the Sargassum, *Bulletin of the Bingham Oceanographic Collection*, 7, Art.I, 1-26.
- HYMAN, L. H., 1955, A further study of the polyclad flatworms of the West Indian region, *Bulletin of Marine Science of the Gulf and Caribbean*, 5, 259-268.
- MARCUS, EV. and MARCUS, ER., 1968, Polycladida from Curaçao and other faunistically related regions, *Studies of the Fauna of Curaçao and other Caribbean Islands*, 26, 1-106.
- MICHELIS, N. and NEWMAN, L. J., 1998, Sex and violence in a hermaphrodite, *Nature*, 391, 647.
- NEWMAN, L. J. and CANNON, L. R. G., 1994, *Pseudoceros* and *Pseudobiceros* (Polycladida, Pseudocerotidae) from Eastern Australia and Papua New Guinea, *Memoirs of the Queensland Museum*, 37, 205-266.
- NEWMAN, L. J. and CANNON, L. R. G., 1995, The importance of the fixation of colour, pattern and form in tropical Pseudocerotidae (Platyhelminthes, Polycladida), *Hydrobiologia*, 305, 141-143.
- NEWMAN, L. J. and CANNON, L. R. G., 2000, A new genus of euryleptid flatworm (Platyhelminthes, Polycladida) from the Indo-Pacific, *Journal of Natural History*, 34, 2.
- PECHENIK, J. A., 1996, The Platyhelminthes, in *Biology of the Invertebrates* (Boston: Wm. C. Brown Publishers), p. 130, figure 7.7.
- PRUDHOE, S., 1985, *A Monograph on Polyclad Turbellaria* (Oxford: Oxford University Press), 259 pp.
- RINEHART, K. L., HOLT, T. G., FREGEAU, N. L., STROH, G. J., KEIFER, P. A., SUN, F., LI, I. H. and MARTIN, D. G., 1990, Ecteinascidin-729, ecteinascidin-743, ecteinascidin-745, ecteinascidin-759a, ecteinascidin-759b, and ecteinascidin-770—potent antitumor agents from the Caribbean tunicate *Ecteinascidia turbinata*, *Journal of Organic Chemistry*, 55, 4512-4515.
- RUPPERT, E. E. and BARNES, R. D., 1994, Chapter 6. Platyhelminthes, Gnathostomulids and Mesozoans. *Invertebrate Zoology*, 6th edn (Fort Worth: Saunders College Publishing), p. 224, figures 6-18.
- RUPPERT, E. E. and FOX, R. S., 1988, Flatworms, in *Seashore Animals of the Southeast* (University of South Carolina Press), pp. 180-182, Pl. B33.
- SCARPA, J., WEIS, B., RUPPERT, E., FRICK, J., FORD, A. and WRIGHT, A., 1996, Direct evidence for planktotrophy in Müller's larvae of the tiger flatworm, *Pseudoceros crozieri*, *American Zoologist*, 36, 107A (Abstract).