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Trimusculotrema schwartzi n. sp. (Monogenea: Capsalidae) from the skin of the stingray *Dasyatis zugei* (Elasmobranchii: Dasyatidae) off Hong Kong, China

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Abstract

Trimusculotrema schwartzi n. sp. (Capsalidae) is described from the skin of the stingray *Dasyatis zugei* (Elasmobranchii: Rajiformes: Dasyatidae) off Hong Kong, China. Only three other species have been placed in the genus *Trimusculotrema: T. micracantha* (Euzet & Maillard, 1967), *T. leucanthemum* (Euzet & Maillard, 1967), and *T. uarnaki* Whittington & Barton, 1990. *T. schwartzi* n. sp. may be differentiated from all known species of *Trimusculotrema* by the length of the anterior hamuli and by the absence of pigment shields over the eye-spots. Its occurrence on a stingray off China represents a northern extension of the geographical range of *Trimusculotrema*.

Introduction

The Capsalidae Baird, 1853 (Monogenea, Monopisthocotylea) constitutes a large taxon of seven subfamilies, including the Encotyllabinae Monticelli, 1892, Capsalinae Baird, 1853, Benedeniinae Johnston, 1931, Nitzschiinae Johnston, 1931, Trochopodinae Price, 1936, Entobdellinae Bychowsky, 1957 and Interniloculinae Suriano & Beverley-Burton, 1979 (Egorova, 1999; Pérez-Ponce de León & Mendoza-Garfias, 2000). According to Kritsky & Fennessy (1999) this family includes more than 40 genera of about 200 species. Capsalids parasitise a wide host spectrum of marine fishes, including elasmobranchs of the orders Squaliformes, Rajiformes and Lamniformes as well as actinopterygians of the orders Acipenseriformes, Anguilliformes, Perciformes, Tetraodontiformes, Zeiformes, Scorpaeniformes and Pleuronectiformes.

A new capsalid species of *Trimusculotrema* Whittington & Barton, 1990 taken from the skin of a stingray, *Dasyatis zugei* (Müller & Henle), off China is proposed. *D. zugei* has a wide geographical distribution, extending from southern Japan to the China Seas and the Indian Ocean (Masuda et al., 1984). Stingrays are observed most commonly lying on the bottom of flats of bays, shoal lagoons and river mouths or on patches of sand between coral heads and often are partly buried in mud or sand with only a portion of the tail, eyes and spiracle exposed (Bigelow & Schroeder, 1953). The new species from China represents a northern extension of the geographical range of *Trimusculotrema* and constitutes the fourth described species.

Materials and methods

In February 1999, while examining two stingrays (*Dasyatis zugei*) taken in February and August 1958 off Hong Kong, China and deposited in the California Academy of Sciences Ichthyology Collection, Dr. Frank J. Schwartz noted the presence of external parasites and submitted samples to us. Presumably the stingrays were preserved initially in formalin and later transferred to 55% isopropyl alcohol. Six monogeneans were removed and transferred to 70% ethanol. Later we removed an additional sixteen specimens and four haptors from the same two stingrays. These were also placed in 70% ethanol subsequent to removal. Morphology was studied in 22 whole specimens and three haptors as temporary mounts in

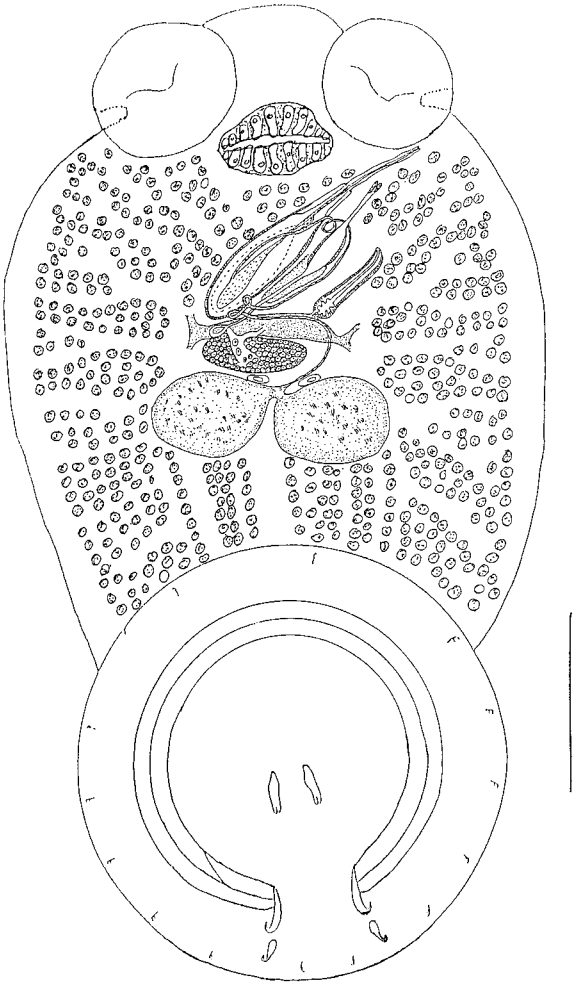


Figure 1. Trimusculotrema schwartzi n. sp. Entire worm (composite, ventral). *Scale-bar*: 600 μm.

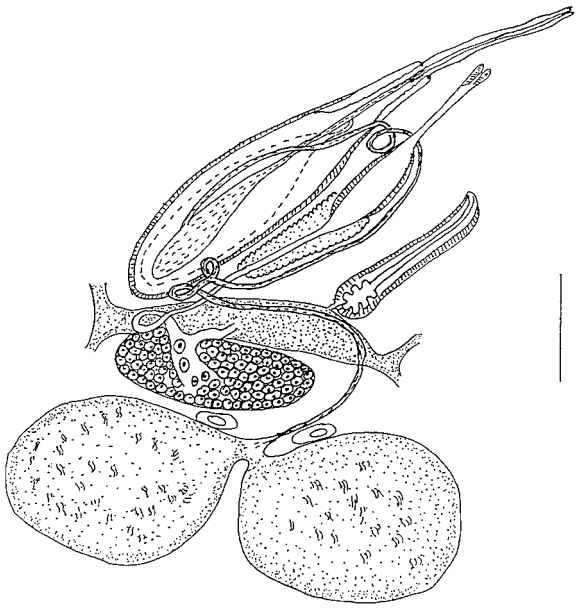


Figure 2. Trimusculotrema schwartzi n. sp. Reproductive system (composite, ventral). *Scale-bar*: 200 μm.

Hoyer's medium. All measurements in micrometres were taken with an ocular micrometer on a compound microscope and are expressed as the mean followed by the range in parentheses. Many specimens were contracted; thus total length and width measurements may not be representative, but other measurements were not affected. Some fixed specimens were stained with Harris' haematoxylin, dehydrated in a graded ethanol series, cleared in beechwood creosote and mounted in neutral Canada balsam. Prepared specimens were examined with a compound microscope, and illustrations were made with the aid of a camera lucida and a microprojector. One fixed specimen was embedded in paraffin wax, sectioned at 4-5 μm and stained with haematoxylin and eosin. One specimen was prepared for scanning electron microscopy by taking the specimen through a graded ethanol series (70-100%) followed by critical point drying and sputter coating with gold-palladium. Two haptors were treated with a modification of the method of Shinn et al. (1993) to obtain haptoral hooks. Haptors were placed in small plastic tubes containing 0.25 mg/ml trypsin in 0.2M Tris HCl buffer (pH 8.0), digested for 65 h at 38 °C, and centrifuged for 5 min at 5,000 rpm. The pellet was resuspended, aliquots were pipetted onto slides and slides were examined with a light microscope to locate hooks. Hooks were cleaned by sonication, air dried on glass slides, and sputter coated with gold-palladium. All specimens were examined with a Hitachi S570 SEM at 20 kV.

Type-specimens were deposited in the California Academy of Sciences, Invertebrate Zoology collection (CASIZ), San Francisco, California and the United States National Parasite Collection (USNPC), Beltsville, Maryland as indicated in the description of the new species.

For comparative purposes the following specimens were examined: 2 paratypes of *Trimusculotrema uarnaki* Whittington & Barton, 1990 from the Queensland Museum, South Brisbane, Australia (QM no. GL 10382 and GL 10383), (the holotype was not available for loan); 3 paratypes of *T. uarnaki* from the US National Parasite Collection, Beltsville, Maryland (USNPC no. 080822.00); 3 paratypes and 1

voucher of *T. micracantha* (Euzet & Maillard, 1967) Whittington & Barton, 1990 and holotype of *T. leucanthemum* (Euzet & Maillard, 1967) Whittington & Barton, 1990 housed in the personal collection of Professor Louis Euzet; and 1 voucher of *Benedeniella posterocolpa* (Hargis, 1955) Yamaguti, 1963 (USNPC no. 038146.02). Host specimens were borrowed from the California Academy of Sciences Ichthyology Collection (CAS 42146 and 42147).

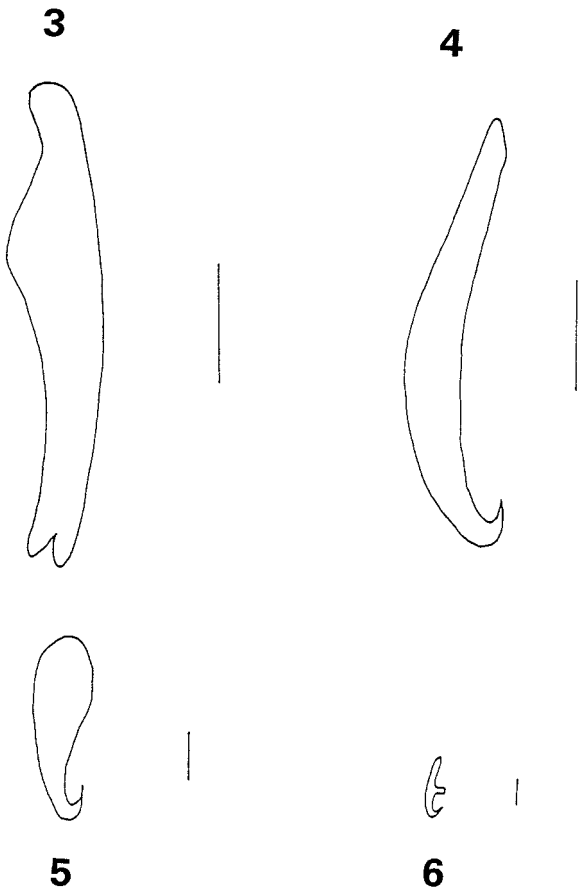
Capsalidae Baird, 1853
Benedeniinae Johnston, 1931
***Trimusculotrema* Whittington & Barton, 1990**

***Trimusculotrema schwartzi*, n. sp.**

Type-material: CASIZ 142479 (holotype); CASIZ 142480 (8 paratypes, type-locality); CASIZ 142481 (4 paratypes, other locality); USNPC no. 090871.00 (3 paratypes, other locality); USNPC no. 090872.00 (4 paratypes, type-locality); pers. coll. of W.J. Poly (2 paratypes, both localities); voucher: CASIZ 142482 (other locality).
Type-locality: At mouth of the Pearl River (Zhujiang estuary), off Hong Kong, Peoples Republic of China (22°22′00"N, 113°54′30"E), 14 August, 1958 (host: *Dasyatis zugei* (CAS 42147); *other locality*: SE of Sha Chau (island) (Zhujiang estuary), off Hong Kong, Peoples Republic of China (22°20′40"N, 113°54′45"E), 13 February, 1958 (host: *D. zugei* (CAS 42146)).
Host: *Dasyatis zugei* (Müller & Henle).
Etymology: The specific name 'schwartzi' is in reference to Dr Frank J. Schwartz, who first noticed and collected specimens on which the description was based and who has contributed much to our knowledge of the biology and taxonomy of elasmobranchs.
Collectors: Rolf Ling Bolin and F.D. Ommanney collected the hosts; Frank J. Schwartz, William J. Poly, and William G. Dyer collected the parasites.

Description (Figures 1-13)

(Based on 18 adults, 4 juveniles and 3 haptors (only) from 2 rays). Body elliptical; length including haptor 2,323 (900-3,425); greatest width 1,710 (470-2,600). Haptor circular in outline 1,161 (380-1,900) in diameter, pedunculate, with 3 concentric haptoral muscle bands (Figures 1,9), aseptate, with radially arranged papillae on ventral surface (Figures 1,8,10). Fibres of muscle bands sometimes connected between adjacent muscle rings; bands not traversing area between



Figures 3-6. Trimusculotrema schwartzi n. sp. 3. Accessory sclerite. 4. Anterior hamulus. 5. Posterior hamulus. 6. Marginal hooklet. *Scale-bars*: 3, 30 μm; 4, 50 μm; 5, 30 μm; 6, 15 μm.

hamuli. Accessory sclerites 95 (48-140) long; proximal end with bifid notch; distal end rounded and directed anteriorly (Figure 3); anterior hamuli 114 (60-205) long, broader in posterior half, tapering to more slender anterior end; posterior terminus recurved forming hook with anteriorly directed point (Figures 4,12,13); posterior hamuli 65 (48-90) long, with rounded proximal end and recurved distal end forming sharp hook (Figure 5); 14 marginal hooklets 16 (10-19); 1 pair between posterior hamuli; remaining hooklets arranged symmetrically along edge of haptor (Figures 1,6). Anterior attachment organs disc-shaped; each 455 (153-640) in diameter. Pigment shields over eye-spots absent. Pharynx 312 (122-460) long, 450 (182-660) wide. Intestinal caeca dendritic medially and laterally; posterior confluency not determined (Figure 7).

Male genital pore ventral, sinistral, submarginal, immediately posterior to left anterior attachment or-

gan (Figure 1). Two testes, ovoid, juxtaposed near body mid-length, intercaecal, penetrated by bands of muscle fibres that extend through organ as described for *Neobenedenia melleni* by Jahn & Kuhn (1932); vas efferentia short, uniting to form vas deferens; vas deferens extending anteriorly along left side of ovary and vitelline reservoir, with dextral loop anterior to vitelline reservoir, forming 3 coils before looping dorsal to distal female duct, then entering dorsal aspect of cirrus-sac where it widens to form pouch-like seminal vesicle; seminal vesicle narrowing to form ejaculatory duct; cirrus-sac diagonal, housing elongate spermatophore matrix reservoir of Whittington & Barton (1990) containing granular substance; distal end of spermatophore matrix reservoir united with ejaculatory duct to form cirrus; cirrus may evert through male genital pore (Figure 2). Glands of Goto not observed; pair of conspicuous elliptical cells present between testes and ovary.

Ovary pretesticular, medial, with mid-ventral zone (= fertilisation zone of Tappenden et al., 1993) containing developing oöcytes; oviduct extending anteriorly ventral to right vitelline duct. Mehlis' gland empties via several ducts into proximal portion of oötype; distal chamber of oötype muscular, leading to uterus. Vagina muscular, opening between mid-line and left body margin below oötype. Seminal receptacle a multichambered (7-8 chambers) muscular organ, at proximal terminus of vagina. Vitellarium occupies almost entire available space of body proper; vitelline reservoir ventral, median may overlap anterior aspect of ovary (Figure 2). Single egg present in oötype of some specimens, 190 (154-215); egg filament proximal, elongate, convoluted at terminus. Pair of raised structures ventrally at anterior end of worm, possibly having sensory function (Figure 11). Stout papillae scattered dorsally at extreme anterior end of worm.

Differential diagnosis

Trimusculotrema schwartzi n. sp. may be differentiated from all previously described species of *Trimusculotrema* in having anterior hamuli that are longer than the accessory sclerites in most cases (Figure 14) and also differs from other congeners by the absence of pigment shields over the eye-spots (Table 1). *T. schwartzi* differs further from *T. leucanthemum* by the shape and length of the accessory sclerites, which are 95 (48-140) in the former compared to 37 (32-42) in the latter and by the shape and length of the anterior hamuli, which are 114 (60-205) in the former compared with 30 in the latter. Furthermore, the haptor of *T. schwartzi* has three concentric muscle bands and no ribs at the periphery of the haptor, whereas that of *T. leucanthemum* possesses only two concentric muscle bands and has radial ribs along the margin of the haptor. *T. schwartzi* may be further differentiated from *T. uarnaki* by the shape and length of the anterior hamuli, which are 114 (60-205) compared to 46 (44-52). The haptoral papillae of *T. leucanthemum* differ from those of the other three congeners in having a scalloped margin (illustrated in Euzet & Maillard, 1967). *T. schwartzi* also differs from *T. micracantha* as to the level of the vaginal opening, which is at the level of the oötype in *T. schwartzi* compared to the posterior level of the left anterior attachment organ in

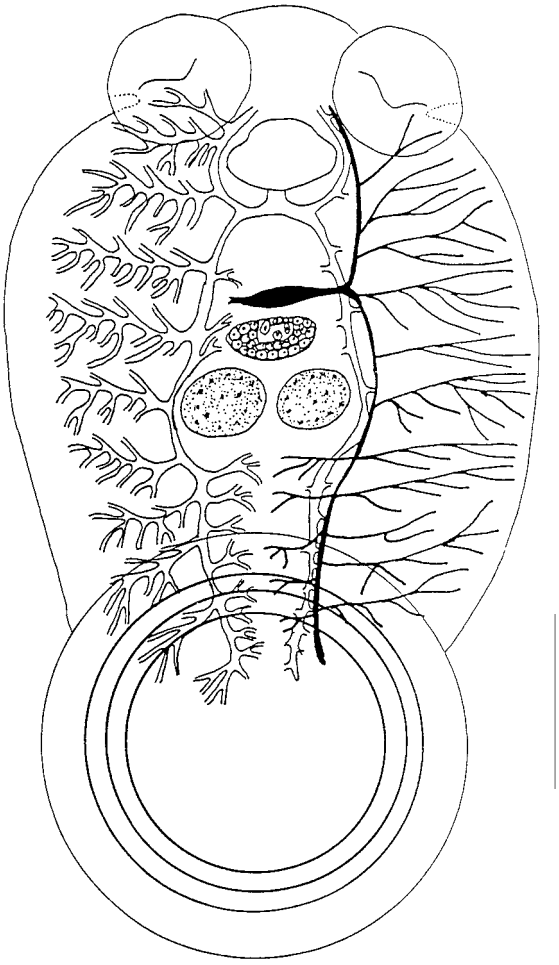
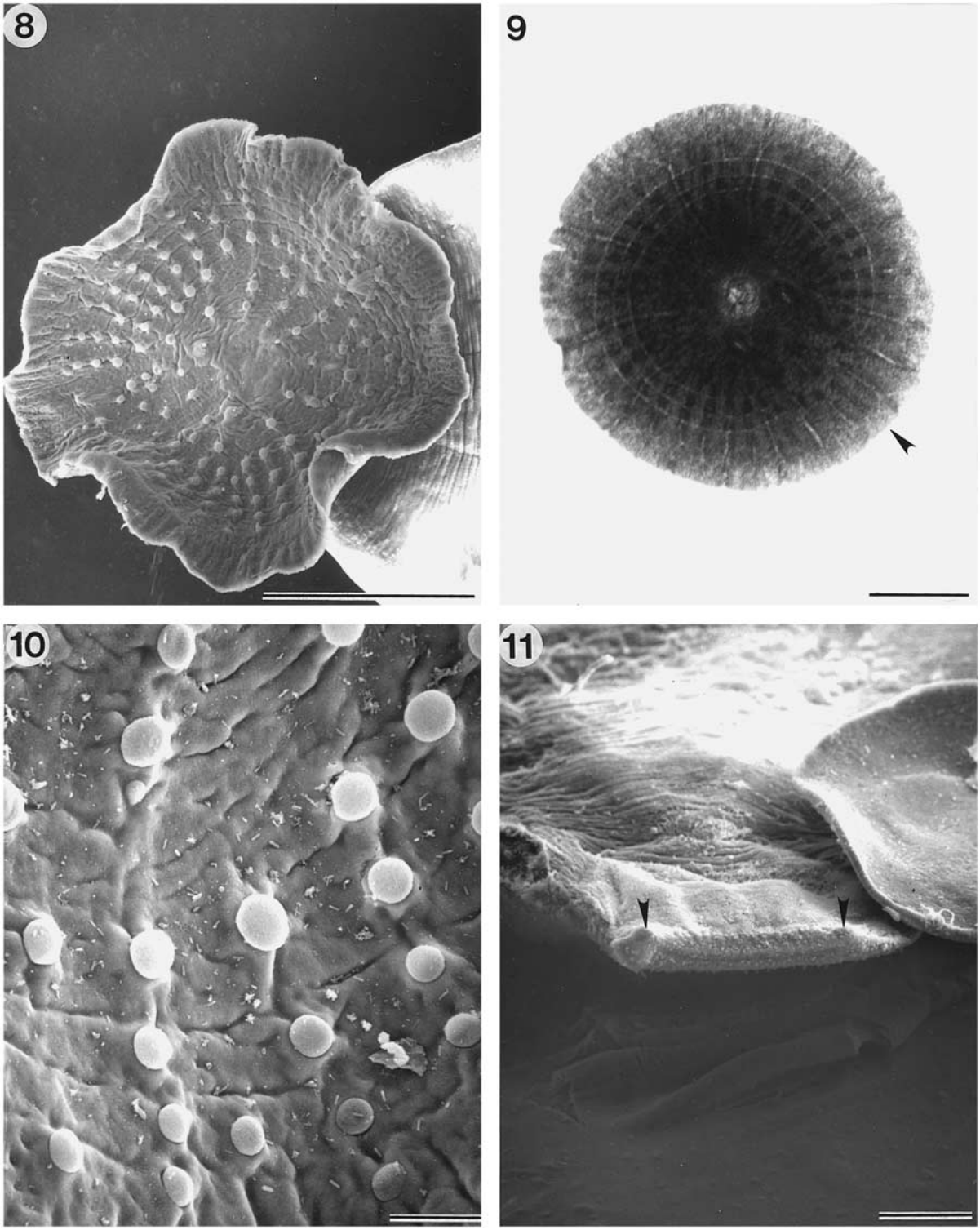
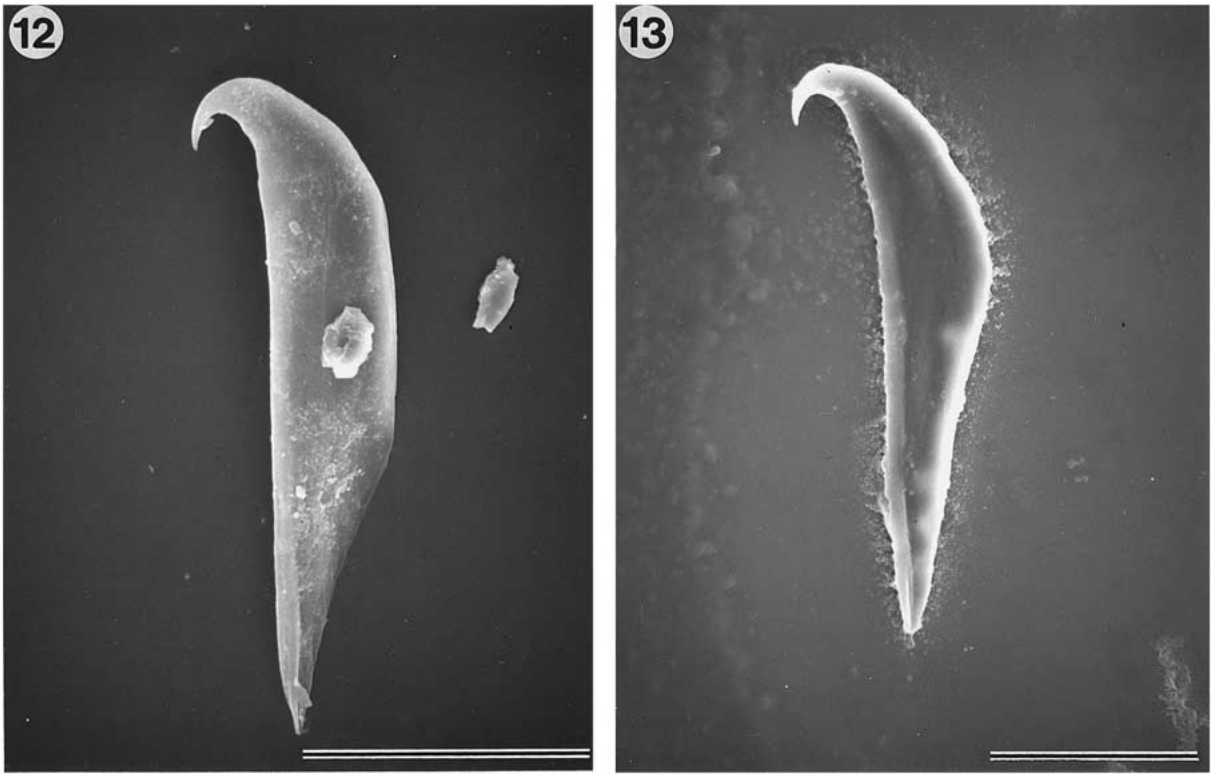


Figure 7. Trimusculotrema schwartzi n. sp. Entire worm (composite, ventral), caecum omitted from right of figure, vitellarium omitted from left of figure. *Scale-bar*: 600 μm.



Figures 8-11. Trimusculotrema schwartzi n. sp. 8. Haptor showing papillae (ventral, SEM). 9. Haptor showing three muscular rings, area between haptoral hooks indicated by arrow (ventral, LM, CASIZ 142481). 10. Papillae on haptor (ventral, SEM). 11. Pair of raised structures at anterior end of worm (ventral, SEM). *Scale-bars*: 8, 500 μm; 9, 500 μm; 10, 50 μm; 11, 100 μm.



Figures 12-13. Anterior hamuli from two haptors of *Trimusculotrema schwartzi* n. sp. *Scale-bars*: 12, 50 μm; 13, 50 μm.

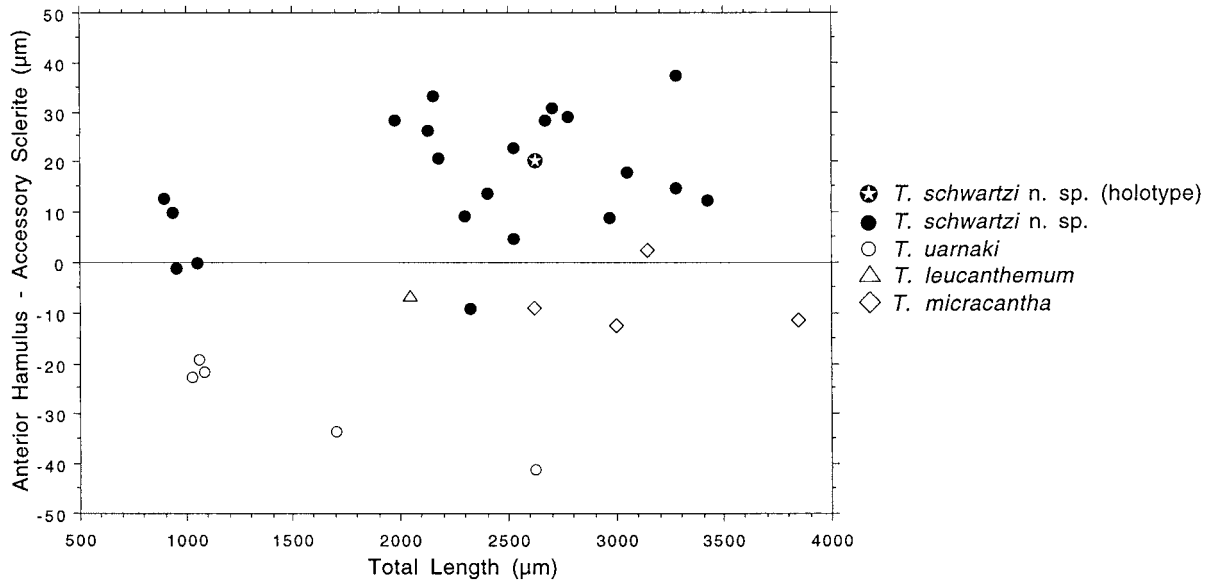


Figure 14. Anterior hamulus length (μm) minus accessory sclerite length (μm) *vs* total length (μm) of four species of the genus *Trimusculotrema: T. schwartzi* n. sp., *T. uarnaki, T. micracantha* and *T. leucanthemum*. All data from this study.

Table 1. Comparative measurements (μm) and other characteristics of four species of *Trimusculotrema.*

Species	*T. micracantha*[a]	*T. leucanthemum*[a]	*T. uarnaki*[b]	*T. schwartzi* n. sp.[a]
Total length	3,156 (2,625-3,850)[†]	2,050[†]	1,983 (1,432-2,661)	2,323 (900-3,425)
Maximum breadth	1,681 (1,275-2,100)	850	1,082 (776-1,354)	1,710 (470-2,600)
Haptor diameter	1,000 (775-1,175)	1,000	720 (515-934)	1,161 (380-1,900)
Accessory sclerite length	84 (75-98)	37 (32-42)	81 (66-105)	95 (48-140)*
Anterior hamulus length	76 (65-85)	30 (30)	46 (44-52)	114 (60-205)*
Posterior hamulus length	44 (32-52)	40	40 (30-45)	65 (48-90)*
Marginal hooklet length	12 (12)	14 (12-15)	16 (15-17)	16 (10-19)**
Anterior attachment organ diameter	388 (320-450)	168 (165-172)	240 (160-320)	455 (153-640)
Pharynx length	368 (290-450)	148	205 (141-297)	312 (122-460)
Pharynx width	382 (340-410)	190	253 (156-328)	450 (182-660)
Pigment shields over eye-spots	2 pairs	2 pairs	2 pairs	absent
Number of concentric muscle bands in haptor	3	2	3	3
Radial ribs around periphery of haptor	absent	present	absent	absent
Number of specimens	4	1	9	22 (25)***
Host	*Dasyatis marmorata*	*Dasyatis marmorata*	*Himantura uarnak*	*Dasyatis zugei*
Site on host	Skin	Gills	Skin	Skin
Geographic locality	Gorée, Senegal, Africa	Gorée, Senegal, Africa	Moreton Bay, Queensland, Australia	Hong Kong, P.R. China

[a]Data from present study.
[b]Data from Whittington & Barton (1990).
*$n = 49$, **n = 89, *** 22 whole specimens and 3 haptors lacking bodies (25 haptors total).
[†]Total lengths of *Trimusculotrema micracantha* and *T. leucanthemum* reported in Euzet & Maillard (1967) and repeated in Whittington & Barton (1990) referred to the body without the haptor, whereas those of *T. uarnaki* were based on the body with the haptor (W. Poly and W. Dyer, pers. observ.). All total length measurements in this table include the body and haptor.

T. micracantha (however, note that this characteristic might have been affected by contraction).

Discussion

The pair of raised structures at the anterior end of *Trimusculotrema schwartzi* might serve a sensory function (Figure 11); similar structures were not observed by us on other species of the genus (the structures were visible with LM on most specimens of *T. schwartzi*). Thus, the structures might be unique to *T. schwartzi. Benedeniella posterocolpa* has a large conical papilla on each anterior attachment organ (McMahon, 1963; W. Poly and W. Dyer, pers. observ.) that also might be a unique sensory structure for this species (McMahon, 1963).

One feature of the genus *Trimusculotrema* is the muscular rings (two or three) in the haptor (Whittington & Barton, 1990). Although we have not examined specimens of *Entobdella apiocolpos* Euzet & Maillard, 1967, three concentric muscle bands were illustrated by Euzet & Maillard (1967, p. 1438) for this species; therefore, the concentric muscle bands may not be a unique character of *Trimusculotrema*. In *T. schwartzi* and *T. leucanthemum* the muscle bands are not continuous through the area between the hamuli. In *T. micracantha* they extend into the area between the hamuli but as smaller groups of fibres and not as cohesive bands. The innermost muscle bands of *T. uarnaki* extend between the hamuli, sometimes becoming discontinuous, but the outermost band usually does not, except as a few small fibres. Whittington & Barton (1990) illustrated all the muscle bands as continuous. Connections between adjacent muscle bands were observed in *T. schwartzi, T. micracantha* and *T. uarnaki*, and were illustrated in the latter by Whittington & Barton (1990).

Conspicuous cells of *T. schwartzi* lie between the ovary and testes, whereas they lie lateral to the ovary in contact with the testes in slide-mounted specimens of *T. uarnaki* which we studied. In addition, one conspicuous cell can be seen in a living specimen (Figure 13 of Whittington & Barton, 1990), lying posterolateral to the ovary in contact with the testis (just above scale-bar in photo).

T. schwartzi is only the third capsalid species with reductions in pigment shields and/or other ocular structures; the other capsalids reported to lack eye-spots are *Pseudoentobdella pacifica* (Guberlet, 1936) Yamaguti, 1963 and *Lagenivaginopseudobenedenia tinrowi* Timofeeva, 1995 (Guberlet, 1936; Price, 1939; Timofeeva, 1995). All four species of *Trimusculotrema* occur on stingrays of the genera *Dasyatis* and *Himantura*. *T. schwartzi* was found on the ventral body surface (skin) of *D. zugei*; other species of the genus occurred on the skin, except *T. leucanthemum*, which was found on the gills of *D. marmorata*. *T. schwartzi* is the only member of its genus that occurs in Asian waters and thus far is known only from the Zhujiang estuary.

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