Zootaxa 1162: 53–64 (2006)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

1162

A new acotylean flatworm, *Armatoplana colombiana* n. sp. (Platyhelminthes: Polycladida: Stylochoplanidae) from the Caribbean coast of Colombia, South America

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Abstract

Armatoplana colombiana n. sp. (Polycladida: Stylochoplanidae), a new species of acotylean flatworm from Colombia, is described. *A. colombiana* is characterized by 6–8 fleshy, anterior knobs, short nuchal tentacles, tentacular and cerebral eyes. A strongly muscularized, interpolated prostatic vesicle and a penis armed with a very long stylet places the species into the genus *Armatoplana.* The female reproductive system is characterized by the presence of a Lang's vesicle and a sinuous vagina with rigid walls. Type material is deposited at the Museo de Historia Natural Marina de Colombia INVEMAR under INV-PLA 0019 and INV-PLA 0020 HS.

Key words: Acotylea, turbellarians, Caribbean biodiversity, species description

Resumen

Armatoplana colombiana n. sp. (Polycladida: Stylochoplanidae), se describe como una nueva especie de gusano plano del orden Acotylea encontrado en Colombia. *A. colombiana* se caracteriza por presentar de 6–8 protuberancias carnosas en la parte anterior, tentáculos nucales cortos y ojos tentaculares y cerebrales. La vesícula prostática es interpolada y esta fuertemente muscularizada y el pene esta armado con un estilete largo lo cual situa esta especie en el género *Armatoplana*. El sistema reproductor femenino esta caracterizado por presentar vesícula de Lang y una vagina sinuosa con paredes rugosas. El material tipo esta depositado en el Museo de Historia Natural Marina de Colombia INVEMAR (INV-PLA 0019 y INV-PLA 0020 HS).

Accepted by W. Sterrer: 17 Feb. 2006; published: 30 Mar. 2006

Introduction

The order Polycladida represents a highly diverse clade of free-living, almost exclusively marine flatworms (Prudhoe 1985). Polyclads are found from the littoral to the sublittoral zone; on coral reefs, among shells and seaweeds, as well as on colonial ascidians. Based on the character presence/absence of a cotyl or sucker, Lang (1884) divided the order into the Cotylea and Acotylea, respectively. Of the two, the Acotylea is the larger group with over 28 families worldwide. Most acotyleans are dull in coloration, negatively phototactic and cryptic in their behavior, hiding in crevices and under coral during the day. Many acotyleans are major predators of commercial bivalves (Galleni et al. 1980, Prudhoe 1985, Littlewood & Marsbe 1990, Newman et al. 1993, Jennings & Newman 1996a, b, O'Connor & Newman 2001).

Despite the fact that many polyclads pose a threat to aquaculture industries, acotyleans and polyclads in general, have received little taxonomic attention. This is due to difficulties in collecting, preserving, and identifying specimens. Polyclads are known to autolyse upon handling, and it was not until Newman & Cannon (1995) developed a new fixation technique, that routine distortion could be avoided during histological processing. In addition, positive species identification of acotyleans requires serial sagittal sectioning of the reproductive system, a process that is time-consuming and reliant on expert knowledge. Thus, it is not surprising that polyclads have largely been ignored, and that only scant information is available on their distributions.

The earliest surveys of polyclads in the Caribbean include those of Prudhoe (1944) in the Cayman Islands, Hyman (1939, 1955a, b) in the US Virgin Islands, Jamaica, Puerto Rico, Bermuda, the Bahamas, Dominica, and Florida, and Marcus and Marcus (1968) in the Lesser Antilles, Puerto Rico, Key Biscayne, and Brazil. More recently, in a survey of the Tayrona National Park in Colombia, Quiroga et al (2004) listed 25 species of Polycladida. Of these, 13 species belong to the Acotylea, bringing the total number of acotyleans recorded for the Caribbean to 78 species.

In this account, we describe a new species of the genus *Armatoplana* Faubel 1983 from the Caribbean coast of Colombia. This species was previously listed as an undescribed species of *Pleioplana* Faubel 1983 (Bolaños et al. 2004, Quiroga et al. 2004).

Material and methods

Polyclads were hand collected in the littoral zone from under rocks at Inca-Inca in Gaira Bay, Santa Marta, Colombia. Animals were measured (measurements given as length mm x width mm) and photographed *in vivo* in the lab, fixed on frozen 10% buffered formalin and preserved in 70 % ethanol. From one specimen, a 3mm x 2 mm segment was dissected containing the reproductive structures. This segment was embedded in paraffin, sagittally sectioned at 5–7 µm, and stained with hematoxylin and eosin. Sections were mounted in

Permount on glass slides. Diagrammatic reconstructions of the reproductive system were derived from the sectioned material. The material has been accessed into the Museo de Historia Natural Marina de Colombia at INVEMAR in Santa Marta as a wet specimen and as serial sections. Taxonomic identifications were done following the classification system of Faubel (1983), which is based on the characteristics of the male reproductive system, specifically the structure of the prostatic vesicle and its orientation and relationship to the ejaculatory duct.

Systematics

Family: Stylochoplanidae Faubel 1983

Genus: *Armatoplana* Faubel 1983

***Armatoplana colombiana* n. sp.**
(Figs. 1–9)

Type material

Holotype, one mature specimen (5.5 mm X 3 mm) in 70% ethanol, INV-PLA 0019; collected in August 2002.

Paratype, one mature specimen (6 mm x 2.5 mm) as serial sagittal sections, INV-PLA 0020 HS, collected in August 2002.

Other Material Examined: one additional mature specimen (6 mm x 3 mm); reproductive system sectioned sagittally.

Type Locality: Inca-Inca (N11° 11'; W74° 14'), Gaira Bay, 6 km southeast of Santa Marta, Colombia.

Etymology

Species name refers to Colombia, the country from which the type specimens were collected.

Synonyms

Pleioplana sp. Bolaños et al., 2004. *Pleioplana* sp. Quiroga et al., 2004.

Distribution

To date, found only at Inca-Inca Bay, Tayrona National Park, Santa Marta, Colombia, from under rocks in the littoral zone.

Diagnosis

Species characterized by non-retractile nuchal tentacles and by 6–8 submarginal knobs at the anterior end. Male stylet extremely long (1250–1500 µm), curved, with very pointed end.

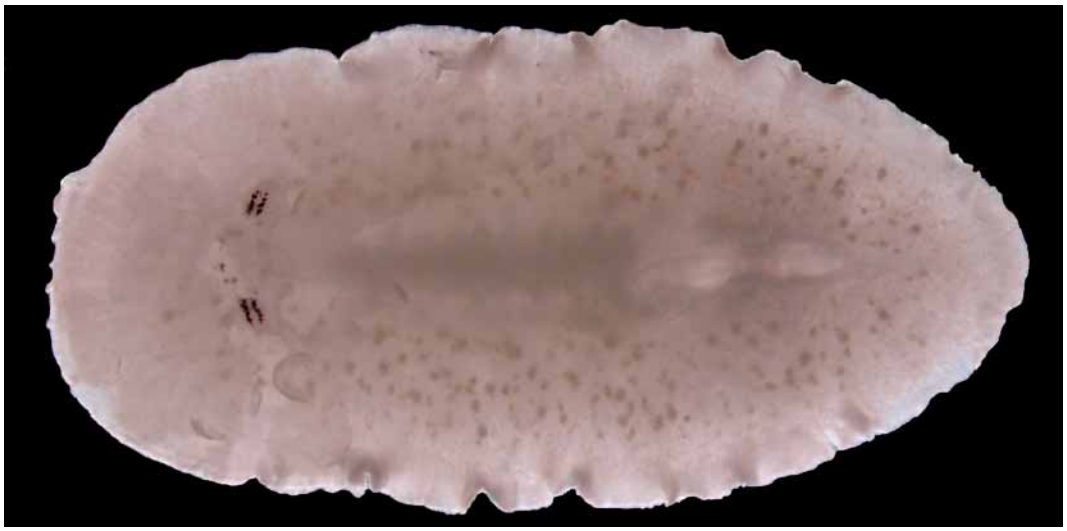

FIGURE 1. Whole, fixed animal, representing general body shape and nuchal tentacles.

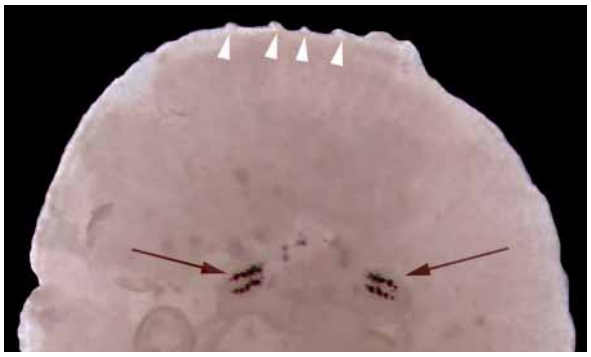

FIGURE 2. Fleshy, anteriorly located knobs (white arrow heads) and nuchal tentacles (black arrows).

FIGURE 3. Sagittal section through the anterior end, indicating position of knobs (arrow); the anterior end is folded subterminally. Scale = 1 mm.

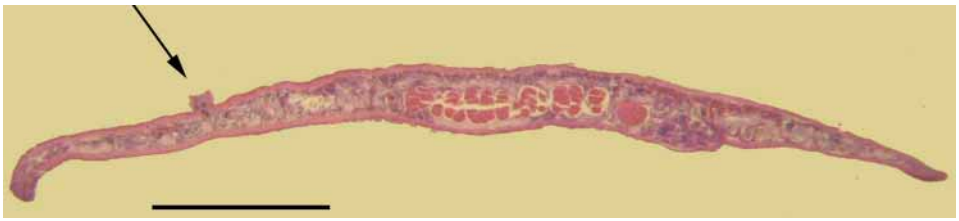

FIGURE 4. Sagittal section with nuchal tentacles (arrows). Scale = 1 mm.

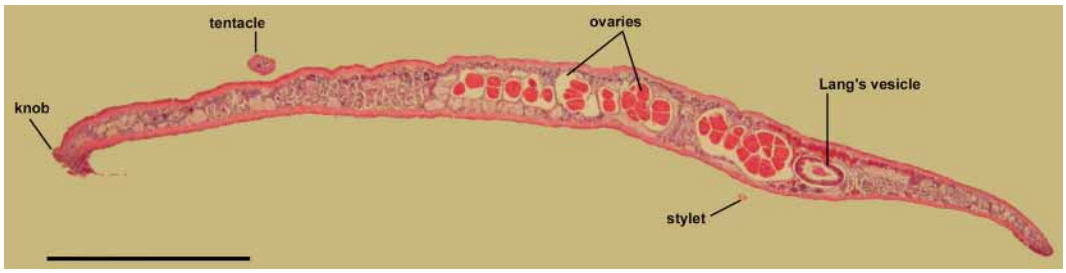

FIGURE 5. Sagittal section showing one anterior knob, tentacle, ovaries, Lang's vesicle, and distal point of extruded stylet. Scale = 1 mm.

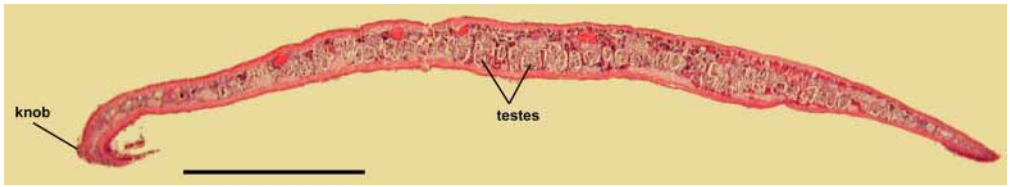

FIGURE 6. Sagittal section showing ventral location of testes. Scale = 1 mm.

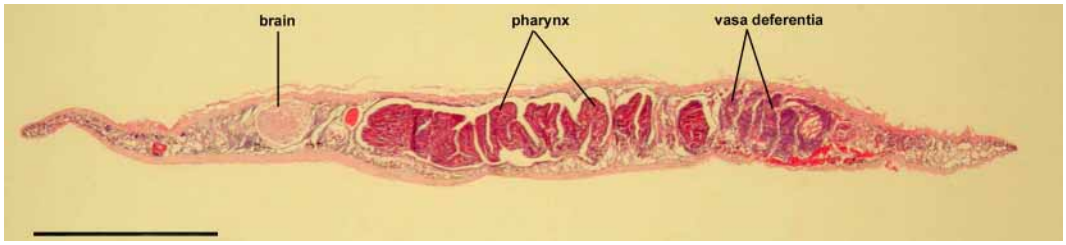

FIGURE 7. Sagittal section displaying anterior brain capsule, voluminous pharynx, and highly sinuous vasa deferentia. Scale = 1 mm.

FIGURE 8. Sagittal section showing prostatic and seminal vesicles, sinuous vagina, and cement glands. Scale = 250 µm.

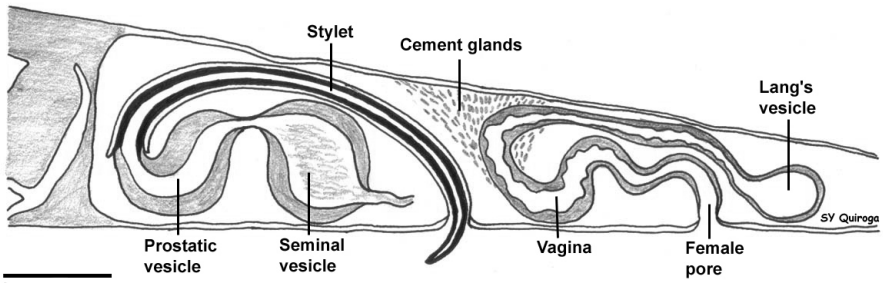

FIGURE 9. Diagrammatic representation of copulatory complex of *A. colombiana*. Scale = 250 µm.

Description

External features: Small worms, of light grayish color, with dorsal surface covered with an irregular distribution of brown spots (Fig. 1). Anterior end rounded and bearing 6–8 fleshy, well-separated knobs (Figs. 2, 3, and 5). Short (200 µm long), non-retractile nuchal tentacles present just lateral to the brain (Figs. 2, 4 and 5). Small eyes scattered at the base of the tentacles and in the cerebral region as three eye clusters. Ruffled pharynx centrally located in anterior third of body, mouth at posterior end of pharynx. Uteri visible through body wall, running anterior, anastomosing just above the anterior end of the pharynx. Male and female gonopores separate and posterior to pharynx. Anterior and posterior heavy concentration of rhabdites in epidermis. Posterior end pointed.

Reproductive anatomy: Measurements refer to lengths in a 4.3 mm long worm. Male copulatory apparatus located anterior to male pore and directed posteriorly. Very deep, male antrum houses a long (1250 x 50 µm) and curved stylet, stylet curves dorsally over the seminal and prostatic vesicles. In the majority of fixed worms, the stylet is extruded from the male pore (Fig. 5). Prostatic vesicle (275 x 225 µm) interpolated, seminal vesicle (275 x 175 µm) joined dorsally to prostatic vesicle (Fig. 9). Both seminal and prostatic vesicles with strongly muscularized walls. Prostatic and seminal vesicles very close to each other, hence it is very difficult to distinguish between them. Prostatic vesicle slightly curved, joined almost directly to the stylet. Testes ventral (Fig. 6); highly sinuous vasa deferentia (Fig. 7) joined dorsally to seminal vesicle. Female reproductive system with very sinuous vagina with ridged walls (Fig. 8); Lang's vesicle present. Uteri highly voluminous. Male gonopore close to female pore. A schematic representation of the reproductive complex is given in Fig. 9.

Taxonomic Remarks

Bock's (1913) seminal work on the Polycladida divided the Acotylea into three sections based mostly on the arrangement of the eyes, namely the Emprosthommata, Craspedommata, and Schematommata. Prudhoe (1982) in turn, emended these divisions into three superfamilies, the Cestoplanoidea, Stylochoidea, and Planoceroidea, respectively. Using mostly characters of the male reproductive system, Faubel (1984)

revised the three superfamilies to Ilyplanoidea (true prostatic vesicle lacking), the Stylochoidea (prostatic vesicle free), and the Leptoplanoidea (prostatic vesicle interpolated), respectively (Table 1; see also Tyler et al. 2005). Within the Leptoplanoidea, Faubel (1984) established three new families, one of which, the Stylochoplanidae, he validates with the characters "true prostatic vesicle present, its glandular lining smooth and the glands of which mostly extravesicular."

TABLE 1. Comparison of the changing nomenclature of the three acotylean superfamilies.

Authority	Superfamily	Superfamily	Superfamily
Bock 1913	Craspedommata	Schematommata	Emprosthommata
Poche 1926	Stylochoidea	Planoceroidea	Cestoplanoidea
Marcus & Marcus 1966	Craspedommatidea	Schematommatidea	Emprosthommatidea
Prudhoe 1982	Stylochoidea	Planoceroidea	Cestoplanoidea
Faubel 1983	Craspedommatidea	Schematommatidea	Emprosthommatidea
Faubel 1984	Stylochoidea	Leptoplanoidea	Ilyplanoidea
Tyler et al. (2005)	Stylochoidea	Leptoplanoidea	Ilyplanoidea

Within this family, the genus *Stylochoplana* (Stimpson 1857) consists of a heterogeneous assemblage of numerous species. Recognizing the need for a more appropriate classification, Marcus & Marcus (1968) had separated the genus into groups based on presence or absence of tentacles and of a stylet. Their group C2, characterized by the presence of tentacles and an armed penis, contains three species, *S. divae*, *S. vesiculata,* and *S. evelinae* (Marcus & Marcus 1968).

Since then, Faubel (1983) erected the genus *Armatoplana,* including those species of *Stylochoplana* characterized by an armed penis and the presence of Lang's vesicle. Species with an unarmed conical penis papilla were retained in *Stylochoplana*. Faubel (1983) distinguishes species of *Armatoplana* from species in other genera of the family by the following combination of characters: lack of tentacles, presence of serial cerebral and tentacular eyes, an anteriorly located pharynx, presence of a true seminal vesicle or spermiducal bulbs, and an armed penis with a long, sharp stylet. Lang's vesicle and a true vagina bulbosa are present in the female copulatory apparatus. However, the character "presence/absence of nuchal tentacles" may be of little systematic value, because Faubel (1983) moved two species with head tentacles, *S. divae* and *S. vesiculata* of group C2 (Marcus & Marcus 1968) into *Armatoplana.* Based on this, we believe it is appropriate to emend *Armatoplana* to include worms with or without nuchal tentacles. This is further supported by the fact that nuchal tentacles may be difficult to discern in poorly fixed material and may have been overlooked in the past.

With the exception of the presence of nuchal tentacles in our specimens, the newly described species *Armatoplana colombiana,* agrees with all the characteristics of the genus

as defined by Faubel (1983). However, as stated above, the presence of tentacles may not be of great taxonomic significance. Initial identifications that had placed this species into the genus *Pleioplana* were based mostly on a general arrangement of reproductive structures and the presence of a long, pointed stylet (Bolaños et al. 2004, Quiroga et al. 2004). Since then, it has become clear that the prostatic vesicle of *Pleioplana*, containing well-defined tubular chambers, is very different from the prostatic vesicle observed in our specimens. The only other genus within the Stylochoplanidae that is characterized by an armed penis, the presence of Lang's vesicle and tentacles is *Interplana.* However, in species of *Interplana*, the stylet does not curve dorsally over the prostatic and seminal vesicles as it does in species of *Armatoplana.*

The presence of anterior marginal knobs (Figs. 2, 3 and 5) which are lacking in *A. divae* and *A. vesiculata*, clearly separates *A. colombiana* from these congenerics, as do differences in reproductive system structures, i.e., *A. colombiana* has a much longer and more curved stylet, the prostatic vesicle is more rounded, and Lang's vesicle is bigger (Table 2). In addition, no mature specimens of *A. divae* and *A. vesiculata* are known that are less than 1cm in length.

The shape and nature of the male reproductive systems of *A. lactoalba*, *A. leptalea*, and *A. panamensis* show close similarities with those of *A. colombiana.* However, based on the presence of tentacles and marginal anterior knobs in *A. colombiana*, the new species can be reliably separated from these three species (Table 2). Additionally, coloration and size can be used to differentiate among these species. Finally, live specimens of *A. colombiana* may be confused with *Styloplanocera fasciata* because coloration and color patterns (light grey with isolated dark brown spots and a network of brown pigmentation covering the dorsal surface) of the two are almost identical. However, a closer examination of *S. fasciata* will show that knobs are present all over its surface, whereas they are limited to the anterior end in *A. colombiana.* Additionally, *S. fasciata* of such a small size would not be mature individuals. Internally, of course, the male reproductive systems of *S. fasciata* and *A. colombiana* are completely different, again emphasizing the importance of histological sections for positive species identifications in Acotylea.

Acknowledgements

We thank Nestor E. Ardila for helpful advice, and INVEMAR for logistic support and the curation of types. This work was supported in part by NSF grant DEB0412932, and is Scientific Contribution No. 2291 from the New Hampshire Agricultural Experiment Station.

TABLE 2. Comparison of morphological features of existing valid and proposed new species of the genus *Armatoplana* (characters according to Faubel, 1983)

Species	Coloration	Sub-marginal knobs	Tentacles	Tentacular eyes	Cerebral eyes	Seminal vesicle	Prostatic vesicle	Penis stylet	Lang's vesicle
A. colombiana n.sp	greyish transparent, with brown spots distributed irregularly over dorsal surface	6-8 knobs present at anterior end	non-retractile nuchal tentacles	small eyes scattered at base of tentacles	three small clusters	muscular joined to prostatic vesicle dorsally	interpolated, muscular with slight curvature and located parallel to seminal vesicle	very long and curved	small, rounded
A. affinis (Palombi, 1940) Faubel 1983	yellow with brownish dots over dorsal surface	absent	absent	two groups, next and behind of cerebral eyes	one big, dense group	oval, muscular	oval, muscular	small, slender and long	oval, small with two spherical accessory vesicles
A. divae (Marcus, 1947) Faubel 1983	olive green with brown intestinal ramifications extending from middle to periphery	absent	short nuchal tentacles	one group at base and surrounding each tentacle	two small, dense groups	large, elongate below prostatic vesicle	Interpolated and dorsal and parallel to seminal vesicle	conic and long	small, rounded
A. lactea (Laidlaw, 1903) Faubel 1983	white with minute grey dots scattered sparsely over dorsal surface	absent	absent	two small groups with a few eyes	small group with few eyes wide spread over brain area	two elongated seminal vesicles with thick muscular walls	reduced	small, armed and curved	small, muscular
A. lactoalba (Verrill, 1900) Faubel 1983	translucent milky white	absent	absent	two longitudinal bands with numerous eyes enlarged at the level of the brain	absent	oval, large, very muscular	oval, chambered	long slender	large, long
A. leptalea (Marcus, 1947) Faubel 1983	translucent	absent	absent	two longitudinal bands of few eyes extended to pre-cerebral region	small group of few eyes	elongate, muscular, anterior to male gonopore	interpolated, anterior to seminal vesicle	muscular, slender and long	narrow, long
A. panamensis (Plehn, 1986) Faubel 1983	grey	absent	absent	two groups of about 12-15 eyes each, some large, others small	two groups with numerous eyes, not distinctly separated from tentacular groups	oval, muscular and joined directly to prostatic vesicle	long, narrow; directly joined to seminal vesicle	armed, long and curved	extraordinary large, elongated

TABLE 2 (continued).

Species	Coloration	Sub-marginal knobs	Tentacles	Tentacular eyes	Cerebral eyes	Seminal vesicle	Prostatic vesicle	Penis stylet	Lang's vesicle
A. rabita (Marcus & Marcus, 1968) Faubel 1983	light yellow with two longitudinal chestnut brown bands	absent	absent	two forward converging rows but only a few distinct eyes	small group of tiny eyes directed dorsally and ventrally	small, oval and anterior to prostatic vesicle	interpolated, large, elongated and posterior to seminal vesicle	short and conical	short, rounded
A. reishi (Hyman, 1959) Faubel 1983	pale	absent	absent	definite clusters of about six eyes in each group	one group of 18-20 eyes extending forward linearly	fusiform, muscular below prostatic vesicle	oval and above seminal vesicle	conical penis papilla and long stylet	small, crescent shape
A. robusta (Palombi, 1928) Faubel 1983	dark chestnut brown	absent	absent	two groups, large and of irregular form	two small groups with numerous eyes irregularly arranged	sac-shaped, directly joined to prostatic vesicle	elongate and directly joined to seminal vesicle	short	small, short, extending forward
A. snadda (Marcus & Marcus, 1968) Faubel 1983	unknown	absent	absent	two dense clusters directed peripherally	one group anterior and medial of brain, extending anteriorly; plus single cerebral eye behind each tentacular cluster	large, circular, muscular and below prostatic vesicle	small, rounded above seminal vesicle	slender and long	small, moniliform
A. taurica (Jacubova 1909) Faubel 1983	unknown	absent	present	two dense groups covering tentacles	two long rows with few eyes extending anteriorly	small, elongate, muscular, directly joined to prostatic vesicle	long, elongate, posterior to seminal vesicle	short, small	small, rounded
A. tenuis (Palombi, 1936) Faubel 1983	dark brown	absent	absent	two dense rows extending beyond brain level	one irregularly arranged group not distinctly separate from tentacular groups	elongate, directly joined to prostatic vesicle	small, oval, elongate and bent towards posterior end	small, conic penis without stylet	small, rounded
A. vesiculata (Palombi, 1940) Faubel 1983	grey and yellow, clearer in the pharynx area	absent	absent	two groups	two long, dense groups in middle of tentacular eyes	small, elongate, muscular	piriform	small penis with strong stylet	elliptical, large with two spherical accessory vesicles

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