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New model for the formation and function of sagittocysts: *Symsagittifera corsicae* n. sp. (Acoela)

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Abstract. This study is focused on the formation and function of sagittocysts, which are secretions typical of members of the acoel family Sagittiferidae. The needle-shaped sagittocysts are produced in specialized gland cells (sagittocytes) whose distal necks are often surrounded by muscle mantles. Contraction of the muscle mantle ejects the sagittocyst. We establish a model for the development of sagittocytes and muscle mantles out of the stem cell pool of the new acoel species *Symsagittifera corsicae.* We used various techniques, especially interference and phase-contrast microscopy of living specimens as well as labeling of the body-wall musculature, for species characterization. In addition to the morphological features, we provide the third complete sequence of the 18S rDNA gene in the family Sagittiferidae.

Additional key words: Turbellaria, Platyhelminthes, Acoelomorpha, *Symsagittifera poenicea*

Sagittocysts are needle-shaped secretory products and range in length from 5 to 50 μm and in diameter from 1 to 5 μm. They are very often combined with special muscle mantles connected with the epidermis. Sagittocysts can be extruded by contraction of the muscle mantle after physical or chemical stimulation (Yamasu 1991; Gschwentner et al. 1999). The diversity of sizes and arrangements of sagittocysts—e.g., sagittocytes with single or with proximal and terminal sagittocysts, with or without muscle mantles—points to a long evolutionary history of these extrusomes. Sagittocysts occur in 12 acoel species all classified in a single family, the Sagittiferidae (Geddes 1879; Kato 1951; Ivanov 1952; Beklemishev 1957; Marcus 1957; Yamasu 1982, 1991; Winsor 1990; Kostenko & Mamkaev 1990a,b; Mamkaev & Kostenko 1991; Kozloff 1998; Gschwentner et al. 1999; Hooge & Tyler 2001).

Sagittocysts consist of a central filament, surrounded by an intermediate electron-lucent layer, and an external cylinder of specialized matrix (Gschwentner et al. 1999). Central filaments occur in two forms: one has a rectangular profile, the other is round. Rectangular profiles have been seen in *Sagittifera sagittifera, Symsagittifera bifoveolata* (Mamkaev & Kostenko 1991), *Praesagittifera shikoki* (Yamasu 1991), and *Symsagittifera corsicae* n. sp. A clearly round profile of the central filament has so far been found only in *Convolutriloba longifissura* (Gschwentner et al. 1999). This suggests different types of central filaments in the subfamily Convolutrilobinae (round), and in the Praesagittiferinae and Sagittiferinae (rectangular).

In the literature, the term sagittocyst is used for three structures: (1) the gland cell (sagittocyte) producing an elongated secretory product in a vacuole, (2) the sagittocyst secretion itself, and (3) a unicellular muscular discharge apparatus that may contain the distal neck of the sagittocyte with a sagittocyst. Graff (1891) used the term sagittocyst for the single-cell muscular discharge apparatus with a sagittocyst in its center. We have restricted this term to the elongated secretory product in the sagittocyte.

More than rhabdites (see lit. in Smith et al. 1982) the structure of sagittocysts is of significant interest for comparison with cnidarian nematocysts. This is not to say that sagittocysts and nematocysts are homologs, but they may represent convergent structures important for the onset of true gastrodermal digestion of larger food items.

In the acoel *C. longifissura,* both sagittocytes and muscle cells differentiate from stem cells (neoblasts) within a few days (Gschwentner et al. 2001). Neoblasts in Platyhelminthes are held to be the sole source responsible for the renewal of all cell types during development, growth, and regeneration, a unique situation in the animal kingdom (Baguñà 1981; Ehlers 1985; Palmberg 1990; Baguñà et al. 1994; Hori 1997; Rieger et al. 1999). Neoblasts are characterized by a high nucleo-cytoplasmic ratio, basophilic cytoplasm, few mitochondria, free ribosomes, and a relatively small amount or even lack of endoplasmic reticulum.

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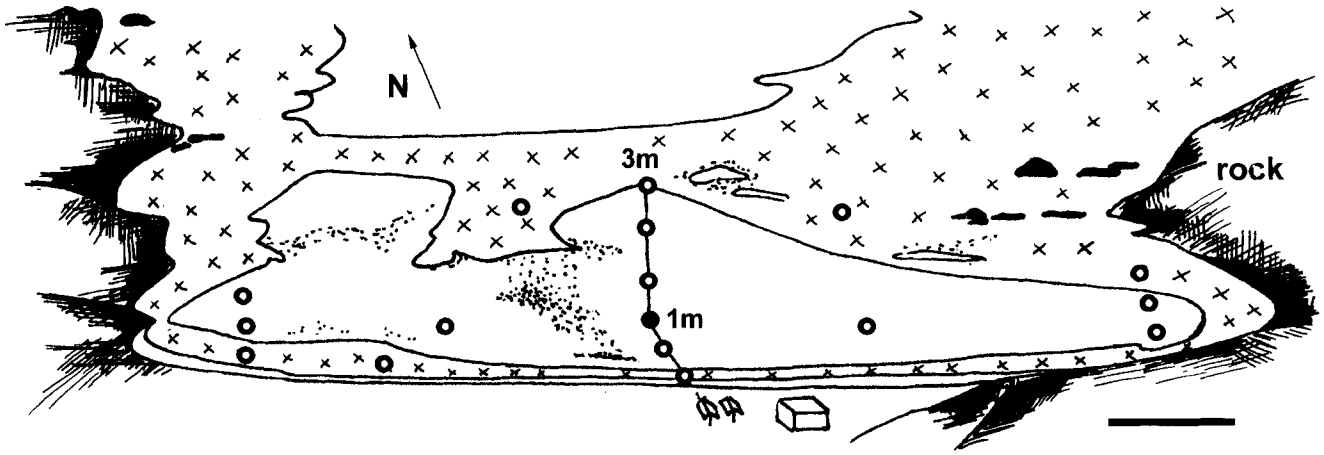

Fig. 1. Drawing of type locality of *Symsagittifera corsicae* n. sp. at the sandy beach of the Revellata peninsula. Fine sand samples were taken along a median line up to 3 m water depth along two lateral lines and in additional scattered spots (○). At 1 m water depth, 7 quantitative cores were taken (●). Sand always covered by decaying seagrass (*Posidonia oceanica*) during our study is indicated by x's. Sand where decaying seagrass was not always present is indicated by dotted areas. The remaining large area is sandy beach without seagrass. Scale bar, 40 m.

We discovered a new species of the genus *Symsagittifera* on a sandy beach on Corsica in the Mediterranean Sea. In this paper, special attention is given to a new model explaining the development, function, and terminology of sagittocysts, and the new species is described. Also, we sequenced the complete 18S rDNA gene from *Symsagittifera corsicae* n. sp. to allow future phylogenetic comparisons within the Acoela.

Methods

More than 1000 specimens of *Symsagittifera corsicae* were collected from the type locality (see species description and Fig. 1). The animals were found in relatively clean sand by skimming the topmost portion and were washed out using a solution of magnesium chloride isotonic to seawater for relaxation of the worms. The worms were cultured in petri-dishes containing artificial seawater and small amounts of sand, in Innsbruck. More than 60 specimens were studied in detail, in live preparations and from serially sectioned material. Living worms were studied in sqeezed preparations using interference and phase-contrast optics, and were fixed in Bouin's solution before being embedded in Spurr's low-viscosity resin (Spurr 1969). Four semi-thin 2-μm serial sections (transverse and longitudinal) were cut and stained at 80°C using the procedure of Richardson et al. (1960) and examined with a Reichert Jung Polyvar microscope.

Genomic DNA was extracted from 5 specimens of *S. corsicae* using the Chelex 100 method (Walsh et al. 1991) and from 50 specimens by proteinase K digestion, sodium chloride extraction, and isopropanol precipitation as described by Bruford et al. (1998). The complete 18S rDNA gene was amplified by the polymerase chain reaction using primers 1F and 5R, 5F and B (Carranza et al. 1996; Littlewood et al. 1999), and 4F (AAAGCATCCTTTGGGATTGA) and 6R (CCCTTCCGTCAATTCCTTTA). PCR reactions were set up in 17-μl volumes containing 50 mM Tris pH 8.3, 3 mM $MgCl_2$, 250 μl/ml bovine serum albumin, 1 μmol of the forward and 1 μmol of the reverse primer, 0.5 mM of each deoxynucleoside triphosphate, 1 μl DNA (quantity was not determined), and 0.4 U of the AccuTaq™ LA DNA Polymerase (Sigma). PCR was performed in microcapillary tubes on the RapidCycler® (Idaho Technology Inc., USA) with an initial denaturation step, 2 min at 94°C; 38 cycles with 0 sec at 94°C, 0 sec at 50°C, and 15 sec at 72°C; and a final extension step, 30 min at 72°C. Purified PCR products were ligated into PCR®-Blunt II-TOPO plasmid vectors of the Zero Blunt™ TOPO™ PCR Cloning Kit (Invitrogen) and transformed into TOP10 One Shot™ competent cells. After purification of plasmid DNA, inserts were sequenced directly by applying the universal M13 Forward and M13 Reverse primers and the BigDye Terminator Cycle Sequencing Kit™ (Applied Biosystems) following the manufacturers protocol. Sequences were visualized on the automated sequencer ABI 373A (Applied Biosystems). A BLAST search (Altschul et al. 1997) was performed to identify the species most closely related to *Symsagittifera corsicae* on the basis of 18S rDNA sequences. Sequences were aligned by eye and pair-wise distance values were calculated.

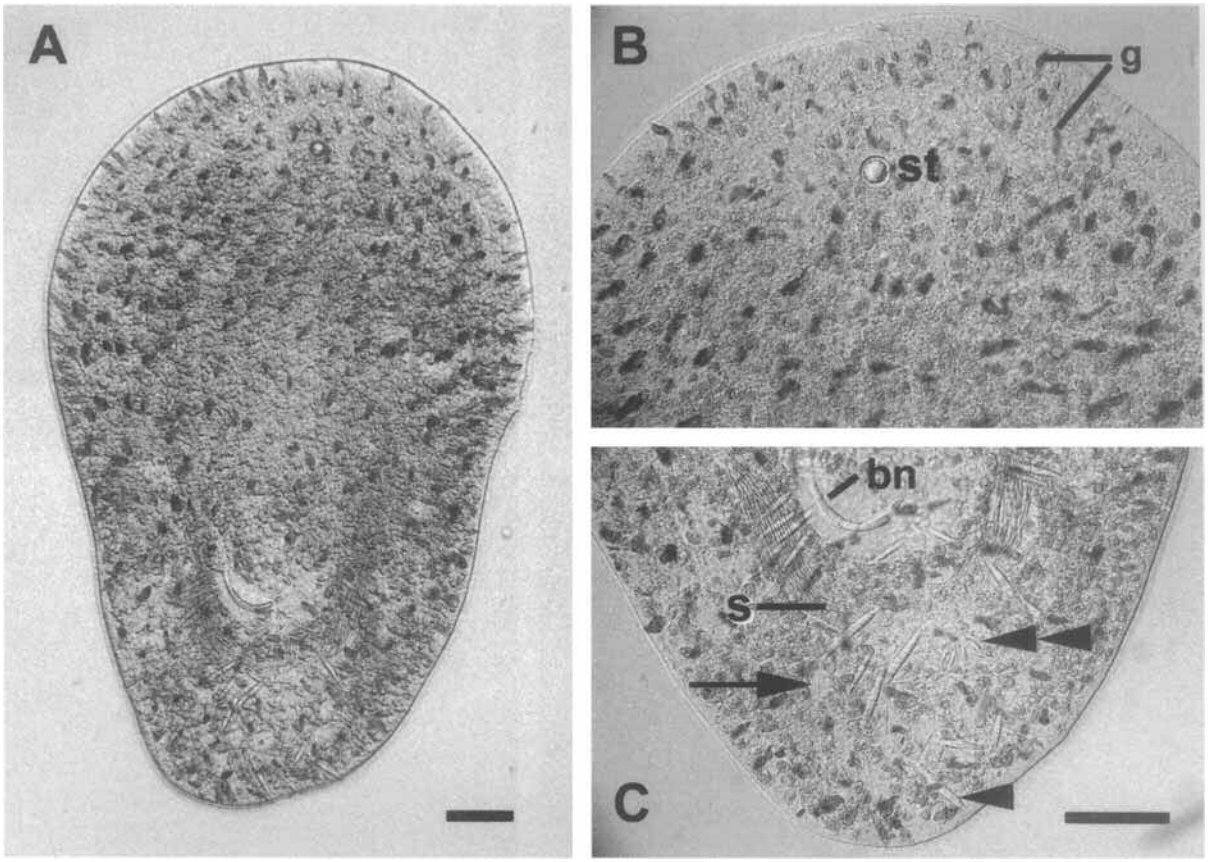

Fig. 2. *Symsagittifera corsicae* n. sp., interference contrast micrographs of squeezed living animal. Dorsal view. **A.** Mature animal. **B.** Anterior end showing glands with lancet-shaped rods (g) and statocyst (st). **C.** Posterior end. Small muscle mantles (double arrowhead) are located in a small area around the male genital opening, and large muscle mantles (arrowhead) are distributed over the whole caudal third of the animal. Sagittocysts are formed in sagittocytes (arrow). Bursal nozzle (bn), sperms (s). Scale bars, 60 μm.

Results

Sagittocysts

Two groups of sagittocysts were differentiated by their size and their distribution in mature specimens of the worms (Figs. 2, 3). Up to 24 large sagittocysts occurred ventrally in the posterior third of the body; most of them were covered by a muscle mantle (Fig. 2C arrowhead). Almost all animals studied also had sagittocysts forming gland cells (sagittocytes, Fig. 2C arrow). In these gland cells (n = 5–11), no muscle mantle was seen around the sagittocyst. Cross sections of large sagittocysts with a muscle mantle showed a round, thick-walled, secretory product surrounding a rectangular central filament (Fig. 4). Large sagittocysts measured about 30 μm in length and 3–4 μm in diameter. The surrounding muscle mantle was spindle-shaped, about 30–40 μm long and 5–7 μm in diameter, and showed a noncontinous spiral in cross-section (Fig. 4 and unpubl. obs. of TEM material). Animals were observed to extrude the central filament after physical or chemical stimulation (e.g., weak hydrochloric acid)—probably due to contraction of the muscle mantle surrounding the sagittocyst. Muscle mantles without sagittocysts can be seen (Fig. 3A,B arrows).

Small sagittocysts covered by a muscle mantle were found only in the region of the male copulatory organ, arranged more or less radially around the genital opening (Figs. 2C, 3A,B double arrowheads). They measured 5–8 μm in length and 1 μm in diameter. The surrounding muscle mantle resembled that of the large type, but measured only about 10 μm in length and 2–3 μm in diameter. Small sagittocysts are formed in vesicles of special gland cells (sagittocytes) comparable to large sagittocysts. The main occurrence of the sagittocytes with small sagittocysts is close to the male genital opening. Muscle mantles were seen with or without central sagittocysts.

We never found sagittocysts or muscle mantles in small animals (<250 μm long, see Table 1). Sagittocysts were found in some medium-sized animals (250–450 μm long), but only in those developing genital

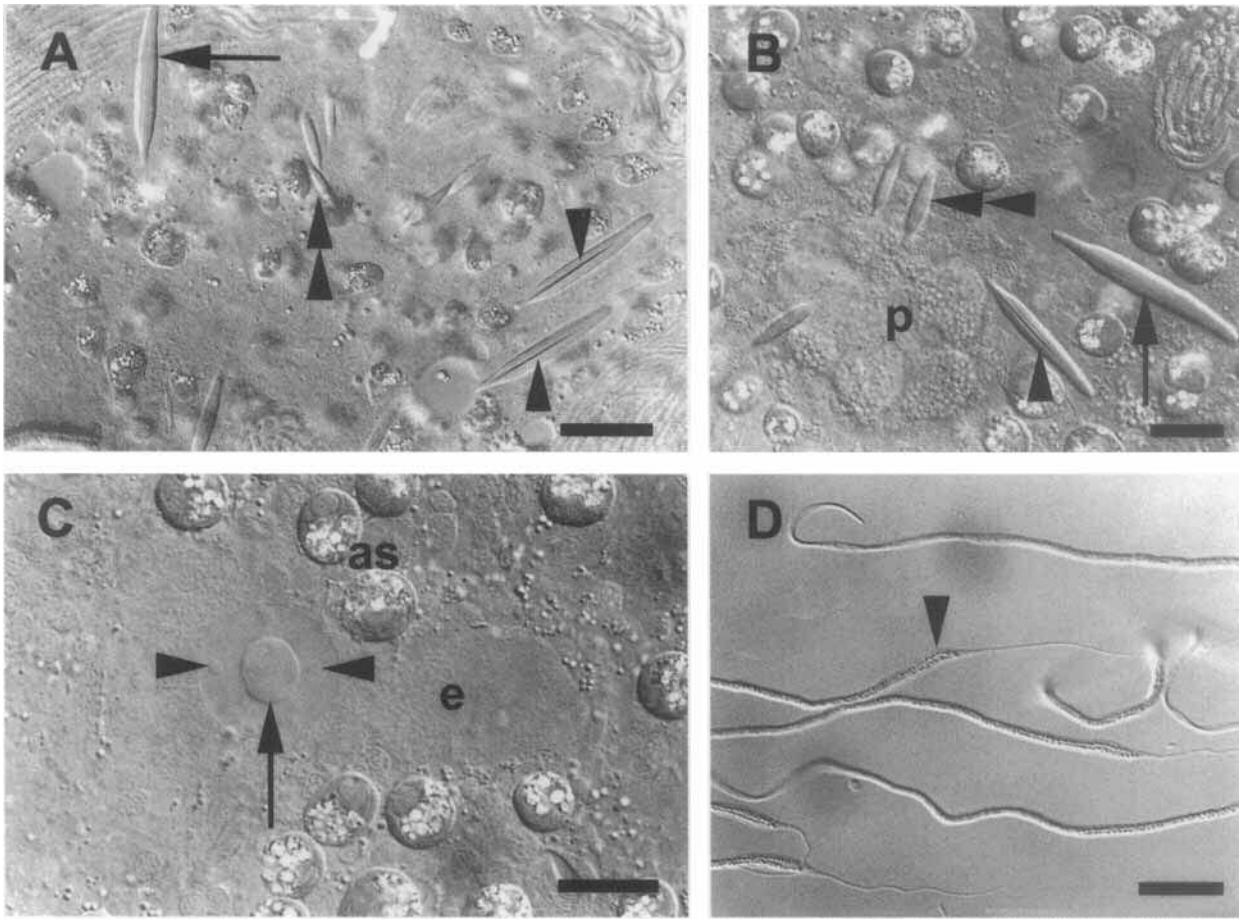

Fig. 3. *Symsagittifera corsicae* n. sp., interference contrast micrographs of squeezed living animal. **A.** Note two different sizes of muscle mantles, small ones (double arrowhead) and large ones (arrow, arrowheads). Some of the large spiral muscles contain sagittocysts (arrowheads). **B.** Small muscle mantles (double arrowheads) are distributed ventral to the penial glands (p). Large muscle mantles show a sagittocyst in the center (arrowhead) or do not (arrow). **C.** Egg (e) with nucleolus (arrow) and two bipolar chromatin structures (arrowheads). Algal symbiont (as). **D.** Living sperms with crowded small granules (arrowhead). Scale bars, 10 μm.

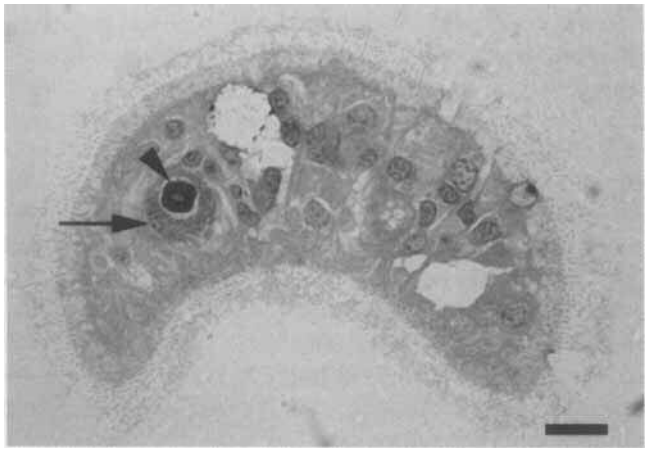

Fig. 4. Semithin cross-section of *Symsagittifera corsicae* n. sp. at caudal third of the animal. Note sagittocyst with rectangular central filament in its center (arrowhead) and the surrounding muscle mantle (arrow). Scale bar, 10 μm.

organs. Large, adult specimens (450–750 μm) always had both sagittocyst types.

Molecular data

Five clones of the first half of the 18S rDNA (positions 1 to 955) and five clones of the second half of the 18S rDNA (positions 939 to 1795) were sequenced, while the intermediate segment (positions 772 to 1157) was sequenced directly from purified PCR products. Sequences of the clones were identical except for uninformative point mutations at 4 positions. We thus created a consensus sequence of the 18S rDNA for *Symsagittifera corsicae* n. sp. The complete 18S rDNA sequence of *S. corsicae* was deposited in GenBank under the accession number AJ319029.

A BLAST search identified *Symsagittifera psammophila* (AF102893) and *Symsagittifera roscoffensis*

Table 1. Number of individuals of *Symsagittifera corsicae* n. sp. at 1 m water depth obtained by 7 core samples (volume 13.5 ml/core). We sorted animals by their length into 3 size classes: large (450–750 μm), medium (250–450 μm), and small (<250 μm). Cores 3, 4, and 6 were washed two times; animals detected after the second wash in parentheses. See Fig. 1 for location of sampling site.

Cores	Number of large animals		Number of medium animals		Number of small animals	Number of all size classes	
1	8		18		27	53	
2	50		71		81	202	
3	17	(3)	—		18	35	(3)
4	71	(24)	157	(25)	21	249	(49)
5	20		25		35	80	
6	12	(4)	19	(6)	1	32	(10)
7	82		40		66	188	
total number →	260	(31)	330	(31)	249	839	(62)

(AJ012530) as the taxa most closely related to *S. corsicae* on the basis of published 18S rDNA sequences. The alignment of *S. corsicae* and *S. psammophila* demonstrated that the first 370 bp of the 18S rDNA were highly divergent, whereas between *S. corsicae* and *S. roscoffensis* no such region could be identified. Pair-wise genetic distances were calculated after excluding the variable region (position 1 to 370) and showed 0.28% sequence divergence (4 base substitutions and 3 indels) between *S. corsicae* and *S. psammophila* and 2.02% sequence divergence (28 base substitutions and 2 indels) between *S. corsicae* and *S. roscoffensis*.

Symsagittifera corsicae n. sp.

Type locality

A sandy beach of the Revellata peninsula near the marine biological station STARESO (42°34′48″N; 8°43′2″E) in Calvi, Corsica, Mediterranean Sea. Specimens of *Symsagittifera corsicae* were found only in the top 2 cm of the sediment, most in the top 1 cm. Worms were most abundant in areas with exposed sand and least abundant in zones where decaying seagrass (*Posidonia oceanica*) was present on the sediment surface (Fig. 1). During the time of our sampling, we observed little change in tidal levels of the sea. In relation to water depth, animals were distributed from 0.3 to 3 m with their greatest abundance at 1 m in fine sand. Here we found up to 298 animals in a 13.5 ml sediment sample (see Table 1).

Morphology

The drop-shaped adult animals measure up to 850 μm long and 350 μm wide, with the greatest width at about one-third of the body length from the anterior end (Fig. 2A). The worms mostly remain in tight contact with the substrate in the culture dishes; sporadically they swim up into the water column following a spiral path. Up to 1400 green algal symbionts are distributed over the whole mature animal under a ciliated, partially insunk epidermis (Fig 2). The spherical symbionts measure 6–10 μm in diameter and are filled with green chloroplasts and yellow to brown granules (Fig. 3C). Occasionally we found juvenile worms without algal symbionts. Statocyst and frontal organ are present (Fig. 5A); the latter opens almost terminally. Stiff sensory cilia, longer than the general epidermal cilia, are mainly distributed in the anterior and posterior quarter of the body. The mouth opening is situated slightly in front of the mid-body and leads into the central digestive syncytium (Fig. 5A). Glands containing lancet-shaped, small, red rods about 2.5 μm long and 1 μm thick (Fig. 2) occur all over the dorsal body, but are missing ventrally. Testicular and ovarian follicles are positioned laterally in paired strings and show increasing maturity towards the genital pores (Fig. 5A). We have not observed special structures that delimit the follicles, even when the follicles appear discrete.

The 5–7 testis follicles (each 50–70 μm in diameter) include mature sperms (~140 μm long, Fig. 3D). The caudalmost testis follicles enlarge and fuse, forming a U-shaped mass of sperms (Fig. 2A,C). The caudal end of this sperm bundle appears to lie within the seminal vesicle, which would mean that the latter is open laterally. The copulatory organ is a bulbous structure (60 μm in diameter) limited by a layer of muscle cells that terminate at the epidermis (Fig. 5C). It is attached directly to a small groove (male antrum) in the epidermis. No central pore opening to the outside was observed. The epidermis in that groove contains 5–11 small sagittocysts, each with a muscle mantle. Anteriorly, small cyanophilic glands are building the penis

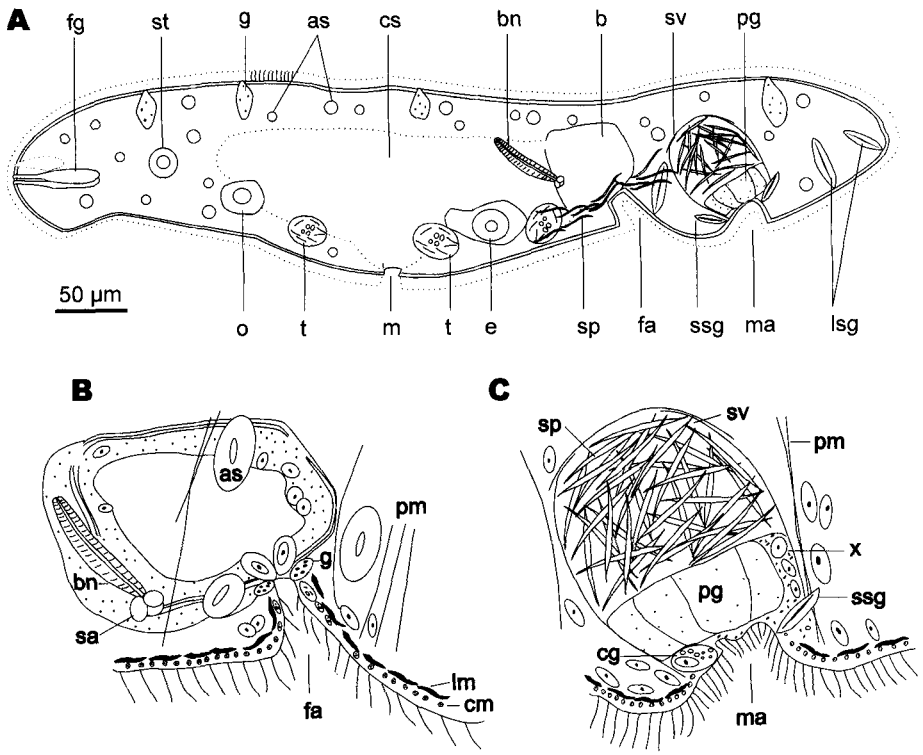

Fig. 5. Sagittal reconstruction of adult individual of *Symsagittifera corsicae* n. sp. **A.** Whole organism. Entire surface is ciliated (cilia shown on only a small part). **B.** Female genital organs. **C.** Male genital organs. Algal symbionts (as), bursa (b), bursal nozzle (bn), cyanophilic glands (cg), circular muscles (cm), central syncytium (cs), egg (e), female antrum (fa), frontal organ (fg), gland (g), longitudinal muscles (lm), large sagittocysts in spiral muscles (lsg), mouth opening (m), male antrum (ma), ovary (o), penial gland (pg), parenchymal muscles (pm), sorting apparatus (sa), sperm (sp), small sagittocysts in spiral muscles (ssg), statocyst (st), seminal vesicle (sv), testis (t), three nuclei of insunken epidermal cells (x).

(Figs. 3B, 5C). We never observed sclerotized components of the penis. The dorsal two-thirds of the copulatory bulb represents a seminal vesicle, open laterally to the caudal mass of fused testis follicles and sperms; it was not filled with sperms in all specimens. Below the seminal vesicle are 10–12 penial glands (Fig. 3B). From serial sectioning, we can say that there are 10–12 gland cells and not one gland cell with 10–12 lobes. We have not found nuclei in any of them, and we assume that these spherical portions filled with fine granular material must have an insunken cell body outside of the copulatory organ. Three nuclei of epidermal cells were found deeply insunken alongside the penial glands (x in Fig. 5C).

The ovaries are transparent in transmitted light and easily overlooked. Like the testis follicles, the least mature ovarian follicles lie just posterior to the statocyst (Fig. 5A); 3 or 4 follicles were seen between the anterior region and the bursal organ. We have always found only one large oocyte in the developing follicles (Fig. 3C). The bursal organ has a short, epidermal vagina that connects to the delicate bursa (Fig. 5B). The bursa itself shows a small ventral orifice which is surrounded by cyanophilic glands. To the left and right of the bursa, stronger dorso-ventral muscles frequently are observed. As in the male copulatory organ, there seems to be no direct connection of this delicate musculature of the bursa with the body-wall musculature. However, the latter clearly extends along the short vagina (Fig. 5B). Contrary to the condition in the male copulatory organ, only a few muscles surround the bursa itself. The bursal nozzle (~55 µm long) is embedded in the anterior part of the bursal tissue (Figs. 2C, 5A,B). The glandular sorting apparatus at the proximal end of the bursa mouth-piece is clearly visible (Fig. 5B). At the connection of the epidermal vagina to the bursa, we found small clusters of eosinophilic glands and above them two large cells that seem to block the entrance to the bursa (Fig. 5B).

Diagnosis of *Symsagittifera corsicae* n. sp.

The new species belongs to the genus *Symsagittifera* because of the following character set: a ciliated saccate male antrum, occurrence of sagittocysts, and occurrence of symbiotic algae. Adults up to 850 µm long and about 340 µm wide; frontal organ terminating apically; statocyst at level 12 (that is, at the level of 12% of the length of the animal measuring from the anterior end); mouth opening at level 46; female opening and male antrum at level 74 and level 84, respectively. Mature animals with up to 1400 algal symbionts; large and small sagittocysts within muscle mantles (10–25) and in sagittocytes (5–11); small muscle mantles only around the male genital pore; sagittocytes mostly near the caudal end. Large sagittocytes extend no farther anteriorly than level 60; juveniles lack sagittocysts and muscle mantles; epidermis partially insunk and with

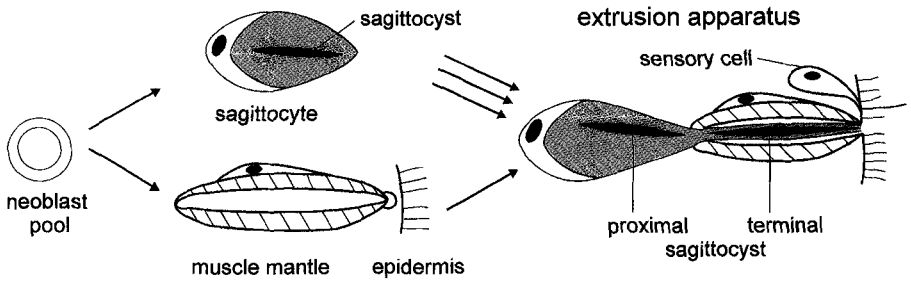

Fig. 6. Scheme of differentiation of sagittocyte and muscle mantle from the neoblast pool. Description in the text.

red rhabdoid glands only dorsally. Paired follicular testes that fuse caudally. Ovaries also paired and follicular. Eggs extremely transparent. Bursa with short epidermal vagina and two special closing cells at the transition between the two and with single mouthpiece ~55 μm long. Copulatory organ distal, with large, swollen gland cell endings and a simple proximal seminal vesicle. Specific cyanophilic glands are associated with the vagina and the epidermis of the sac-like antrum. Sperms 140 μm long.

Type material

Holotype: sagittal section deposited at Naturhistorisches Museum Wien, number: NHMW ZOOEV M 4295.

Paratype: cross section deposited at Naturhistorisches Museum Wien, number: NHMW ZOOEV M 4296.

Discussion

A new model of sagittocyst function and structure

From our observations on morphological and functional features of sagittocysts in *Symsagittifera corsicae* (this work) and *Convolutriloba longifissura* (see Gschwentner et al. 1999) and the differentiation of sagittocytes from BrdU-labeled neoblasts (bromodeoxyuridine, see Gschwentner et al. 2001), we have generated a model for the development and function of sagittocysts (Fig. 6). Both the sagittocyte and the cells of the muscle mantle have their source in the neoblast pool of the animal. Gschwentner et al. (2001) have shown that BrdU-labeled neoblasts differentiate into sagittocytes within 3 days. Generally, for muscle cells, differentiation was observed within 9 days. The sagittocytes must actively connect to the proximal end of the muscle mantle. This connection was observed in semi-thin sectioned material of *C. longifissura* and *S. corsicae* and was also visible in TEM material of *C. longifissura* (see Gschwentner et al. 1999).

Roundish sagittocytes with very small developing sagittocysts separate from the muscle mantle can be observed, especially in live preparations. The mature sagittocysts finally move to the distal neck-region of the gland that projects through the muscle mantle. The latter is always connected to the epidermis and seems to be associated with small sensory cells. Contraction of the muscle mantle leads to a very fast expulsion of the terminal sagittocyst. New sagittocysts are produced in one and the same sagittocyte, and thus the muscle mantle is possibly reloaded with another sagittocyst. In addition, new sagittocytes seem to be able to attach to the same muscle mantle. In this process, the new sagittocyte must replace the one which was previously attached to the muscle mantle. Thus, the muscle mantle functions like a gun that can be reloaded, either when additional sagittocysts are produced within the same sagittocyte or when a new sagittocyte connects to the mantle.

In most of the species of the family Sagittiferidae, sagittocysts are distributed at the ventral side of the body, often in the proximity of genital openings (Graff 1891; Kato 1951; Ivanov 1952; Beklemishev 1957; Marcus 1957; Yamasu 1982; Kostenko & Mamkaev 1990a,b; Mamkaev & Kostenko 1991; Yamasu 1991; Kozloff 1998; this work). In these species the occurrence of sagittocysts is restricted to mature, sexually reproducing animals, which indicates a relationship of sagittocysts to sexual behavior. We assume that especially the sagittocysts around the male genital pore are used to perforate the epidermis of the partner, which makes it easier for sperms to find a way into the partner's body. Another possible mechanism could be the transfer of pheromones. Although nothing is known about the existence of stimulating substances in acoels we cannot exclude this possibility.

In contrast, the genus *Convolutriloba* shows both sexual and asexual reproduction with a clear trend to asexuality. The distribution of sagittocysts in this genus is diverse. While *C. retrogemma* shows sagittocysts around the male genital opening (J. Hendelberg, pers. comm.), the sagittocysts in *C. hastifera* and *C. longifissura* are absent from this region and occur on the dorsal surface and along an arc on the anterior ventral margin (Winsor 1990; Gschwentner et al. 1999). It is more likely that *C. hastifera* and *C. longifissura* use sagittocysts for defense and prey capture than for sexual behavior.

New terminology

The terms spiral muscle, muscle mantle, and muscle layer are used synonymously in the literature for the musculature surrounding sagittocysts (Yamasu 1991; Gschwentner et al. 1999). Whereas the muscle mantle in *C. longifissura* and *S. corsicae* shows a spiral profile (Gschwentner et al. 1999, this paper), the one of *Praesagittifera shikoki* appears circular (Yamasu 1991). Should further studies provide new evidence that *P. shikoki* actually has more than one muscle cell in the extrusion apparatus (a fact not known from the present literature), the single spiral muscle of *C. longifissura* and *S. corsicae* would appear definitely as the derived character stage. Without that knowledge the reading direction of the morphological sequence of the structure of the muscle mantle seems possible also from *C. longifissura* and *S. corsicae* to *P. shikoki.*

The sagittocyst-producing gland cell, or sagittocyte (also called sagittocytoblast: Winsor 1990) may simultaneously contain both a "proximal sagittocyst" located in the sagittocyte cell body and a "terminal sagittocyst" located in the narrow neck of the sagittocyte within the muscle mantle (Fig. 6). Our model suggests that the whole complex micro-organ be called the "extrusion apparatus," which consists of a sagittocyte partly surrounded by muscle mantle and filled with sagittocysts. Adjacent sensory cells could be considered an integral part of the extrusion apparatus.

Systematic discussion

The family Sagittiferidae MAMKAEV & KOSTENKO 1991 can be defined by a special male copulatory organ (saccate antrum) and includes species without sagittocysts (genus *Praesagittifera*), without algal symbionts (genus *Sagittifera*), and with cloning by fission (genus *Convolutriloba*). The genus *Symsagittifera* is defined by a ciliated male saccate antrum, and by the occurrence of sagittocysts and algal symbionts; it does not show asexual reproduction. The photosynthetic activity of the algal symbionts in this genus may be essential for survival of the worms.

Symsagittifera corsicae differs only slightly from two other species described in the genus *Symsagittifera, S. psammophila* (BEKLEMISHEV 1957) and *S. poenicea* KOZLOFF 1998. The original drawing by Beklemishev (1957) as well as the drawings by Kostenko & Mamkaev (1990a) show a caudal distribution of sagittocysts in *S. psammophila* and a lateral distribution along two bands up to the level of the statocyst. No size classes of sagittocysts could be distinguished on the basis of these drawings. In contrast to these drawings, we never observed individuals of *S. corsicae* with sagittocysts or muscle mantles around the mouth opening or in front of it. While Beklemishev (1957) did not specify the size of mature individuals of *S. psammophila,* Kostenko & Mamkaev (1990a) found specimens up to 1400 μm in length. The largest individuals of *S. corsicae* are appreciably smaller, measuring 850 μm in length.

S. poenicea (see Kozloff 1998) is very similar to *S. corsicae* in habitus, size (largest specimens of *S. poenicea* measured 940 μm), and distribution of algal symbionts and sagittocysts, but there are clear differences in their genital apparatus. In *S. poenicea,* the male genital opening leads directly into the glandular region of the penial organ and does not match a saccate antrum by definition. *S. poenicea* also lacks a noticeable orifice of the bursa, whereas *S. corsicae* showed such an orifice, but no opening of the male antrum. These discrepancies indicate that *S. poenicea* and *S. corsicae* are two separated species. It is possible that the discrepancies of the genital structures between *S. poenicea* and *S. corsicae* reflect different developmental stages, although we never found transitional stages. Were such stages formed, *S. corsicae* would become a junior synonym of *S. poenicea.*

Photographic images proved valuable for comparing characters of the new species to other members of the genus. While neither the description nor the line drawing of *S. poenicea* suggests two size classes of sagittocysts, it is evident from the photomicrograph shown for *S. poenicea* that there are the same two size classes as in *S. corsicae.* Interference and phase-contrast microscopy as well as semi-thin sectioning help to distinguish characters of closely related species. Another useful character for taxonomic determination in acoels would be the labeling and comparison of the entire body-wall musculature. Observations on the body-wall musculature after a phalloidin labeling identified *S. corsicae* to be a member of the "Convolutida" muscle-type corresponding to the description of Hooge (2001). Body-wall muscle patterns may be useful to discriminate families within the Acoela (Hooge & Tyler 1999). However, there are not enough data within the genus *Symsagittifera* to distinguish the species by way of muscle patterns.

Sequence comparisons of the 18S rDNA between *S. corsicae* and *S. psammophila* demonstrated 99.72% sequence homology except for a highly variable region in the first part of the gene. This 370 bp long region shows only 69.28% sequence homology, while *S. roscoffensis* lacks such a variable region and exhibits 98.00% sequence homology to *S. corsicae* for the complete length of the gene. Because no other 18S rDNA sequences are available for the family Sagittiferidae, we cannot explain the occurrence of the variable region between *S. corsicae* and *S. psammophila.*

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, & Lipman DJ 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402.

Baguñà J 1981. Planarian neoblast. Nature 290: 14–15.

Baguñà J, Saló E, Romero R, Garcia-Fernàndez J, Bueno D, Muñoz-Marmol AM, Bayascas-Ramirez JR, & Casali A 1994. Regeneration and pattern formation in planarians: cells, molecules, and genes. Zool. Sci. 11: 781–795.

Beklemishev WN 1957. *Convoluta psammophila* sp. n. and the tendency toward juvenile oligomerization of cellular elements in Turbellaria Acoela. [In Russian] Tr. Leningrad. obtch. estestvoisp. 73(4): 5–14.

Bruford MW, Hanotte O, Brookfield JFY, & Burke T 1998. Multilocus and single-locus DNA fingerprinting. In: Molecular Genetic Analysis of Populations. Hoelzel AR, ed., pp. 287–336. Oxford University Press, Oxford, New York, Tokyo.

Carranza S, Giribet G, Ribera C, Baguñà J, & Riutort M 1996. Evidence that two types of 18S rDNA coexist in the genome of *Dugesia (Schmidtea) mediterranea* (Platyhelminthes, Turbellaria, Tricladida). Mol. Biol. Evol. 13: 824–832.

Ehlers U 1985. Das Phylogenetische System der Plathelminthes. Gustav Fischer, Stuttgart.

Geddes P 1879. Observation on the physiology and histology of *Convoluta schultzii.* Proc. Roy. Soc. London 1879: 449–457. [cited by L von Graff 1891]

Graff L von 1891. Die Organisation der Turbellaria Acoela. W. Engelmann, Leipzig.

Gschwentner R, Ladurner P, Salvenmoser W, Rieger R, & Tyler S 1999. Fine-structure and evolutionary significance of sagittocysts of *Convolutriloba longifissura* (Acoela, Platyhelminthes). Invertebr. Biol. 118: 332–345.

Gschwentner R, Ladurner P, Nimeth K, & Rieger R 2001. Stem cells in a basal bilaterian: S-phase and mitotic cells in *Convolutriloba longifissura* (Acoela, Platyhelminthes). Cell Tissue Res. 304: 401–408.

Hooge MD 2001. Evolution of body-wall musculature in the Platyhelminthes (Acoelomorpha, Catenulida, Rhabditophora). J. Morph. 249: 171–194.

Hooge MD & Tyler S 1999. Body-wall musculature of *Praeconvoluta tornuva* n. sp. (Acoela, Platyhelminthes) and the use of muscle patterns in taxonomy. Invertebr. Biol. 118(1): 8–17.

——— 2001. Interstitial acoels (Platyhelminthes: Acoela) from Bermuda. Proc. Biol. Soc. Wash. 114(2): 412–424.

Hori I 1997. Cytological approach to morphogenesis in the planarian blastema. II. The effect of neuropeptides. J. Submicros. Cytol. Pathol. 29: 91–97.

Ivanov AV 1952. Turbellaria (Acoela) from the southern coast of Sakhalin. [In Russian] Trudy. Inst. Zool. Acad. Sci. SSSR 12: 40–132.

Kato K 1951. *Convoluta,* an acoel turbellarian, destroyed the edible clam. Rep. Res. Inst. Nat. Res. Nos. 19–21: 64–67.

Kostenko AG & Mamkaev YuV 1990a. The place of green convoluts in the system of Acoela. Turbellaria. 1. *Simsagittifera* gen. n. Zool. Zhurn. (Moscow) 69(6): 11–21. [In Russian, English summary]

——— 1990b. The place of green convoluts in the system of Acoela. Turbellaria. 2. *Simsagittifera* gen. n. Zool. Zhurn. (Moscow) 69(7): 5–16. [In Russian, English summary]

Kozloff EN 1998. Acoel flatworms (Platyhelminthes) from the ancient punic ports of Carthage, at Salambo, Tunisia. Cah. Biol. Mar. 39: 15–28.

Littlewood DTJ, Rohde K, & Clough KA 1999. The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules. Biol. J. Linn. Soc. 66: 75–114.

Marcus E 1957. On Turbellaria. Ann. Acad. Ciencias 29: 198–191.

Mamkaev YuV & Kostenko AG 1991. On the phylogenetic significance of sagittocysts and copulatory organs in acoel turbellarians. Hydrobiologia 227: 307–314.

Palmberg I 1990. Stem cells in microturbellarians. An autoradiographic and immunocytochemical study. Protoplasma 158: 109–120.

Richardson KC, Jarret L, & Finke EH 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain. Technol. 35: 313–325.

Rieger R, Legniti A, Ladurner P, Reiter D, Asch E, Salvenmoser W, Schürmann W, & Peter R 1999. Ultrastructure of neoblasts in microturbellaria: significance for understanding stem cells in free-living Platyhelminthes. Invertebr. Reprod. Dev. 35: 127–140.

Smith JS, Tyler S, Thomas MB, & Rieger RM 1982. The nature of turbellarian rhabdites: phylogenetic implications. Trans. Am. Microsc. Soc. 101: 209–228.

Spurr AR 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31–43.

Walsh PS, Metzger DA, & Higushi R 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506–513.

Winsor L 1990. Marine Turbellaria (Acoela) from North Queensland. Mem. Queensland Mus. 28(2): 785–800.

Yamasu T 1982. Five new species of acoel flat worms from Japan. Galaxea 1: 29–43.

——— 1991. Fine structure and function of ocelli and sagittocysts of acoel flatworms. Hydrobiologia 227: 273–282.