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## On the Anatomy of *Gnosonesima mediterranea* sp. n. from Corsica (Plathelminthes, Lecithoepitheliata) with Special Emphasis on its Genito-Intestinal Connections

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### Abstract

*Gnosonesima mediterranea* sp. n. from algae in the Bay of Calvi (Corsica) is described. It is the first record of a *Gnosonesima*-species from the Mediterranean.

The pharynx and the genital organs are extensively described at the light microscopic level from 1  $\mu\text{m}$  Epon sections. The male "accessory organ" is revealed to be an andro-genito-intestinal connection with a high similarity with the female genito-intestinal connection. The taxonomic position of the Lecithoepitheliata is discussed.

### A. Introduction

So far five species within the genus *Gnosonesima* are known (see KARLING 1968; SCHOCKAERT & MARTENS 1985). While investigating the turbellarians of the Bay of Calvi (Corsica), one of us (P.M.M.) had the opportunity to collect several

individuals of a new species, *G. mediterranea* sp. n., some of which were fixed for later study by the electron microscope and embedded in epon. One of these was serially sectioned in alternating 1 µm and ultrathin sections. In this first contribution on *G. mediterranea* a description is given on the light microscopic level, based on the 1 µm sections which have the advantage of the high-quality E. M. fixation and of being very thin (a disadvantage however during reconstruction).

All representatives of the genus *Gnosonesima* show a very high degree of similarity in their organisation. Our description only gives detailed information where we consider this necessary or relevant, as a confirmation of or in comparison with KARLING's (1968) description of *G. brattstroemi* Karling, 1968 and his redescriptions of *G. borealis* (Westblad, 1952) and *G. antarctica* Reisinger, 1926. It can be foreseen that later on further data will be given on selected structures at the electron microscopic level.

## B. Material and Methods

One animal studied alive (22. 10. 82) and conserved as whole mount with lactophenol (Holotype). Two animals (30. 06. 82) fixed for E. M. with 2 % glutaraldehyde, postfixed with 2 % OsO<sub>4</sub>, both in cacodylate buffer, and embedded in Epon. One of them serially sectioned in 1 µm sections (paratype) alternating with ultrathin sections on a Reichert Ultracut.

The 1 µm sections were stained with thionine-methylenebleu. The typematerial is deposited in the zoological collection at the Limburg Universitair Centrum, Diepenbeek, Belgium. Samples were taken by SCUBA-diving. The algae were carefully picked and put in a plastic bag. In the laboratory the algae were vigorously stirred and the seawater with animals poured over a 38 µm mesh sieve. The sieve was examined under a stereo microscope and the animals picked out.

Figures without a scale are free-hand drawings, those with a scale are made with the camera lucida.

## C. Description

Locality: Corsica (France), Bay of Calvi. Pointe de la Revellata, on algae at 12 m depth (22. 10. 82) (Typelocality) and on 25 m depth (30. 06. 82).

The fusiform animals, 3–4 mm long and 0.7 mm broad, move slowly about on the algae. They are light-brown and transparent but without pigment in the

epidermis. A group of 3–4 eyespots can be seen at each side, situated rather laterally some distance backwards of the anterior tip (Fig. 1 B).

The epidermis is a cuboidal cellular epithelium with intra-epithelial spherical to lobate nuclei. Dorsally the apical part of the epidermis cells is packed with large corpuscles of varying shapes (rhabdites?), some of which protrude through the surface. The epidermis is about 5 µm high at the dorsal side, the basement membrane 1.5 µm thick and the cilia 2–4 µm long. At the ventral side the epidermis is 6–10 µm thick, the basement membrane 5 µm and cilia 5–7 µm long. So contrary to *G. brattstroemi* Karling, 1968 (KARLING 1968, p. 84) the body ciliation in *G. mediterranea* is longer at the ventral side than dorsally. Several glands, the identity of which has yet to be established, are associated with the epidermis. The inner longitudinal muscle layer is highly developed at the ventral side; most of the fibres attach to the epidermis in a poorly delimited zone just behind the female pore where the basement membrane is extremely thin and many glands are present. This "disc" was, however, not obvious in the living animal.

No sensory pits or bristles were seen in the living animal. Some deep invaginations between (or in) epidermal cells may be considered as the sensory pits. However, no relevant details can be given.

As to the eyes, we can confirm KARLING's (1968) observations on *G. brattstroemi*: they apparently consist of an intraepidermal cell with a cup shaped mass of pigment granules at the base. A report on further details or comments on similarities with e. g. the epidermal eyes of Polycladida (LANFRANCHI et al. 1981; RUPPERT 1978) must be postponed until E. M. data become available.

The pharynx fills the anterior third of the animal (Fig. 1 B). It is a coniform bulb with a small tube in its posterior part (the so-called "internal pharynx"). The pharynx, reconstructed in Fig. 3, is clearly retracted (compare to KARLING 1968, fig. 16 and 17).

The pharynx cavity has weak circular muscles around its anterior part and fine outer longitudinal muscles. A rather weak sphincter is developed around the terminal mouth. The epithelium of the cavity becomes gradually lower near the junction with the cone epithelium and contains several nuclei over its entire length. The epithelium of the cone near the junction is very flattened and has nuclei. More anteriorly this cone epithelium is thick, very vacuolated, devoid of nuclei and filled with spherical coarse secretion granules. The cytoplasm of the epithelium at the apex (in Fig. 3 A partly retracted into the pharynx lumen) is vacuolated and without nuclei, the secretion fine and rodshaped. The secretion in the cone epithelium is evidently produced by gland cells outside the bulb. Sections through the necks can be found between the cone retractor muscles.

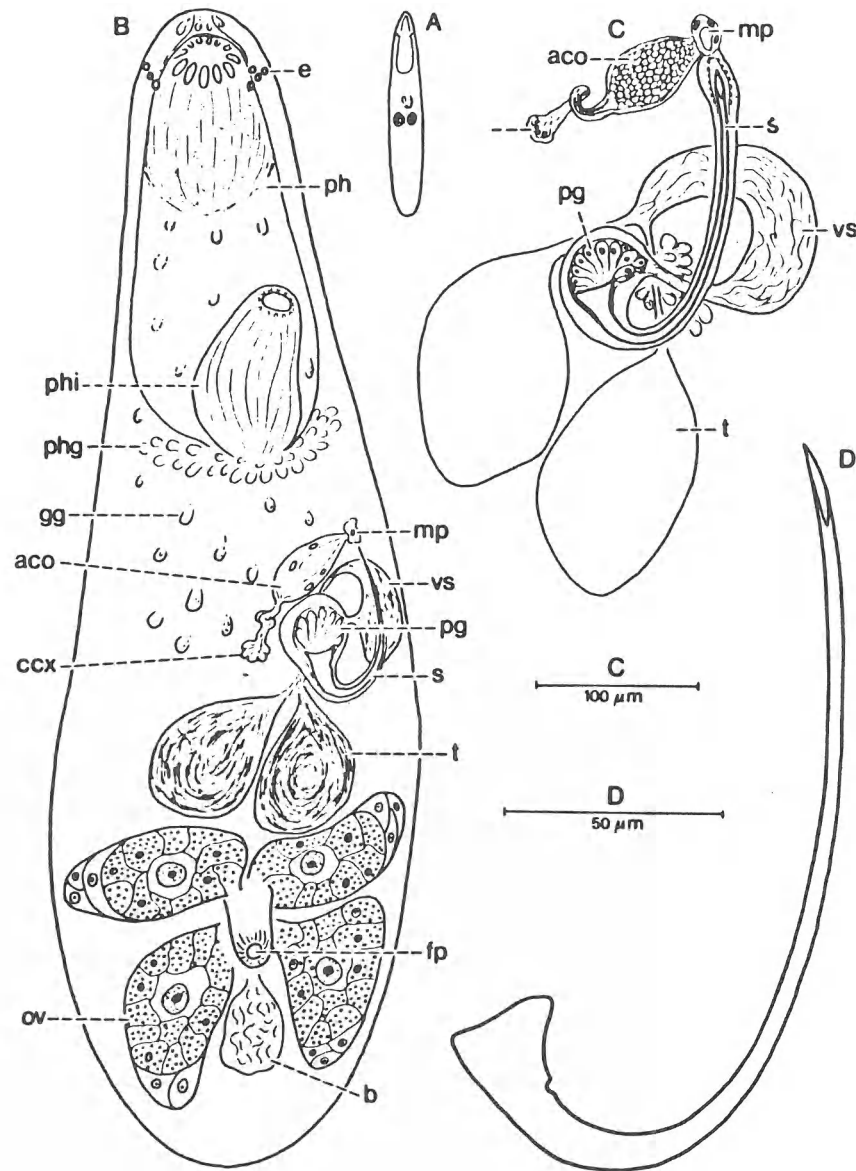


Fig. 1. A. Habitus. B. General organisation in living animal (obliquely from the dorsal side). C. Male genital organs. D. Stylet.

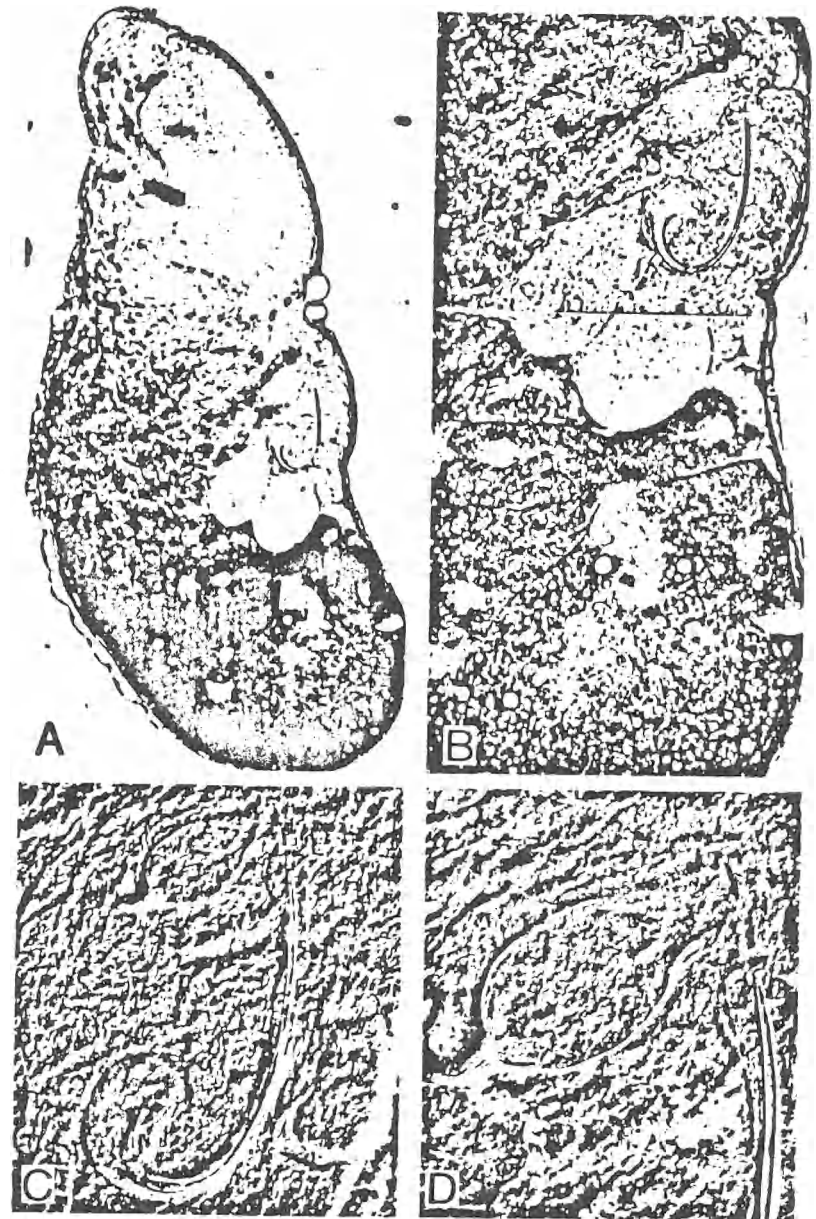


Fig. 2. A. A slightly squeezed living animal. B. Organisation of the genital organs. C. Male genital organs. D. Male accessory organ.

Where these gland necks actually enter the bulb could not be established; most probably at the nodus but possibly also near the junction.

The epithelium of the pharynx lumen is devoid of secretion in its anterior half, with some nuclei about half way. Within the posterior part of the lumen three compartments can be recognized. In the sectioned material the first compartment forms a cone shaped protrusion into the anterior part of the lumen. This cone is thus surrounded by a circular fold (originated by the contraction of fine retractor muscles) in the bottom of which some glands discharge a very fine secretion. The internal canal of this protrusion is surrounded by thick circular muscles. The epithelium is not clearly visible, but here glands discharge a relatively coarse secretion, possibly of different kinds. The next compartment is globular, surrounded mainly by longitudinal muscles while several dilatator muscles attach on its wall. The epithelium is high and nucleated, covered with a thick and intensively stained layer, most probably microvilli. No secretion is seen here. In the most posterior compartment of the lumen, the epithelium is similar to the one in the previous compartment, but has a highly folded basement lamina, due to the contraction of the more anteriorly attaching retractors and some weak circular and dilatator muscles.

The three posterior compartments of the pharynx lumen form the pharynx-within-the-pharynx, (as seen in the living animal) when it is fully extended and protruded into the anterior part of the lumen. It is surrounded by the many necks of several kinds of glands discharging into the epithelium and whose cell bodies are outside the pharynx bulb. This bulb contains thick cone retractor muscles almost parallel to each other, though some weaker oblique muscles can be seen running from the nodus to the external cone wall. Under the cone epithelium with secretion, an unusually thick circular muscle layer is present. Posteriorly, the pharynx bulb is clearly delimited by a diaphragm consisting of muscle fibers. Some of those continue in the longitudinal muscles of the pharynx-cavity. At the junction a strong circular muscle is present and some dilators attach.

The topography of the genital organs as seen in a squeezed individual is shown in Figs. 1 and 2, the reconstruction from serial section in Fig. 4 A. The four "germovitellaries" seem separate in the living animal but in sectioned material those at the left side and those at the right side are broadly interconnected. Each germovitellary contains one large "follicle". The follicle cells (vitellocytes) are filled with (yolk) granules and some similar granules can be found within the oocyte.

From the left and right pairs of germovitellaries a narrow oviduct (the right one much longer than the left one) leads to the distal part of the bursa or seminal receptacle. This receptacle is lined with a nucleated epithelium and is slightly

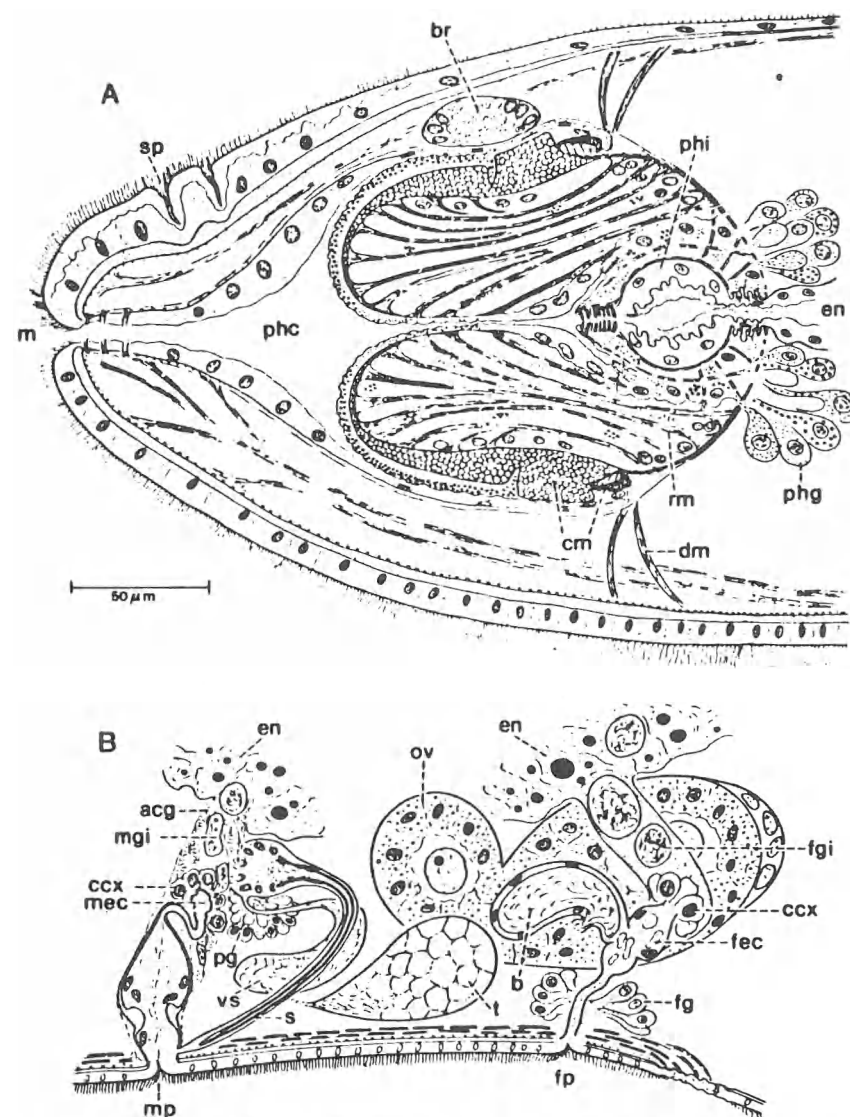


Fig. 3. A. Reconstruction of the pharynx, from serial sagittal 1 µm epon sections. B. Reconstruction of the genital organs, from serial sections (seen from the left).

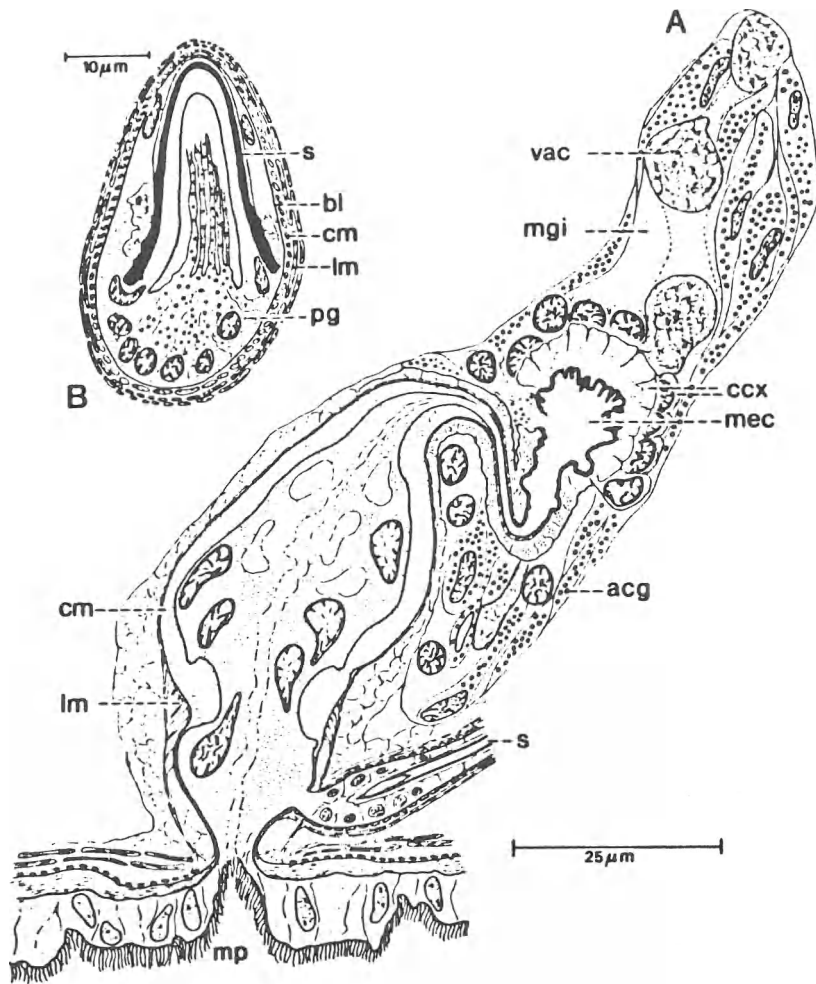


Fig. 4. A. The male accessory organ with the male genito-intestinal tissue, from serial sections. B. Section through the copulatory bulb with the stylet at its proximal opening.

muscular. It contains sperm without any trace of degeneration. The receptacle enters the female duct (slightly broadened here) with a high vacuolated and nucleated epithelium. The female duct narrows towards the female pore and numerous glands enter the duct (regarded as shell glands by KARLING 1968, p. 91).

Proximally to the entrance of the receptacle into the female duct the latter is enlarged, forming what can be called the ending cavity (fec). This ending cavity

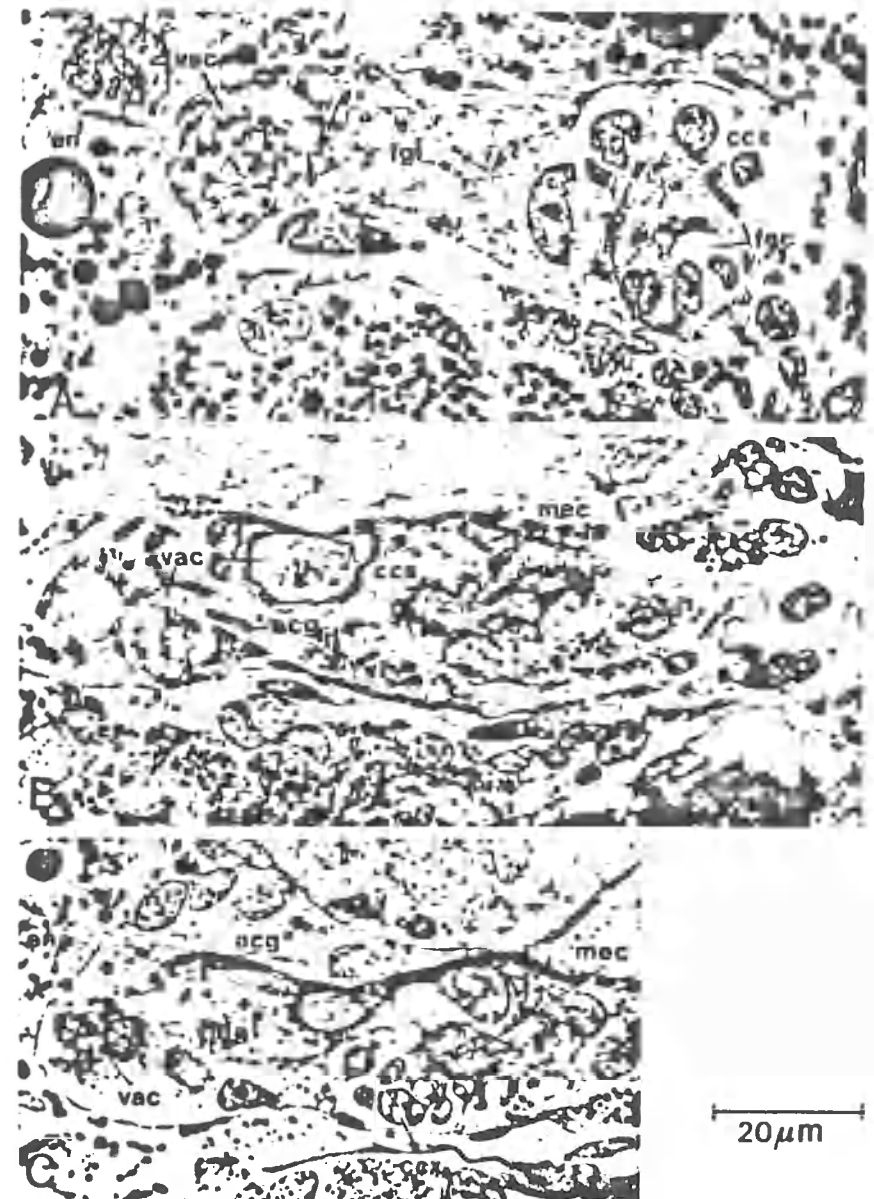


Fig. 5. A. Section through the female genito-intestinal connection with its cellular complex, female ending cavity and genito-intestinal tissue. B-C. Sections through parts of the male accessory organ with its duct (in B), the male ending cavity, the cellular complex, (with nuclei in C) and the genito-intestinal tissue.

is surrounded by a cellular complex: a girdle of cells with a hyaline cytoplasm and large spherical nuclei. The cellular complex (and so the ending cavity) is enclosed in a parenchymal tissue that is neatly delimited from the surrounding parenchyma though devoid of a proper wall or muscles. Dorsally this parenchymal tissue is embedded in the gastrodermis and constitutes the genito-intestinal connection. Inside the genito-intestinal tissue large vacuoles occur with what is most probably degenerating alien sperm. Such vacuoles have also been found in the cells of the gastrodermis.

The vasa deferentia leading from the ventrolateral pair of testes fuse to form a bent pyriform and slightly muscular seminal vesicle. The seminal duct from this vesicle enters the small copulatory bulb (or prostate vesicle) at its ventral side. The prostate secretion is produced by outside glands, but numerous nuclei are also to be found inside the bulb. From the prostate vesicle originates the stylet, 240  $\mu\text{m}$  long, 25  $\mu\text{m}$  broad at its proximal opening and 4  $\mu\text{m}$  broad distally. The oblique terminal opening is 21  $\mu\text{m}$  long (all measurements on the holotype). The stylet bends over 180° in its proximal part and is slightly curved in its distal part. From sections through the proximal funnel it can be derived, even on the light microscopic level, that the stylet is an intracellular formation (Fig. 4 B).

As in the other representatives of the genus, an accessory organ joins the male canal near the porus. In a living *G. mediterranea* this organ is recognized as a pyriform "reservoir" proximally leading to a narrow canal that ends in a rosette of glandlike cells (see Fig. 1 A, C). In the sections the "reservoir" appears to be surrounded by a thick layer of circular muscles (forming a sphincter distally) and thin longitudinal muscles. Its lumen is lined by a high and highly vacuolated epithelium with dark stained elongate nuclei. No secretion was found. Proximally there is a gradual transition to the canal that opens into an end cavity (mec). In the canal the epithelium gradually becomes thinner and fades. In the ending cavity no distinct epithelium, but a dark structureless material was visible. The basement lamina is thick, intensively stained and strongly folded, surrounded by some small muscle fibers. The cavity is surrounded by a complex of cells with a hyaline cytoplasm and large spherical nuclei; this complex may be the insunk parts of the epithelium cells. It is this cellular complex that is seen as a rosette of glandlike elements in the living animal. The ending cavity has a narrow opening through which the lumen communicates with an elongated parenchymal tissue that is proximally embedded in the gastrodermis. This tissue is neatly separated from its surroundings, and within there is an irregular lumen of interconnected vacuoles which contain a degenerating material of unknown nature. Numerous gland cells and gland ducts can be seen within the parenchymal tissue, but their site of discharge could not be established. Obviously the whole accessory organ thus constitutes a genito-intestinal connection associated with the male-copula-

tory organ, and thus is comparable in many respects to that of the female system (see discussion below).

## D. Discussion

*G. mediterranea* is the first species described from the Mediterranean, but representatives of the genus have been observed by P. Ax at Napels (Ax, pers. comm.). Though the *Gnosonesima*-species are rarely encountered, the genus seems nevertheless widely distributed (Antarctic: REISINGER 1926; N. Atlantic: WESTBLAD 1952, KARLING 1968; E. Africa: SCHOCKAERT & MARTENS 1985). The new species can easily be recognized by the size and shape of the stylet (among other characteristics).

In the pharynx the main differences between the species are differences in ciliation, the location of the nuclei of the epithelia, the degree of differentiation in the posterior part of the lumen . . . (see KARLING 1968, p. 85–90, 92–93, 97). According to KARLING (op. cit. p. 90) the posterior diaphragm "seems to be devoid of muscles" (in *G. brattstroemi*). In *G. mediterranea* muscles (with different orientation) are present in this diaphragm and in the pharynx of *G. borealis* as well (as KARLING's fig. 29 suggests).

KARLING postulates that the *Gnosonesima*-pharynx has originated from a plicatus-like "simple" pharynx (= the "internal" pharynx) around which a cone has developed (for details: see KARLING 1968, p. 100 and fig. 46–48; 1974, p. 10). We disagree with that. The pharynx-within-the-pharynx is nothing but a peculiar differentiation of the posterior part of the pharynx lumen that can be protruded in the anterior part by a synergic action of the different muscles. Two other peculiarities differentiate the *Gnosonesima*-pharynx from other pharynxes (bulbous or plicate): the secretory nature of the external pharyngeal epithelium (conex epithelium) and the arrangement of the internal muscles which evidently function as retractors of the pharynx lips (the cone). In our opinion these differences, however, spectacular they may be, do not support the hypothesis that this type of pharynx should have evolved "independently" from the other types of composite pharynxes.

We consider the whole pharynx as a pharynx bulbosus (or variabilis), i. e. a pharynx the internal parenchyma of which is separated from the body parenchyma and we are inclined to believe that this pharynx in its whole has evolved from a pharynx plicatus by the development of a septum or diaphragm (see REISINGER 1926; AX 1961, 1963; KARLING 1963), possibly along an independent line.

As far as the female genital organs are concerned it is now clear that all species

have a genito-intestinal connection with a "parenchymal bursa", interposed between the female duct and the gastrodermis (only for *G. brattstroemi* KARLING 1968, p. 92 expresses some doubt) most probably to eliminate (and digest) excess of alien sperm.

The resemblance of the male accessory organ in *G. mediterranea* with the female genito-intestinal connection is striking in many respects: a proximal ending cavity surrounded by a cellular complex followed by a (resorbing) parenchymal tissue (genito-intestinal tissue) partly embedded in the gastrodermis. In the male as well as in the female parenchymal tissue large vacuoles with degenerating material are present. The content of these vacuoles has yet to be identified but looks very much like degenerating sperm, at least in the female system. In living specimens the male accessory organ is only seen up to the rosettelike cellular complex (Fig. 1 B, C; SCHOCKAERT & MARTENS 1985, fig. 1 A) and in paraffin sections the parenchymal tissue is easily overlooked (the most proximal part of the organ in *G. borealis* may perhaps be interpreted as the parenchymal tissue (see KARLING 1968, fig. 34, 39)).

REISINGER (1926) regarded the male accessory organ as a bursa copulatrix, a function denied by KARLING and WESTBLAD. As in the other species studied on sections (see KARLING 1968) no connection whatsoever with the female system has been found in *G. mediterranea*. At the present state of our knowledge speculations on the function of this organ or on homologies with any structure of other taxa (e. g. the prepenial bursa of the Monocelididae, REISINGER 1926, p. 436) are premature. Nevertheless our observations suggest that it might have a resorbing function and perhaps a glandular function of some kind.

Some comments can be given on systematics. From our discussion of the pharynx it appears that we do not consider the coniform pharynx as "a type of its own". So the pharynx does not provide an argument for the diphyletic origin of the Lecithoepitheliata (KARLING 1968, p. 105, 1974, p. 10) (which does not mean that we exclude the possibility of a diphyletism!). On the other hand KARLING 1974, p. 10) uses the idea of an independent origin of the pharynx in *Gnosonesima* and of *Prorhynchus* (from a pharynx simplex) as the main argument "to let the Lecithoepitheliata branch of from the archoophoran stem". In our opinion at least this argument is invalid and the precise phylogenetic position of the Lecithoepitheliata is left open. We can hope that new data e. g. on the ultrastructure of eyes and other sense organs, ovaries, rhabdites... may help us to elucidate the taxonomic position of *Gnosonesima* and the function of the enigmatic male accessory organ.

## Abbreviations in the Figures

acg	glands of the accessory organ	m	mouth
aco	accessory organ	mec	male ending cavity
b	bursa	mgj	male genito-intestinal tissue
bl	basal lamina	mp	male pore
br	brain	ov	ovary
ccx	cellular complex	pg	prostate glands
cm	circular muscles	ph	pharynx
dm	dilatator muscles	phc	pharynx cavity
e	eye	phg	pharynx glands
en	intestine	phi	"internal" pharynx
fec	female ending cavity	rm	retractor muscles
fg	female glands	s	stylet
fgi	female genito-intestinal tissue	sp	sensory pit
fp	female pore	t	testes
gg	glands	vac	vacuole
lm	longitudinal muscles	vs	seminal vesicle

## Acknowledgements

The first author is much indebted to Dr. D. Bay, adjunct-Director of Stareso (Station de Recherches Sous-marines et Océanographiques in Corsica of the Liège University), his wife and staff for assistance and research facilities. He also thanks Dr. L. Snoecks for his valuable help.

Ms. M. Uytterhaegen is acknowledged for correcting the English of the manuscript, Miss E. Plum for preparing the section, Mr. M. Withofs for printing the photographs and Mrs. H. Zurings for typing the manuscript.

## Literature

- AX, P. (1961): Verwandtschaftsbeziehungen und Phylogenie der Turbellarien. *Ergebn. Biol.* 24, 1-68.
- (1963): Relationships and phylogeny of the Turbellaria. In: *The lower Metazoa. Comparative biology and phylogeny.* Ed.: Dougherty E. C. University of California Press, Berkeley, Los Angeles, 191-224.
- KARLING, T. G. (1963): Some evolutionary trends in turbellarian morphology. In: *The lower Metazoa. Comparative biology and phylogeny.* Ed.: Dougherty E. C. University of California Press, Berkeley, Los Angeles, 225-233.
- (1968): On the genus *Gnosonesima* Reisinger (Turbellaria). *Sarsia* 33, 81-108.
- (1974): On the anatomy and affinities of the turbellarian orders. In: *Biology of Turbellaria.* Eds.: Riser, N. W. & M. P. Morse. New York, McGraw-Hill, 1-16.
- LANFRANCHI, A., C. BEDINI & E. FERRERO (1981): The ultrastructure of the eyes in larval and adult polyclads (Turbellaria). *Hydrobiologia* 84, 267-275.
- REISINGER, E. (1926): Zur Turbellarienfauna der Antarktis. *Dt. Südpol.-Exped. 18 Zool.* 10, 415-462.
- RUPPERT, E. E. (1978): A review of metamorphosis of turbellarian larvae. In: *Settlement and metamorphosis of marine invertebrate larvae.* Eds.: Chia & Rice. Elsevier North-Holland Biomedical Press, 65-81.

- SCHOCKAERT, E. R. & P. M. MARTENS (1985): Turbellaria from Somalia III. Lecithoepitheliata and Typhloplanoida. *Monitore zool. Ital. (N. S.) Suppl.* 20, 27-41.
- WESTBLAD, E. (1952): Some new "Alloecoels" (Turbellaria) from the Scandinavian west coast. *Univ. Bergen Arb.* 1952, Naturv. rekke 7, 1-27.