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CONTRAMYZOSTOMA BIALATUM (ANNELIDA: MYZOSTOMIDA), A NEW GENUS AND SPECIES OF PARASITIC MYZOSTOME INFESTING COMATULID CRINOIDS

I. Eeckhaut and **M. Jangoux**

ABSTRACT. - The external and internal morphology of a new genus and species of parasitic myzostome is described from the territorial waters of Singapore. *Contramyzostoma bialatum*, new genus and new species, infests the arms of the crinoid *Comaster gracilis* where it occurs in burrow-like integumental cavities. *Contramyzostoma bialatum* is characterized by a dorsal body field bearing all the external appendages (viz., five pairs of parapodia, four pairs of lateral organs, two pairs of cirri, and an ano-genital cone) and by two large wing-like body extensions. The digestive system comprises a pharynx included in an introvert, several salivary gland cells, a stomach from which originate two loop-shaped digestive tubules, and an intestine. All individuals examined were females possessing two ovaries and a branched oviduct. The infestation by *C. bialatum* produces significant modifications of the host epidermis and dermis.

INTRODUCTION

The Myzostomida are represented by about 140 species of obligate symbionts of echinoderms which have unsettled taxonomic affinities. Most of them look superficially like flatworms based on their dorsoventrally flattened body shape, but their parapodia and cirri suggest that they are close to Polychaeta (see Stummer-Traunfels, 1926; Jägersten, 1940; Prenant, 1959).

The last significant taxonomic paper dealing with the higher classification of the Myzostomida was by Jägersten (1940) who divided them into two orders and seven monogeneric families. Out of these seven families, only the Cystimyzostomatidae includes individuals that induce tissular abnormalities, i.e., galls and cysts, on their crinoid hosts (Jägersten, 1940). Gallicolous species reside in the tissue of crinoid arms or pinnules where they induce conspicious deformations of the original host ossicles which considerably enlarge

I. Eeckhaut - Laboratoire de Biologie marine, Université de Mons-Hainaut, 19 ave. Maistriau, B-7000 Mons, Belgium. **M. Jangoux** - Laboratoire de Biologie marine (C.P. 160/15), Université Libre de Bruxelles, 50 ave. F. D. Roosevelt, B-1050 Bruxelles, Belgium.

to finally surround the myzostome (Graff, 1884; Jangoux, 1990). Cysticolous species live in the tissue of crinoids where they do not deform the original skeleton but sometimes induce the formation of minute skeletal plates reinforcing the walls of their shelter (Jangoux, 1990). The present paper describes a cysticolous myzostome, *Contramyzostoma bialatum*, new genus and species, which infests the arms of the comatulid crinoid *Comaster gracilis* (Hartlaub, 1890) and considers the tissular deformations it induces on its host.

MATERIAL AND METHODS

Contramyzostoma bialatum, new genus and species, were collected with their hosts *Comaster gracilis* by SCUBA diving around Pulau Satumu (Raffles Lighthouse), Singapore, in August 1992. Adults of *C. bialatum* were detected on their hosts under binocular microscope.

For histological observations (semi-thin sections), individuals were fixed for 3 hours at 4°C in a solution of 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.3). They were washed in buffer, postfixed for 1 h with 1% osmium tetroxide in 0.1 M cacodylate buffer and washed again in buffer. After dehydration, specimens were embedded in Spurr and cut into 1 µm thick serial sections. Sections were stained in a 1:1 solution of Methylene Blue/ Azur II according to the procedure of Ganter & Jollès (1969-1970).

For TEM observations, individuals were fixed as before, embedded in Spurr, sectioned using a LKB V ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined with a Zeiss EM 10 transmission electron microscope.

For SEM observations, individuals were fixed in Bouin's fluid for 24 h, then dehydrated in graded concentrations of ethanol and dried by the critical point method using $C0_2$ as transition fluid. Samples were mounted on aluminium stubs, coated with gold in a sputter coater and observed with a JEOL JSM 6100 scanning electron microscope.

Descriptions of the parapodial hook and support rod were based on individuals whose soft tissues were digested in weak bleach. Once most of the tissues were dissolved, hooks and support rods were dried and mounted on slides. Microscopic observations were made with an Olympus IMT-2 contrasting phase microscope.

TAXONOMIC AND MORPHOLOGICAL ACCOUNTS

Contramyzostoma, new genus

Diagnosis. - Body bean-shaped, very much wider than long. All external appendages situated on small dorsal body field. Two pairs of posterior cirri. Five pairs of acirrate parapodia. Parapodial hook and support rod of the same length. Manubrium ovoid and very small. Four pairs of lateral organs. Introvert large without buccal papilla. Anal and female genital pores opposite to mouth, opening at top of ciliated ano-genital cone. Vibratile ciliature reduced, only present on dorsal body field. Digestive system with pharynx included in introvert, stomach, intestine, and one pair of loop-shaped tubules which start from the stomach and come back to it. Ventral nervous chain condensed. Only female stage known. Female genital system with branched oviduct opening to exterior through female genital pore and two diffuse ovaries.

Type species. - *Contramyzostoma bialatum*, new genus and species, by monotypy.

Contramyzostoma bialatum, new genus and species

Material. - 85 specimens of *C. bialatum* were collected from 17 *C. gracilis* out of the 32 collected (frequency of the infestation: 53%). There were 1 to 18 myzostomes per host. The holotype (female) and 3 paratypes (females) were deposited in the Zoological Reference Collection of the Department of Zoology, National University of Singapore (catalogue number: ZRC 1994-2593 for the holotype; ZRC 1994-2594, 2595, 2596 for the paratypes), Department of Zoology, National University of Singapore, and 3 paratypes (females) were deposited at the Royal Belgian Institute of Natural Science (catalogue number: IG 28070).

Etymology. - The generic name is a combination of the Latin *Contra*, opposite, referring to the unusual upside down external morphology, and of *Myzostoma*, a genus of Myzostomida. The specific name is a combination of *bi*, two, and *alatum*, wing, referring to the two lateral body extensions that characterize the species. Gender is neuter.

Type locality. - The species was collected with its host around Pulau Satumu (Raffles Lighthouse) in the territorial waters of Singapore between 0 and 20 m depth.

Diagnosis. - See generic diagnosis.

External anatomy. - The length of the holotype is 0.63 mm, its width 1.69 mm. Paratype lengths vary from 0.47 to 0.67 mm, widths from 1.37 to 1.96 mm. The body is formed of a small, ovoid field ca 400 µm in diameter that bears all the external appendages and two large lateral wing-like extensions (Figs. 3, 4, 5, 6). In the sagittal plane are the anterior introvert (ca 300 µm when extended), which can protrude or retract into an anterior pocket, and a posterior ciliated cone ca 50 µm high at the tip of which open the anus and the female genital pore (Figs. 3, 4, 5, 6). On the sides of the ovoid field lie five pairs of parapodia, four pairs of lateral organs, and two pairs of cirri arranged in such a way that the cirri are the closest appendages from the midpoint of the ovoid field, then the lateral organs, and finally the parapodia (Figs. 4, 5, 6). The parapodia are integumental cones ca 50 µm long from the apex of which protrudes the tip of a hook-like seta (Figs. 4, 6). The parapodial hook and support rods of digested individuals were both about 80 µm long (Fig. 7). The tip of each seta curves and forms a weak hook (Fig. 7). The support rod is slightly curved and possesses at its apex a small, somewhat ovoid, manubrium (Fig. 7). The tips of the setae point in a direction opposite to the myzostome sagittal plane (Fig. 6). Lateral organs are small star-shaped holes which alternate with the parapodia (Figs. 4, 6). The most posterior pair of cirri lies lateral to the ano-genital cone and is the longest pair (ca 80 µm long), while the more anterior pair is thinner and smaller (ca 50 µm long) (Figs. 5, 6). Vibratile cilia of 10 µm long occur in tufts on the dorsal field while the two lateral extensions are glabrous (Figs. 3, 4, 5).

Live individuals of *C. bialatum* are orange and their relative translucency reveals the two bright red, loop-shaped, digestive tubules.

Epidermis and cuticle. - The epidermis is a pseudostratified epithelium of 3 to 7 µm high which lies above a parenchyma that completely fills the spaces between the internal organs (i.e., the digestive and genital organs) (Fig. 8). The epidermis is built on two types of cells: covering cells and myoepithelial cells (Fig. 8). Covering cells are flattened cells 3 to 7 µm high for 10 to 15 µm long (Fig. 8). Apically, they bear microvilli which cross a 500 nm high cuticle and end in bulges (Figs. 8, 15). Myoepithelial cells are round and elongated cells of ca 2 µm in diameter for several tens of µm long (Fig. 8). They lie under covering cells in such a way that their longitudinal axis is perpendicular to the antero-posterior axis of the myzostome. Their cytoplasm includes coarse and thin myofilaments and sarcoplasmic reticulum (Fig. 8). Their nucleus lies always above the myofilaments.

Digestive system. - The digestive system consists of a pharynx included in a protrusible introvert at the apex of which opens the mouth, several salivary gland cells, a stomach from which two loop-shaped tubules start and to which they return, and an intestine ending at the anus (Figs. 9, 10). Each digestive organ is formed of an epithelium surrounded by musculature. The musculature is well developed at the level of the pharynx but very thin in the other organs (Fig. 10). Around the junction between the pharynx and the stomach lie the salivary gland cells; they are intraparenchymal spherical cells from which protrude long cell processes which end at the level of the mouth. The pharynx, the stomach and the intestine form a single continuous tube but are made of different epithelia: pharyngeal cells are cylindrical, stomachal cells are club-shaped while intestinal cells are flat (Fig. 10). From each side of the stomach one tubule goes out into the wing-like body extension, bends at its end, and comes back to re-open in the stomach just before the beginning of the intestine (Figs. 9, 11). The epithelium of the tubules is made of cylindrical cells in which the cytoplasm contains large vacuoles (Fig. 11). The anus is situated at the top of the ano-genital cone.

Nervous system. - A ventral nerve cord lies in the sagittal plane of the body, under the stomacho-intestinal tube (Fig. 10). It is opposite to the body field that bears all the external appendages. Anteriorly, at the end of the pharynx, tiny nerve processes form a small peripharyngeal collar. Lateral nerves (probably five) that spread out into thin nerve processes start from each side of the ventral nerve cord.

Genital system. - All collected specimens were females. The female genital system consists of two ovaries and one branched oviduct that opens to the exterior through the female genital pore.

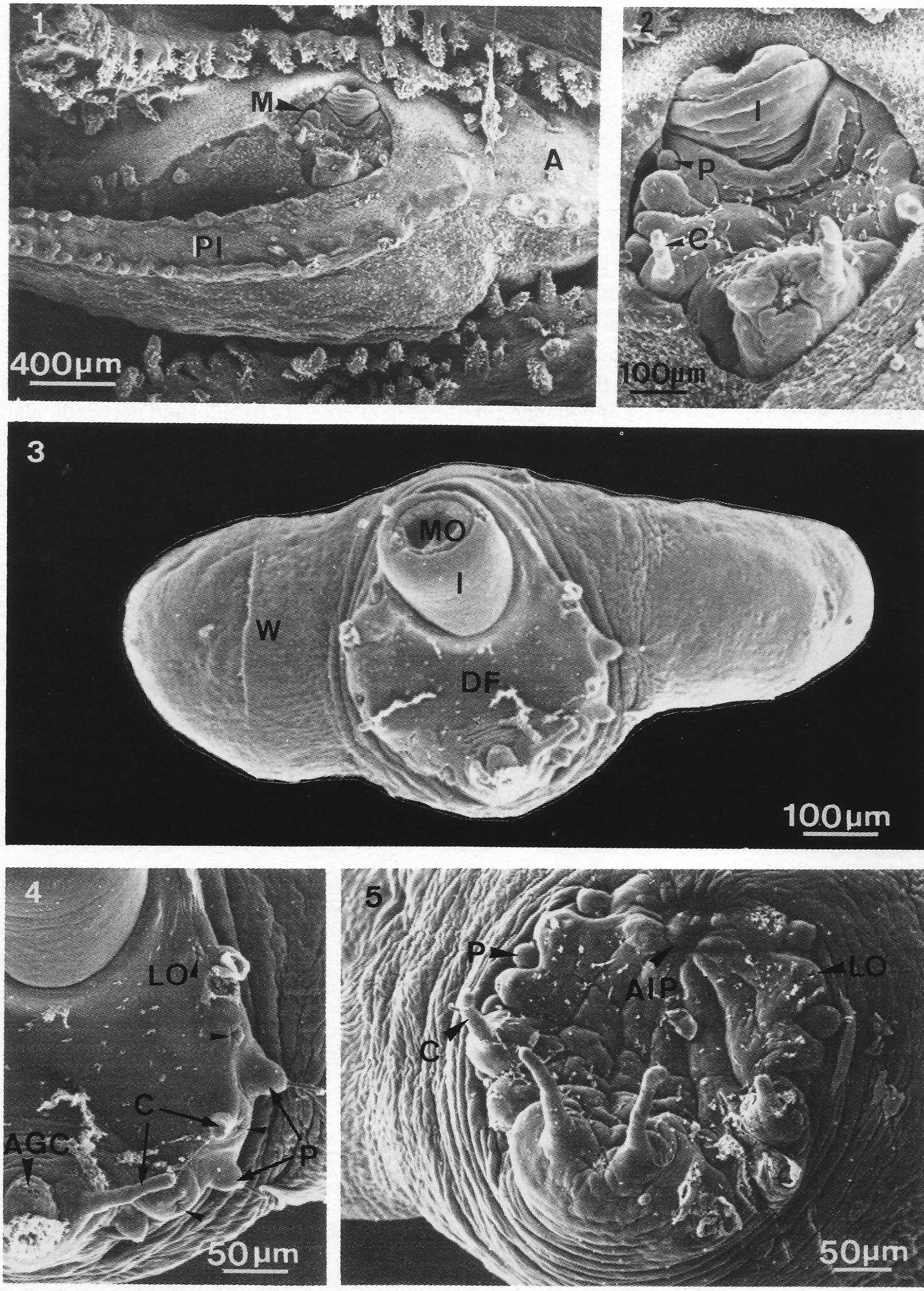
Each ovary is situated in one of the wing-like body extensions (Figs. 9, 11). It is not limited by any epithelium but only consists of female gametes at different stages of development which lie in the parenchyma that fills the myzostome body between the organ systems (Figs. 9, 11).

The oviduct is made of two loop-shaped branches that surround and enclose the two loop-shaped digestive tubules (Figs. 9, 11). In the sagittal plane, the 2 branches of the oviduct fuse together dorsally to the digestive system to form a single channel which opens to the exterior through the gonopore (Figs. 9, 10).

Some morphological observations allow us to infer the mode of reproduction of *C. bialatum*. All individuals were fertilized females: their parenchyma included spermatic syncytia, i.e. spermatozoons surrounded by a polynucleated cytoplasmic sheet (Fig. 12) (for further explanations of the myzostome reproduction see Eeckhaut & Jangoux, 1991; see discussion below).

Excretory system. - No excretory system has been observed.

Injuries induced to the host. - *Contramyzostoma bialatum* lives in an open-ended



Figs. 1-5. External morphology of *Contramyzostoma bialatum*, new genus and species (SEM views). General and detailed views of the myzostome as observed *in situ* (1 and 2, respectively); dorsal view of one individual once extracted from its burrow (3) and detailed views of its dorsal body field with the introvert relaxed and contracted (4 and 5, respectively). Abbreviations. A: host arm; AGC: ano-genital cone; AIP: aperture of the introvert pouch; C: cirrus; DF dorsal body field; I: introvert; LO: lateral organ; M: myzostome; MO: mouth; P: parapodium; PI: host's pinnule; W: wing-like body extension.

spindle-shaped burrow-like cavity dug into the integument of its host. Burrows are always located in the comatulid arms, their long axis being parallel to the host ambulacral groove and the inhabiting myzostomes positioned in such a way that their ovoid body field grossly corresponds in size and location to the aperture of the burrow (Figs. 1, 2, 13). Each burrow includes no more than one myzostome which attaches to the host with its hook-like setae (Fig. 2). All other appendages emerge from the burrow aperture with the introvert always facing the host ambulacral groove (Fig. 2).

The host integument is markedly modified at the level of the burrow. The host epidermis is extremely flat and appears as cytoplasmic sheets above which lies a cuticle (Figs. 14, 15). The host dermis is modified over a thickness of ca 10 µm and is formed of two superimposed layers not present at the level of the regular (i.e., non modified) dermis: the first and closest layer below the epidermal sheets is 3 µm thick and is composed of pseudopodial cells surrounded by a fibrillar matrix; the second and innermost layer is 7 µm thick and is composed of several superimposed strata of collagen fibers being alternately parallel and perpendicular to the long axis of the burrow (Figs. 14, 15).

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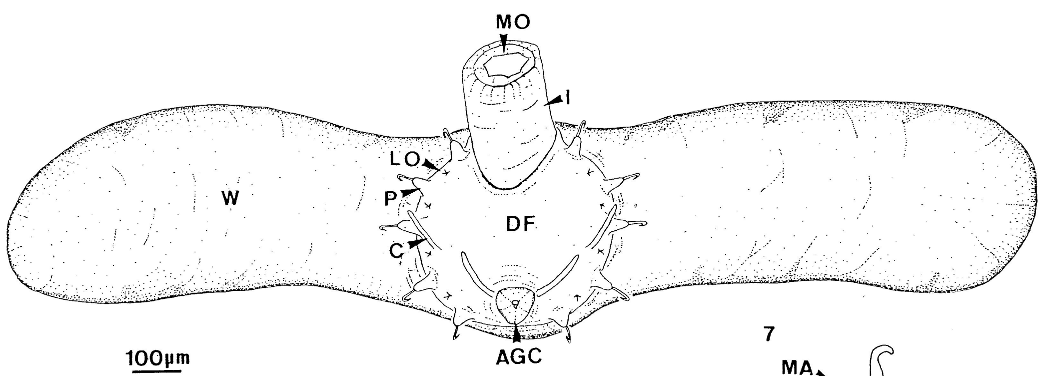

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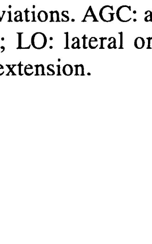
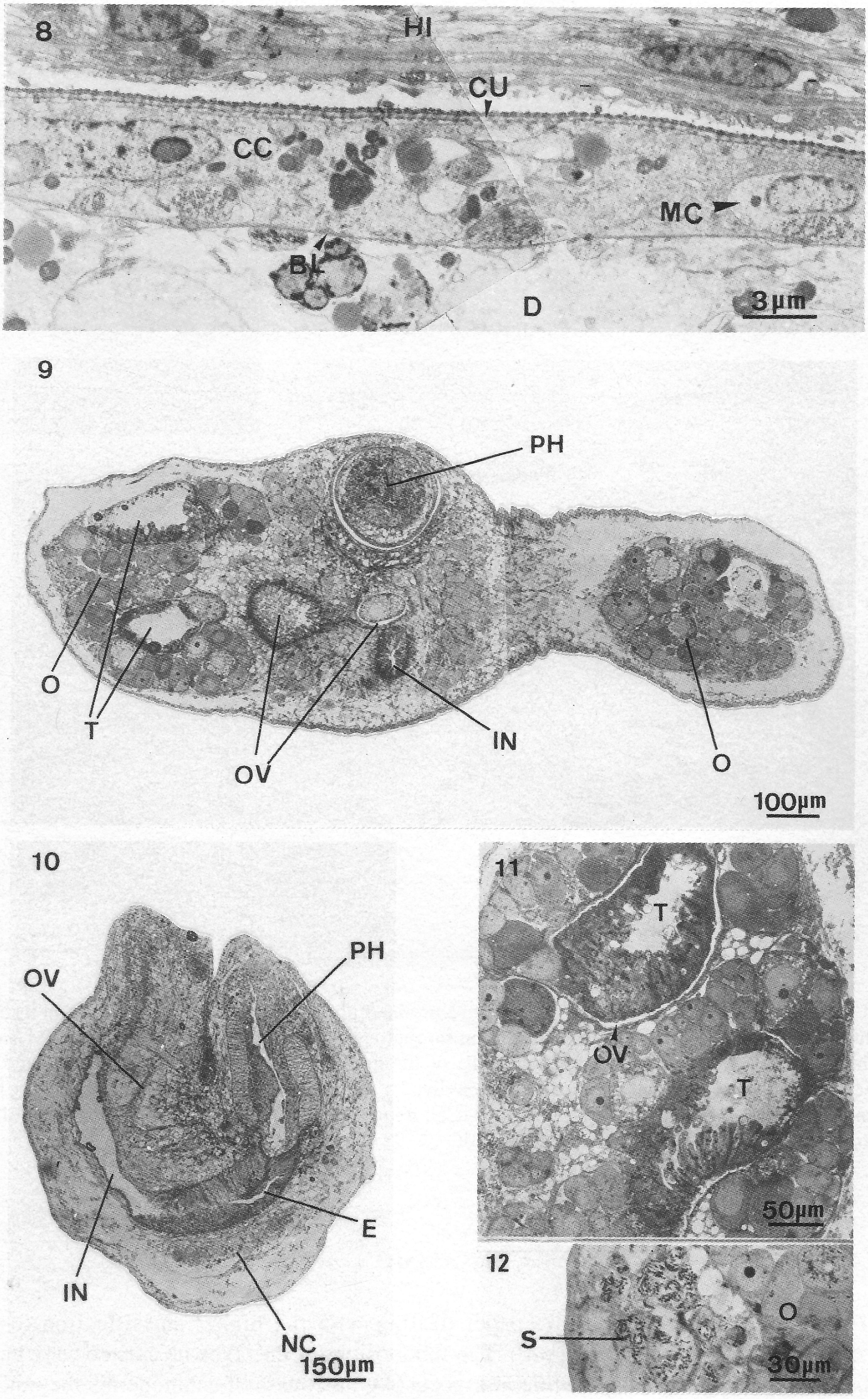
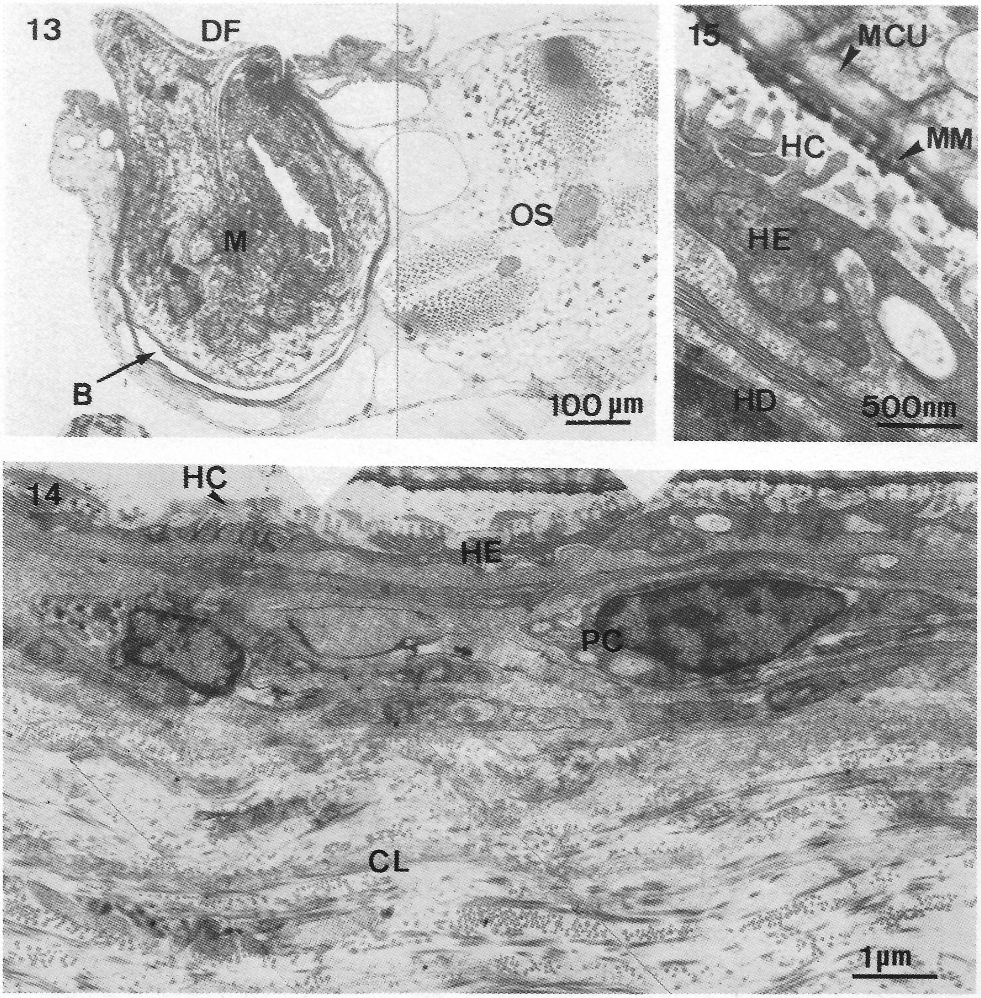


Figs. 6-7. Drawings of *Contramyzostoma bialatum*, new genus and species. Dorsal view of an individual (6) and details of the parapodial hook and support rod (7). Abbreviations. AGC: ano-genital cone; C: cirrus; DF: dorsal body field; H: parapodial hook; I: introvert; LO: lateral organ; MA: manubrium; MO: mouth; P: parapodium; SR: support rod; W: wing-like extension.



Figs. 8-12. Internal morphology of *Contramyzostoma bialatum*, new genus and species. TEM view of the epidermis (8); light microscopic views of frontal and sagittal sections through entire individuals (9 and 10, respectively), and of parasagittal sections through a wing like extension showing the ovary, the oviduct and the two branches of one loop-shaped digestive tubule (11), and spermatic syncytia (12). Abbreviations. BL: basal lamina; CC: covering cell; CU: cuticle; HI: host's integument; D: dermis; E: stomach; IN: intestine; MC: myoepithelial cell; NC: nerve cord; O: ovary; OV: oviduct; PH: pharynx; S: spermatic syncytium; T: tubule.



Figs. 13-15. Deformations induced by *Contramyzostoma bialatum*, new genus and species, on its host. Light microscopic view of a transversal section through an infested host's arm (13) and TEM views of the deformed host's integument with details of the deformed epidermis and cuticle (14 and 15, respectively). Abbreviations. B: burrow-like cavity; CL: collagen layer HC: host cuticle; HD: host dermis; HE: host epidermis; M: myzostome; MCU: cuticle of the myzostome; MM: microvilli of the myzostome; OS: ossicle; PC: pseudopodial cell.

DISCUSSION

The last significant taxonomic paper dealing with the higher classification of the Myzostomida was by Jägersten (1940). The subdivisions of the taxon he created were based on both morphological and behavioural aspects (e.g., the class of echinoderms they infest, the organs they infest). Subdivisions of this taxon need revision and *Contramyzostoma bialatum*, new genus and species, is placed within the family Cystimyzostomatidae because it includes all the species that deform the integument of crinoids (Jägersten, 1940). Up to now, Cystimyzostomatidae was represented by one genus, viz. *Cystimyzostomum* (Jägersten, 1940). *Contramyzostoma bialatum* differs from all *Cystimyzostomum* species but also from all the species of other myzostomidan genera by both its external and internal morphology. One of the most extraordinary features of *C. bialatum* is that all the external appendages lie on a small body field while the rest of the body is stretched out bilaterally. Moreover, the body field is opposite to the ventral nerve cord, thus indicating it forms the dorsal part of the body, while in all other myzostomes which possess developed external appendages, at least the parapodia and lateral organs are always ventral. Viewed externally, two features of *C. bialatum* demonstrate that its body field is dorsal. Firstly, the appendages closest to the midpoint of the body field are the cirri, then the lateral organs, and finally the parapodia are outermost, while in other myzostomes with developed appendages, those closest to the ventral body midpoint are the parapodia, then the lateral organs and finally the cirri. Secondly, in the Singaporean species, the tips of the parapodial setae point away from the sagittal plane while in other myzostomes, the tips point toward this plane. These morphological features suggest that *C. bialatum* must have evolved from an "ancestral myzostomidan worm" which possessed ventral external appendages and was probably an ectocommensal with an external morphology similar to the extant representatives of the genus *Myzostoma*. In the myzostomes, as in other invertebrate taxa, the external morphology of the parasites is always highly different from that of non parasitic species: e.g., the body of *Cystimyzostomum* species is bean-shaped, those of *Protomyzostomum* species and *Mesomyzostoma* species are worm-like. In the case of *C. bialatum*, the parasitic condition would have brought about morphological changes thus allowing the myzostomes to live in long open-ended burrow-like cavities situated at the level of the arms of crinoids. These morphological changes would have been the migration of all the external appendages to a reduced dorsal body field (allowing probably the myzostomes to keep contact with the external medium) and the development of the former mid-ventral region situated between the parapodia of the "ancestral worm" into two large, wing-like body extensions (allowing probably the myzostomes to be wider and thus to develop their genital organs).

With the exception of *Stelechopus hyocrini* Graff, 1884, the digestive system of the myzostomes comprises a pharynx included in an introvert, a stomach from which blind digestive caeca originate, and an intestine. Salivary gland cells have also been reported in numerous genera but not in the *Cystimyzostomum* (Jägersten, 1940). While the different kinds of gut epithelia and the surrounding musculature in *C. bialatum* appear similar to those observed in *Myzostoma cirriferum* Leuckart, 1836 (Platel 1962), the gross anatomy of its digestive system is unique within the Myzostomida: it includes one pair of loop-shaped tubules while in all other myzostomes, there are caeca that dichotomize into a series of blind branches.

Cystimyzostomum species are considered to be protandrous hermaphrodites, with a few exceptions which are simultaneous hermaphrodites (Jägersten, 1940). *Contramyzostoma bialatum* and *Cystimyzostomum* species have the same type of female genital system.

Reproduction of myzostomes is only known in a few species of the genus *Myzostoma* (Jägersten, 1939; Kato, 1952; Eeckhaut & Jangoux, 1991, 1992). It occurs by the emission of a spermatophore from a mature myzostome to any part of the integument of another one; the spermatophore pierces the integument and a spermatic syncytium flows through and extends into the parenchyma of the receiver, the fertilization being internal (Eeckhaut & Jangoux, 1991). We have observed numerous spermatic syncytia in individuals of *C. bialatum* and, consequently, the reproductive process of this species should work in the same way: a male individual or a younger male stage, which should be ectocommensal, probably emits a spermatophore onto the dorsal field (viz., the only part of the female that is accessible from the exterior) and the spermatophoral content would flow through the integument.

Contramyzostoma bialatum was found to infest only *C. gracilis*, although we examined four other comatulid species in the same area during the same period (Eeckhaut et al., 1994). The main advantage the myzostome gets from the parasitism is to be under shelter at the best place for feeding: being located at the level of the host ambulacral groove allows it to divert food particles caught by the comatulid. The infestation by *C. bialatum* causes significant integument modifications on the host: the epidermis is extremely flattened and the dermis includes a wall of pseudopodial cells, not observed on the non infested dermis. These cells could be invading amoebocytic coelomocytes as they look similar to these cells which are commonly observed in the coelom of echinoderms (see Smith, 1981).

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