Cycliophora is a new phylum with affinities to Entoprocta and Ectoprocta

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have, on the other hand, remained relatively scarce. The essence of our primary argument has been that if one accepts that the Earth's mantle is such a system, then an understanding of the physics behind the observation contained in Fig. 1 follows with few assumptions in between. □

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Cycliophora is a new phylum with affinities to Entoprocta and Ectoprocta

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THE mouthparts of the Norway lobster *Nephrops* are colonized by an acoelomate metazoan, *Symbion pandora* gen. et sp. nov. Sessile stages continually produce inner buds replacing feeding structures. They also produce one of three motile stages: (1) larvae containing new feeding stages, (2) dwarf males, which settle on feeding stages, or (3) females, which settle onto lobster mouthparts, and eventually degenerate, giving rise to dispersive larvae. All motile stages are short-lived, and do not feed. The structure and function of the cilia suggest a phylogenetic position in Protostomia, while some aspects of inner budding and brooding of larvae are similar to those of Entoprocta and Ectoprocta. The dispersive larva possesses a mesodermal supporting chordoid structure, otherwise absent in protostomian larvae. We believe that all the above features of this previously undescribed species warrant the recognition of a new phylum with affinities to Ectoprocta and Entoprocta.

Cycliophora, new phylum

Diagnosis. Acoelomate, marine metazoans with bilateral symmetry and differentiated cuticle. Compound cilia engaged in filter feeding working as a downstream-collecting system. Sessile, solitary feeding stages with multiciliated cells in the gut, anus just behind a ciliated mouth ring, and internal budding with extensive regeneration of feeding apparatus. Feeding stages alternating with brief, non-feeding, free-swimming stages. Brooding of asexual larva (Pandora larva), male and female. Females brooding chordoid larva with a mesodermal, ventral rod of plate-like muscle cells (chordoid structure) and a pair of protonephridia with multiciliated terminal.

Etymology. Cyclion and phoros are Greek for 'a small wheel' and 'carrying' referring to the circular mouth ring.

Eucycliophora, new class

Diagnosis. Same as the phylum.
Etymology. Eu is Greek for 'well', referring to the splendid symmetrical mouth ring.

Symbiida, new order

Diagnosis. Same as the phylum with metagenesis in the life cycle.

Symbiidae, new family

Diagnosis. Same as the phylum.

Symbion gen. nov.

Diagnosis. Same as the phylum.
Type species. *Symbion pandora*, new species by designation (Figs 1–4).
Etymology. Syn and bios are Greek for '(together) with' and 'living', referring to the intimate life with the crustacean host; masculine gender.

Symbion pandora sp. nov.

Diagnosis. Same as the phylum.
Etymology. The feeding stage which contains both an inner bud and a Pandora larva with a miniature feeding stage inside (Fig. 4, stage 9) reminds one of Pandora's box.
Type material. The holotype is a feeding stage to which the allotype, a mature dwarf male, is attached (Fig. 1). Both are as total preparations on a microslide. This slide, together with several hundred paratypes, are deposited in the Zoological Museum of Copenhagen (ZMUC CYC-0001–CYC-0300), Denmark. The holotype and allotype occurred on the mouth appendages of *Nephrops norvegicus*, taken off Frederikshavn, North Kattegat, Denmark (57°26′ N, 10°42′ W), at a depth of 20–40 m, 19 August 1991. Additional paratypes were collected from *N. norvegicus* taken at Frederikshavn, Denmark; Gullmarfjord, Sweden; and Kaldbak Fjord, Faroe Islands, North Atlantic Ocean. Part of this material is placed in ZMUC and in the National Museum of Natural History, Smithsonian Institution (USNM), Washington DC, USA.
Holotype. Length, 347 μm; width, 113 μm. Body consists of buccal funnel, trunk, and stalk with disc attached to a host seta. The bell-shaped buccal funnel is constricted at the base and opens anteriorly to form a circular mouth circumscribed by a ciliated mouth ring. This ring is extended in the normal feeding position. The funnel is bent over the anus towards the host's seta. The ovoid trunk is borne on a short (63 μm) stalk which expands to an attachment disc (73 μm across). The funnel, trunk, stalk and attachment disc are lined by a cuticle exhibiting a characteristic pentagonal or hexagonal sculpturing. The stalk and attachment disc consist exclusively of cuticular material.
The buccal funnel narrows into a short S-shaped oesophagus, which continues into the U-shaped alimentary tract. The descending part of the alimentary tract is an enlarged stomach. The stomach wall consists of large, ciliated cells. The stomach is filled with a cluster of granular, secretory cells without cilia, reducing the stomach lumen to lacunar spaces. The ascending part of the alimentary tract consists of a ciliated intestine and a short rectum. In the trunk, the space between the epidermal layers is totally occupied by large vacuolated mesenchyme cells. There is no coelom. The holotype contains a single internal bud which has developed a new mouth ring and ciliated gut. This has pushed the old digestive system forward. Two clusters of cells are situated lateral to the inner bud. We interpret these as early embryos, supported by observations on paratypes.
Allotype (dwarf male). The allotype is a mature male attached to the trunk of the holotype. Total length, 84 μm; maximum width, 42 μm. The male has an ovoid trunk (67 μm), a short stalk (17 μm) and a large attachment disc (27 μm across). Buccal

funnel and anus are absent in the male. The sculpture of the cuticle is the same as that of the holotype, but the polygonal markings are finer. The stalk and disc consist only of cuticular material.

An alimentary tract is absent. Distal to the adhesive disc, two large compartments lined by very thin cuticle are present. Each compartment contains a body of differentiated cells surrounded by a thin cuticle, except in two V-shaped areas with ciliated epithelia. Each body contains different stages of spermatids or spermatozoa and a tubular, cuticular structure, here interpreted as a penis. A cluster of undifferentiated cells is situated near the stalk. The rest of the interior is filled with vacuolated mesenchyme cells.

Paratypes. The mouth ring consists of alternating multiciliated and myoepithelial cells. The latter form two sphincters (peripheral and central) involved in closing the mouth. In live specimens the compound cilia of the mouth ring were observed to collect particles with a downstream collecting system. The lining of the buccal funnel consists of multiciliated cells with compound cilia. The anus is a distinct transverse fissure (about 25 μm long) on a small elevation on the trunk close to the buccal funnel. Transmission electron microscopy (TEM) studies show the 1–2-μm-thick cuticle to be composed of a very thin trilaminate epicuticle and a thick fibrillar procuticle. TEM of the alimentary tract shows multiciliated cells with separate cilia of the digestive tract, a cuticular lining of the rectum and a tuft of long cilia at the entrance to the stomach. In living specimens these long cilia move in an undulatory way and the contents of the intestine rotates. Two long muscle fibres connect the base of the buccal funnel on the anal side with the opposite side of the trunk. These muscles are probably responsible for the only movement observed: a nodding with the buccal funnel. Some muscles are cross-striated, others obliquely striated. A ganglion (the brain) is situated between the oesophagus and the rectum in the trunk region. The position of the brain means that the buccal funnel is not homologous with a true head. The two clusters of cells situated lateral to the inner bud, as described in the holotype, might be early embryos. This is supported by observations of a series of paratypes with intermediate stages leading to a single, fully developed embryo (Fig. 4, stages 7–12–15–16). The development of an inner bud is shown in Fig. 4, stages 4–7. In stages without an internal bud, the alimentary tract extends to the stalk. As the internal bud develops further inside the feeding stage, the old digestive system totally disintegrates and the buccal funnel is finally cast off (Fig. 4, stage 8). The new buccal funnel and alimentary tract formed from the inner bud subsequently emerge and start feeding. Inner budding is repeated several times in the lifetime of an individual.

The male cuticle is ultrastructurally similar, but thinner than that of the holotype. Male paratypes with one and three compartments were also observed. In the cellular parts of the compartments a ring of well-developed, cross-striated muscles underlies the epidermis. Other cross-striated muscles attach to the base of the penis. A large two-lobed ganglion is located in the cellular body inside each compartment. Continuing TEM analysis of males attached to feeding stages could suggest that

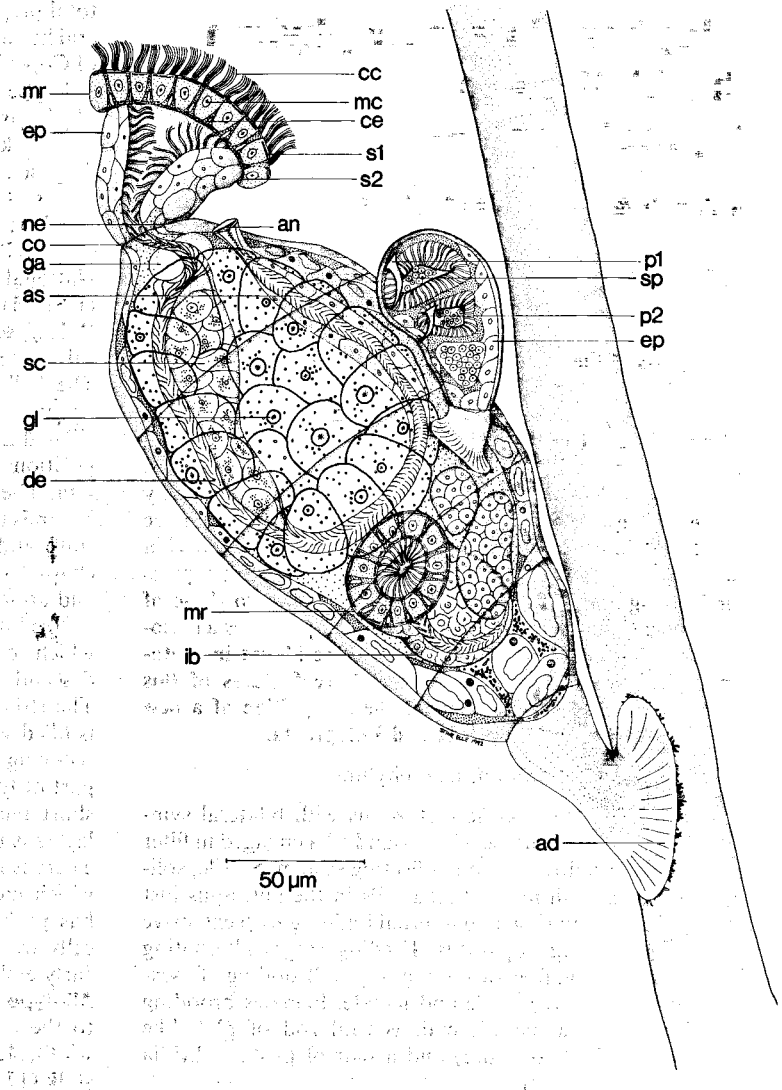

FIG. 1 *Symbion pandora*, new species, holotype and allotype (ZMUC CYC-0001). The holotype is an asexual feeding stage, attached to a seta of a mouth limb from *Nephrops norvegicus*. The allotype is a mature male, a dwarf male attached to the holotypic feeding stage. The specimens were relaxed with $MgCl_2$ before fixation in formalin. ad, adhesive disc; an, anus; as, ascending branch of the digestive system; cc, compound cilia; ce, ciliated epidermis; co, constriction (or 'neck'); de, descending branch of digestive system; ep, epidermis; ga, ganglion; gl, gut lining cell; ib, inner bud; mc, myoepithelial cell; mr, mouth ring; ne, nerve; p1, penis 1; p2, penis 2; sc, stomach cells; sp, sperm; s1, sphincter 1; s2, sphincter 2.

each compartment actually represents a brood chamber for an extremely reduced secondary male formed by inner budding.

The life cycle proposed in Fig. 4 is based on: (1) live studies of different free-swimming individuals liberated from feeding stages of *S. pandora*, (2) TEM of fully developed stages still inside the feeding stages and (3) the temporal occurrence of different stages of *S. pandora* relative to the phases of the host's moulting cycle; for example, chordoid larvae, males and females are numerous on *Nephrops* close to moulting, and absent on those newly moulted.

All ciliated epithelia of *S. pandora* consist of multiciliated cells as in spiralian larvae and rotifers[1]. In the feeding stage, the bands of compound cilia work as a downstream collecting system[2], indicating that *S. pandora* belongs to protostomians[3]. The attached dwarf male of *S. pandora* reminds one of some rotifers. This similarity is apparently superficial, as rotifers do not have a true cuticle[4], lack regenerative powers and have dimorphic spermatozoa[5,6], which have not been observed in *S. pandora*. The cuticle of *S. pandora* is similar to that found in some Nematoda[7] and Gastrotricha[8]. Continuing TEM analysis of the chordoid larva shows that the mesodermal chordoid structure resembles similar tissue found in some free-living Gastrotricha[9], such as *Chordodasys*. There is no evidence for any homology with the dorsal chorda of Chordata, because the chordoid structure in *S. pandora* is situated ventrally. Internal budding, a salient feature of *S. pandora*, is absent in all Aschelminthes, which in general lack powers of regeneration. Nevertheless, Manylov[10] recently described regeneration in the gastrotrich *Turbanella*. We would advocate that a relationship exists with the phyla Entoprocta and Ectoprocta united by Nielsen[3,11] in the superphylum Bryozoa. The fact that all three groups share a U-shaped gut is of minor importance, as this is common among other sessile taxa. Although nephridia have never been reported in Ectoprocta, both Entoprocta and the chordoid larva of *S. pandora* possess protonephridia with multiciliated terminal cells. Budding is characteristic of all Entoprocta and Ectoprocta. Acceleration of the development of feeding structures occurs in the larvae of freshwater ectoprocts in the order Phylactolaemata[12,13] and in certain Entoprocta such as *Loxosomella vivipara* and *Loxosoma jaegersteni*[11]. This is reminiscent of the advanced development

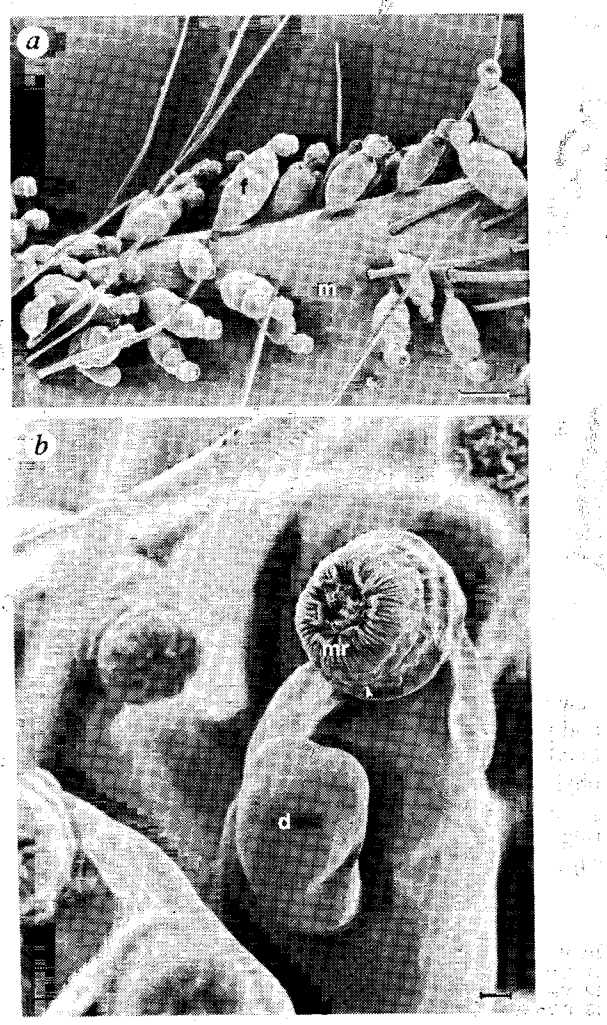

FIG. 2 *Symbion pandora*, new species. SEM of paratypes from Kaldbak Fjord, Faroe Islands. *a*, Overview of *Nephrops* mouthpart (m) with numerous feeding stages (f). Scale bar, 100 μm. *b*, Detail of a feeding stage with a closed mouth ring (mr); arrowheads point to basal border of the mouth ring. Compare with Fig. 1 illustrating the open mouth ring in feeding position. One dwarf male (d) is attached to the trunk of the feeding stage. Often several males are attached to a single feeding stage. Scale bar, 10 μm.

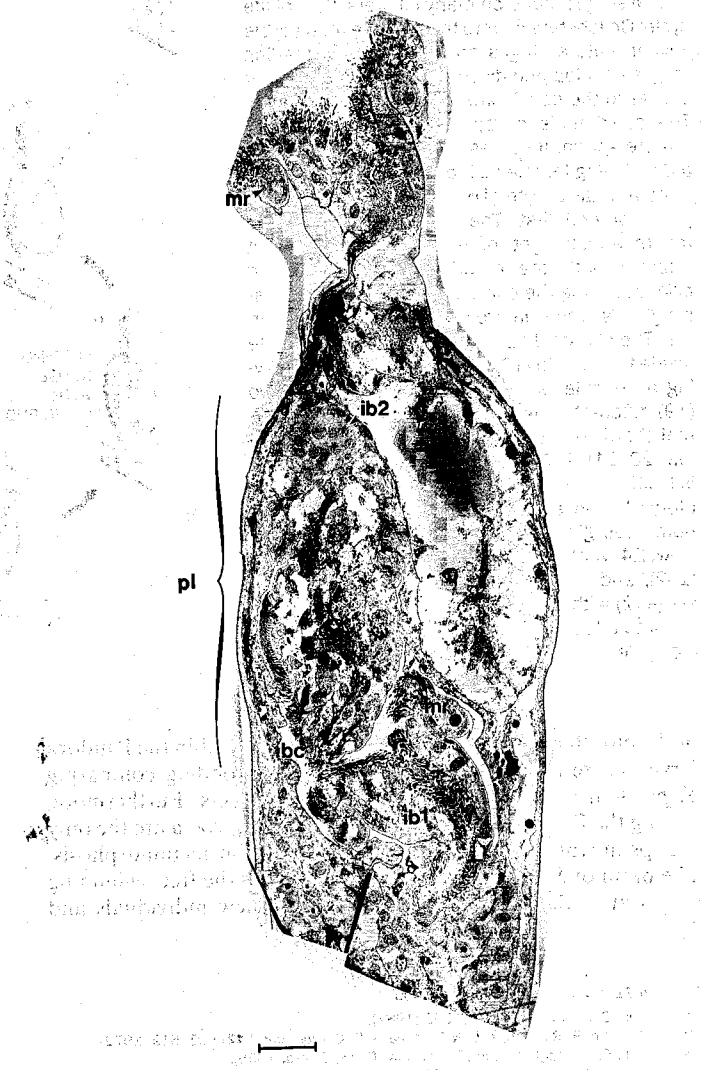

FIG. 3 *Symbion pandora*, new species. TEM mosaic micrograph of paratype from Kaldbak Fjord, Faroe Islands. A longitudinal section of a feeding stage with a Pandora larva (pl) and an inner bud (ib1). The larva and the bud are developed in a brood chamber (bc), lined with cuticle. The inner bud has differentiated into a distinct mouth ring (mr) and ciliated buccal funnel, like those of the maternal feeding stage. Inside the Pandora larva, another inner bud (ib2) later develops into a new feeding stage. Scale bar, 20 μm.

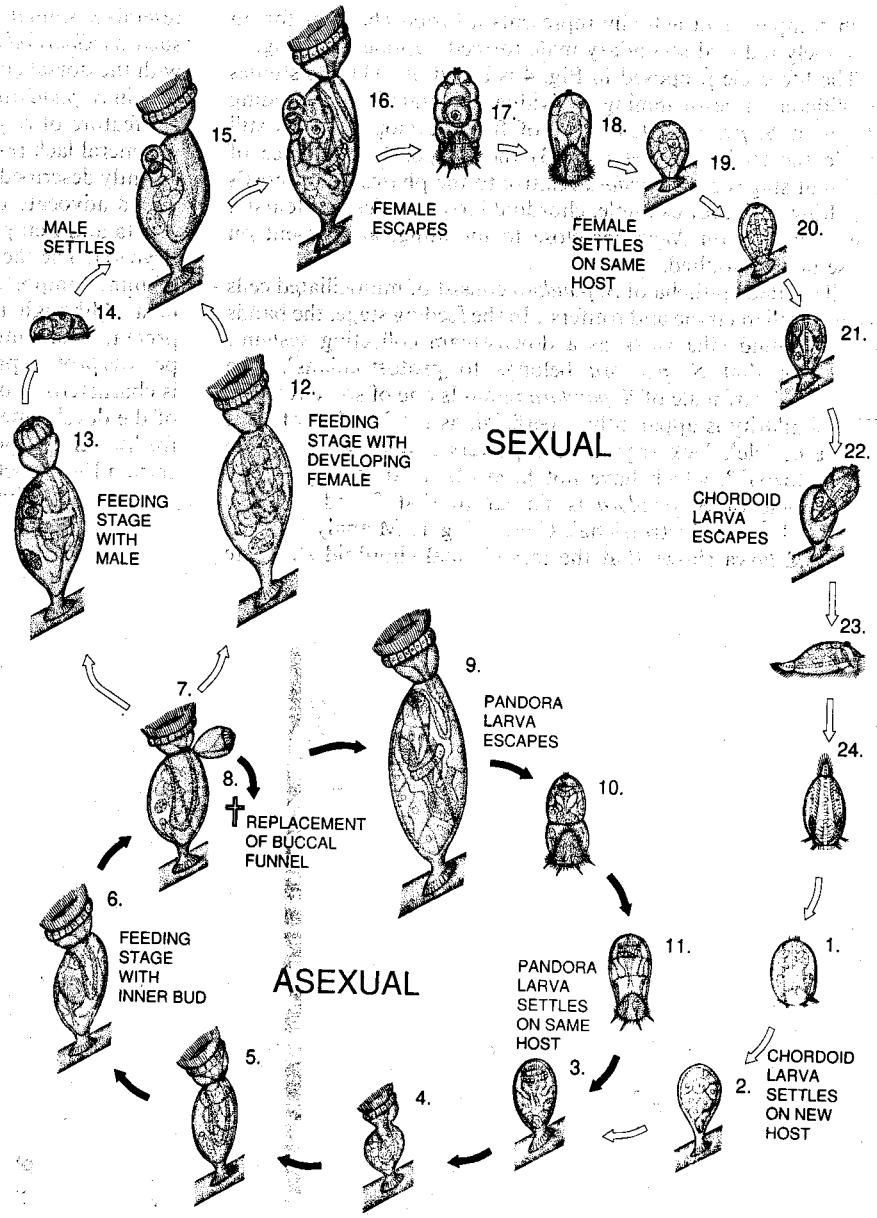

FIG. 4 Hypothetical life cycle of *Symbion pandora* new species. A free-swimming chordoid larva with internal buds settles on the mouth appendages of the crustacean host (1–2). The chordoid larval body degenerates. Asexual life cycle: The bud develops into a small feeding stage (3). When the mouth funnel emerges (4) feeding begins. Growth of the feeding stage is followed by successive inner buddings with total regeneration and replacement of buccal funnel, alimentary tract and nervous system (5–8). Reaching a certain size (maturity), the feeding stage develops asexually a Pandora larva in a brood chamber (9). A small new feeding stage develops through budding inside the Pandora larva, which is still inside the brood chamber (9). At the same time another inner bud is developed (9), also inside the maternal feeding stage; this bud eventually differentiates into a new buccal funnel (9). The Pandora larva (10–11) escapes, settles and repeats growth as described (3–8), finally producing a new Pandora larva (10). This asexual part of the life cycle explains the large populations of feeding stages with no mature sexual stages found on many lobsters. Sexual life cycle: On lobsters near to the end of a moult cycle sexual mature stages are found. Some feeding stages develop one dwarf male (13) in a manner similar to the development of the Pandora larva. The dwarf male escapes (14), now filled with mature sperm. It settles on a feeding stage with a developing female inside (15). The female contains a large oocyte (16), and escapes with the posterior end first. The female settles on the mouth appendages of the crustacean host. It attaches with the anterior, ventrally oriented cilia, secreting the contents of numerous adhesive glands. The cilia are withdrawn and degenerate. The chordoid larva develops from the zygote inside the female (17–18). The female dies, leaving a cuticular shell with a developing embryo (19) differentiating into a larva with a mesodermal chordoid structure and a pair of protonephridia (20–21). This chordoid larva hatches (22); it is lecithotrophic, equipped with extensive locomotory ciliation, a pair of dorsal ciliated organs and numerous glands of different types (23, lateral view; 24, ventral view). The chordoid larva settles (1–2), and metamorphosis leads to the sessile stage (2) with a developing bud. This bud develops into a feeding stage (3) initiating the asexual life cycle.

of the mouth ring and gut of the feeding stage within the Pandora larva. There are also some similarities in brooding comparing *S. pandora* with the phylactolaemate ectoprocts. Furthermore, among the Protostomia, Entoprocta and Ectoprocta are the only groups in which the larval brain disappears[3] at metamorphosis. The brain of *S. pandora* also disappears in all the free-swimming stages at settlement. Inner buds generate new individuals and new nervous systems in the chordoid larva, the Pandora larva and perhaps the male. The colonies of Ectoprocta also arise by budding[3]. We propose that the Cycliophora represents a new phylum with the following autapomorphies: dwarf males, metagenesis in the life cycle, a chordoid larva and a ciliated mouth ring. The sister group of Cycliophora might be Entoprocta or superphylum Bryozoa. □

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