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The history of Entoproct research and why it continues

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1. Introduction

The history of entoproct research encompasses a period of more than 250 years. The observations on the taxon were naturally influenced and restricted by the technical instruments and methods that were available at certain times. Early researchers were much challenged by investigating the miniature entoprocts with only a magnifying glass or a simple light microscope. First details of entoproct morphology were discovered when light microscopes became an established tool. Basic studies about entoproct reproduction, life history, and anatomy were made in the years between 1880 and 1960. The use of sectioning techniques in combination with scanning electron microscopy was not established in entoproct research until the late 1960s, when ultrastructural details of certain body parts as tentacles, digestive tract, or attachment structure were uncovered. In the 1990s, molecular studies were initiated to evaluate lophotrochozoan and metazoan relationships; however, only few studies were designed to resolve entoproct evolution. Entoproct research at the beginning of the 21st century can be characterized by (1) investigations of the anatomy (e.g., neuromuscular system) by applied electron microscopy and immunocytochemistry combined with confocal microscopy, (2) the first cladistic analyses including entoproct morphological characters, and (3) on-going research of the entoproct molecular body plan.

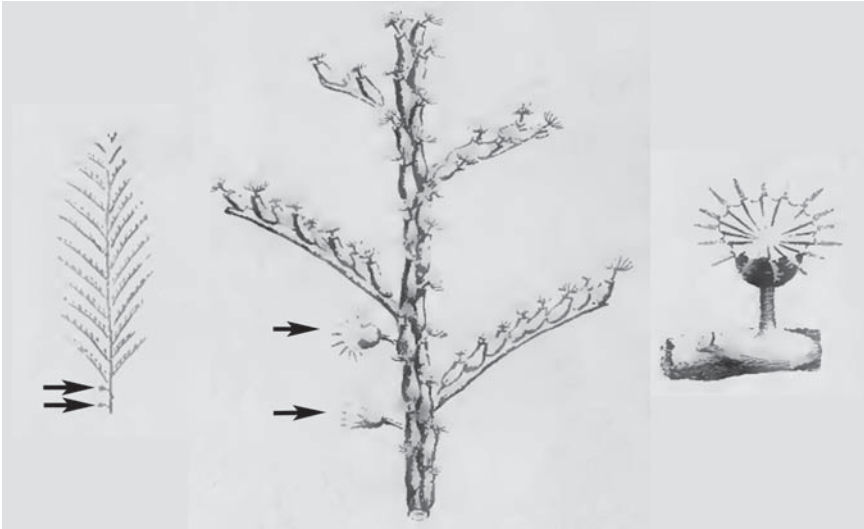


Figure 1. First drawings of a colonial entoproct growing on a hydroid stem (from Ellis, 1755).

It is probably not an overstatement to remark that Entoprocta are amongst the most enigmatic animals on our planet. Questions concerning the systematics of the taxon are as old as its first discovery and they have continuously been applied to entoproct research throughout the centuries. The question now is “how far have we come?” and instead of giving an answer at this point, the following review will shed some light on this issue.

2. Early findings

To determine when research on entoprocts began, one has to go back to the literature of the late 18th century. In John Ellis’ work *Natural history of the Corallines and other marine productions of the like kind* published in 1755, it is intriguing to read the author’s dedication to the Princess Dowager of Wales, which includes the following:

The minutest works of creation are not always the least wonderful: and though the element, of which these are natives, prohibits an exact enquiry, yet enough of order, contrivance, and regularity, appear in their structure, to convince us, they are the workmanship of that Almighty power, whom, exalted as you are in dignity of sentiment, as well as station, you think it your highest honour to reverence and obey.

Although the idea of the Almighty has by now rather disappeared from the minds of modern biologists, many would probably agree that they share with Ellis and other early biologists the sudden emotional excitement when the astonishing complexity of a living organism is observed. Modern zoologists also share with early biologists that this initial excitement subsequently leads to a more detailed observation of the organism and the parts it is made of. Although the short description and drawings of the “polyps of a red

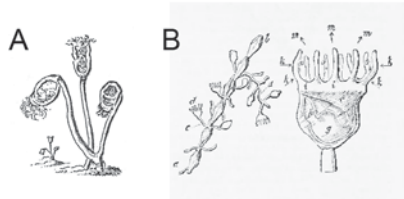


Figure 2. *Pedicellina*. A. Drawing by Pallas (1778). B. Drawings from Sharpey (1836), showing a colony and the calyx of a specimen to describe the feeding mechanism.

colour and a particular kind” in Ellis’s book are not much more than a passing comment, they are considered the earliest report of an entoproct in literature (Figure 1). It was later confirmed that Ellis’s organism matches that of the colonial entoproct genus *Pedicellina*.

Some years later, Pallas (1774, 1778) published the first recognizable, and somewhat longer description of a *Pedicellina* species together with a drawing in his “Novel and strange animal species”. However, Pallas himself identified the entoproct species as the rotifer *Brachionus cernuus*, a kind of a strange, marine polyp, related to hydroids (Figure 2A).

In the following years, several biologists made entoproct findings in nature, however, little focus was placed on their thorough description. Observing these small creatures in detail was still impossible due to the lack of modern light microscopes. In Bosc’s “Natural history of worms” from 1830, a drawing and description of a *Hydre jaune* appears, which later also was suggested to be an entoproct, and Lister in 1834 devoted an entire page of description to a zoophyte (clearly an entoproct) and noted its characteristic bending motion. Lister referred to Ellis’s description and presented a more detailed drawing. Milne-Edwards connected the descriptions of Ellis, Lister, and Bosc, and stated that “*Hydre jaune*” (*Hydra coronata* in Fleming, 1828) is a polype and certainly not a *Hydra* and he proposed a new genus for it, which he named “*Lusie (Lusia)*” (Deshayes and Milne-Edwards 1836).

The problem in these early years of entoproct research was how to determine the taxonomic position (and name) of these strange animals in respect to where they should be placed in the tree of life. This dilemma is reflected in the work of Lister for example, who wrote “the species seemed to be intermediate between such animals of *Flustra* as I had met with, and the pedunculated compound *Ascidia*...”. A zoophyte, closely resembling that described by Lister, was observed by Sharpey, and in 1836 he described the feeding mechanism of this zoophyte which he illustrated (Figure 2B).

The first entoproct genus, *Pedicellina*, was erected by Sars in 1835, who described two species *P. echinata* (now considered to be *P. cernua*) and *P. gracilis* (now *Barentsia gracilis*). The genus name he derived from the shape and movement of the animals, which reminded the author of the pincer-like appendages *pedicellariae* of some echinoderms. Sars stated in his description, that the general form and position of the digestive tract of *Pedicellina* was similar to that of *Vorticella*, and the tentacles resembled those of other polyps. For him it seemed that *Pedicellina* was the link between Infusoria (now mostly

Protista) and the polyps. In the following year, Wiegmann stated that the description of *Pedicellina* would define them as a naked bryozoan, and not *Vorticella* (Wiegmann 1836).

Generally, in these early years of biological research, a systematic understanding of invertebrate animals was being developed and this was a chaotic, but fruitful process. Within this changing hierarchy, the position of *Pedicellina* (the name Entoprocta was not yet invented) was unclear and the insecurity regarding the position of these strange animals was expressed by almost all researchers. An example which illustrates the dynamic development of the invertebrate system in the early 19th century is that Johnston in the first edition of his book *British Zoophytes* (Johnston 1838) mentioned the “entoproct” described by Lister (1834, see above) in the division Zoophyta Ascidioida and the family Vesiculariadae. In the second edition of this book published nine years later in 1847, the same entoproct can be found instead in the class Polyzoa (together with ectoprocts) and within its own family Pedicellinae. Note that the name Polyzoa was first coined in 1830 by John Vaughan Thompson who included only ectoproct species.

3. Description of new genera

Van Beneden (1845) gave the first detailed description of an entoproct. He investigated the species *Pedicellina belgica* (now *Barentsia gracilis*) and although he was not able to see the nervous system, he could observe the general hermaphroditism in entoprocts, and the cleavage and development of the larva of this species. The author stated that the natural relationship of *Pedicellina* was neither in the Vorticelles nor in the ascidians, but in the Bryozoa instead. Note that the name Bryozoa was introduced in 1831 by Ehrenberg who did not include entoprocts.

Subsequently observations of “*Pedicellina*” (*Pedicellina* and *Barentsia* according to recent taxonomy) were made in different regions. When a larval stage of a solitary entoproct was found, it was erroneously described as a “new animal form” under the name *Cyclopelma longociliatum* (Busch 1851).

A new “entoproct” genus (the name Entoprocta was still not invented) was described for the first time from a freshwater habitat near Philadelphia by Leidy in 1851, when he introduced the freshwater species *Urnatella gracilis*. He described the characteristic colonies of *Urnatella* comprising erect, articulated stalks growing from a common attachment base, without seeing the actual soft body parts, which he observed in later studies. However, Leidy suspected *Urnatella* belonged to the Polyzoa. Allman (1856) understood the necessity of searching for homologies instead of analogies, in order to find the allies of entoprocts. He explained similarities of entoprocts to other Polyzoa and also mentioned their uniqueness. Allman included *Urnatella* and *Pedicellina* in the Polyzoa (which he decided contained the two orders Phylactolaemata and the Gymnolaemata). *Urnatella* was tentatively included in the Gymnolaemata and *Pedicellina* in the Phylactolaemata. He realised the similarities between *Urnatella* and *Pedicellina* but decided the taxonomic position of *Urnatella* had to be provisional due to the lack of sufficient anatomical details of the species.

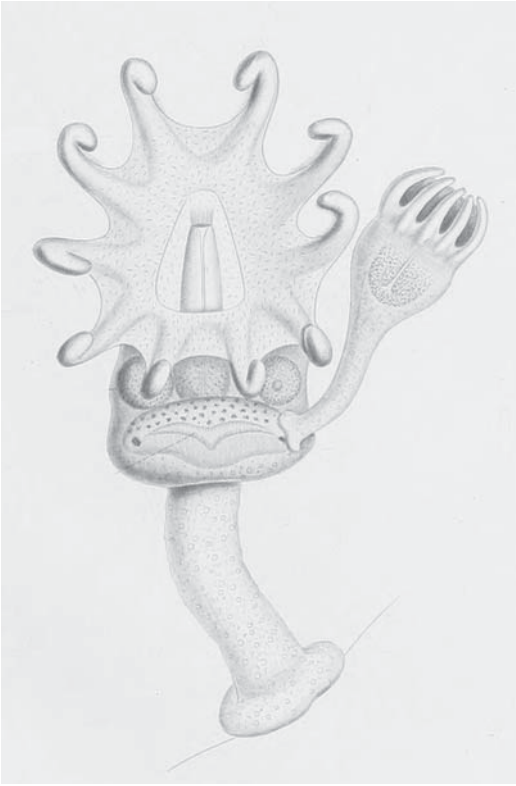


Figure 3. One of the first drawings of a solitary *Loxosoma* by Claparède (1863).

The findings of the 19th century were significantly expanded with the observation of a third entoproct genus, the solitary *Loxosoma*, which we know now, constitutes the most species-rich group of Entoprocta, and most species are found in epi-symbiotic relationships with other invertebrates. Nevertheless, in the original description (Norman 1861), the author did not realize *Loxosoma* was an independent organism but described them as posterior, club-shaped appendages of the “new echinoderm *Strephenterus claviger*” instead. (According to the description, the echinoderm is a sipunculid, a common host for some solitary entoprocts).

Later publications on entoprocts dealt with the description of new species, observations of different life cycle stages of *Loxosoma*, and included observations

of the developmental biology of entoprocts (e.g., Uljanin 1869). One of the early drawings of a *Loxosoma* species is shown in Figure 3.

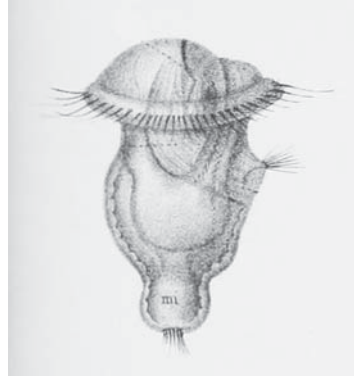
4. A natural group called Entoprocta

An important step in the history of entoproct research was made with the publications of Nitsche (1869, 1875). Nitsche reviewed much of the previous work and his own publications are augmented with useful anatomical illustrations. Through previous and his own studies Nitsche concluded that the genera *Urnatella*, *Pedicellina*, and *Loxosoma* were a natural grouping. He split them off from Bryozoa and proposed the name Entoprocta, leaving the taxon Ectoprocta as a separate group (Bryozoa *sensu stricto*). The family Entoprocta was defined by Nitsche (1869) as “Mouth and anus lie inside the tentacle crown, the anterior part of the body wall is not retractable, thus a tentacle sheath is lacking. The tentacles are arranged in a bilateral manner, not retractable but can just be infolded.”

In the next few decades researchers investigated entoprocts with much focus on embryonic development and the larval stage, and important observations of budding in entoprocts were made (Nitsche 1875). Barrois and Hatschek investigated the entire group

Figure 4. Drawing of a developing larva of *Pedicellina cernua* by Barrois (1877).

of Bryozoa, including Entoprocta and Ectoprocta. In his great monograph “Investigations of the embryology of Bryozoa”, Barrois described the larvae of the entoprocts *Pedicellina* and *Loxosoma* as well as many ectoprocts based on live observations. He observed the spiral cleavage in entoprocts, the radial cleavage of ectoprocts, and provided beautiful drawings of the development of some entoprocts and ectoprocts (Barrois 1877: Figure 4). Barrois considered entoproct and ectoproct larvae to be very similar and stressed the similarity of entoproct larvae and rotifers, based primarily on similarities of appendages between the groups. Hatschek’s work (1877) on the development of *Pedicellina* gives a comprehensive picture of the early developmental events. He observed total unequal cleavage in *Pedicellina* and also described the asexual budding process. Hatschek discussed the germ layer development with regard to other Bilateria, a matter which became of great importance for the scientific community at that time. Hatschek mentioned the similarity of the larva of *Pedicellina* with the cyphonautes larva and he considered entoprocts to be basal bryozoans, and argued that a close relationship between bryozoans and Rotifera could be true, if the bryozoan ganglion and the supraesophageal ganglion were homologous. On the other hand, the bryozoan larval form would put them at the base of the Mollusca.



Major works on the development and anatomy of *Loxosoma* and *Pedicellina* were published by Harmer (Harmer 1885, 1886). His monographs included descriptions of the metamorphosis of the entoproct larval stage as revealed by sectioning, and the first descriptions of parts of the entoproct nervous system and entoproct protonephridia. Harmer described both the larvae and adult entoproct as specialised trochospheres and considered the larvae of entoprocts to be similar to the cyphonautes larva due to both forms bearing a well developed digestive tract. Harmer also pointed out the similarity between *Loxosoma* and molluscan larva (e.g., the ciliated ring of entoprocts could be the velum, and the foot gland the shell gland), as well as that of larvae of chaetopod annelids, and referred to striking similarities between Entoprocta and Rotifera, as characters of the nervous system and muscle fibres. Harmer concluded that Polyzoa seem closest related to Mollusca. Salensky (1877) also noticed the possible homology of the posterior sensory organs of *Loxosoma* and the “antennae” of rotifers as did Barrois.

5. Controversy over entoproct systematics

The following years were characterized by discussions about the systematic position of Entoprocta, which continued alongside the ongoing studies of entoproct morphology. Most new findings fueled these controversies.

In 1888, Hatschek was the first to propose that the Entoprocta were a separate class within the lower worms (Cladus Scolecida; a group also containing Rotifera and flat worms *sensu lato*). Bryozoa (Ectoprocta) were grouped with Brachiopoda and Phoronida in the clade Tentaculata (= Molluscoidea). Hatschek based his view on the similarity of the entoproct larva and the trochophora of Mollusca and Annelida. Stiasny (1905), who described the nephridia of *Loxosoma* and *Pedicellina* in detail, agreed for the moment with Hatschek's classification, since the structure of entoproct nephridia would strictly separate entoprocts from ectoprocts (note: entoprocts have protonephridia, while ectoprocts have no excretory organs). The three main arguments that were put forward at that time in favour of a diphyletic origin of Entoprocta and Ectoprocta, were (1) the position of the anus inside versus outside the tentacle crown, (2) the presence or absence of a body cavity, and (3) protonephridia versus metanephridia. Note that the three arguments are valid until today, except that it has been shown that ectoprocts lack metanephridia.

Hatschek's opinion, however, was not adopted by several other researchers such as Ehlers (1890), Prouho (1832), Davenport (1893), Seeliger (1906), or Cwicklitzer (1909). Their arguments were based on morphological similarities between Entoprocta and Ectoprocta. Davenport refuted the three main arguments separating entoprocts from ectoprocts (see above) by (1) the closure of the tentacular corona between mouth and anus would be late in development, (2) the conditions would be continuous between ento- and ectoprocts (spaces in the entoproct parenchym would represent the ectoproct coelom), and (3) "the existence of an excretory tubule" in entoprocts would "at the presence not even be probable". The authors Seeliger and Cwicklitzer focused on the investigation of the larval stage. Seeliger (1906) concluded that entoprocts were the basal bryozoans. His arguments for this statement were the similar budding processes in entoprocts and ectoprocts and the similar anatomy of the larvae, especially of the larval nervous systems.

6. Dawning of a new age

In the years between 1920 and 1970, entoproct research reached a new level due to major findings regarding early embryology, larval stage, feeding mechanisms, regeneration and budding in entoprocts. Entoprocta were clearly recognized as unique taxon and previous work was reviewed and re-evaluated. New hypotheses regarding entoproct relationships were developed. Regarding entoproct taxonomy, three families were recognized in this period, the solitary Loxosomatidae, the colonial Pedicellinidae (including *Pedicellina*, *Barentsia* and some other genera) and the Urnatellidae.

In the early 1920s, a new name for entoprocts, Calysozoa (meaning "calyx-animals"), was proposed in the "New classification of animals", to distinguish them better from Bryozoa *sensu stricto* (= Polyzoa) (Clark 1921). Nevertheless, the similar name Calycozoa was already used for cnidarian stauromedusae and Cori (1929) chose a new name "Kamptozoa" for entoprocts. The latter name refers to the characteristic bending movement of the animals (from the Greek 'campto', I bend). Cori argued for a separation of entoprocts from bryozoans, with the former following the main body plan of lesser worms

Figure 5. Lateral view of 56-cell stage of *P. cernua*; cell-lineage study of Marcus (1939).



(Scolecida) on one hand, and Bryozoa *sensu stricto* (= Ectoprocta) following the common plan of Coelomata on the other hand. Several reasons for this view were listed by Cori (1936) and contain acoeli versus coelomate body plan, protonephridia versus unknown state, the ganglion being subesophageal as against supraesophageal. One main reason that Cori did not regard Entoprocta as ancestral to gymnolaemate bryozoans, as proposed earlier, was that in such a scenario, one would expect to find transitional forms between a sessile acoelomate and a sessile coelomate. Obviously, such a state is not represented. He saw the entoprocts rather, as the most developed form of Scolecida, and did not exclude the relationship of entoprocts to rotifers. Cori's two main publications on Entoprocta in 1929 and 1936 are of special value since they comprise an elaborate review of previous works combined with the author's own studies. The works cover detailed aspects of the history of research, entoproct reproduction, development, anatomy, regeneration, ecology, taxonomy and phylogenetic perspective.

Further studies provided additional support for Entoprocta/Kamptozoa independence of Bryozoa. The feeding mechanism of entoprocts differs broadly from that of Ectoprocta (Atkins 1932). The intestine in larval and adult *Pedicellina* does not suggest a close relationship of entoprocts and ectoprocts Becker (1937). In 1939, Marcus was able to observe the cleavage and embryology of *Pedicellina cernua* and his results of the cell-lineage and cell-fate remain the only source of entoproct cell-lineage data until today (Figure 5).

Entoprocts are certainly not missing in Hyman's great work of invertebrate animals (Hyman 1951). She summarized knowledge about the phylum Entoprocta and rejected the possibility of a close relationship between entoprocts and ectoprocts due to several reasons; the condition of no coelom versus coelomic cavity was considered by her to be the most decisive. Affinities of entoprocts were instead drawn with the pseudocoelomate groups and the closest relatives were thought to be the Rotifera. The latter shared with entoprocts, similarities as glands in a stalk, protonephridia, similarity of parts of the digestive tract, and Hyman considered the antennae of rotifers homologous with the pair of preoral organs in entoproct larvae and loxosomatid adults, as proposed by earlier investigators.

Prenant and Bobin (1956), however, treated entoprocts and ectoprocts as two classes of Bryozoa in their book *Faune de France*. However, their most useful contribution was to key the entoprocts in a detailed form with useful drawings and descriptions.

Jägersten (1964, 1972) developed new hypotheses of metazoan evolution. The essence of his work was to focus on the entire life cycle of animals and his aim was to get more insights in metazoan evolutionary relationships through implementing their comprehensive life-histories. Regarding entoprocts, Jägersten considered their evolution, including pedomorphosis. The entoproct larval stage retained some features of an ancestral free-

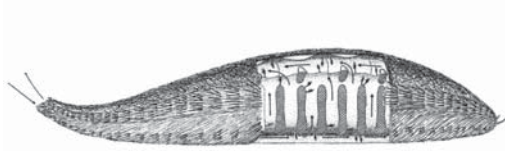


Figure 6. *Loxosmatid entoprocts, Loxosomella fauveli, on their polychaete host Aphrodita aculeata. Arrows show the direction of the respiration water current produced by the host. From Nielsen, 1966.*

living adult, as for example, the ciliated creeping sole, as well as it gained truly larval organs. This larva then gave rise to a secondary, benthic adult. Concerning intraphyletic relationships, Jägersten considered all similarities between Entoprocta and Bryozoa as convergences due to a sessile life and thus strongly rejected a close relationship between the taxa.

7. A new generation of entoproct researchers

Throughout the entire history of entoproct research, only a few researchers have concentrated their studies on Entoprocta. As the examples above have shown, many biologists treated entoprocts as part of broader faunistic studies. Clear exceptions are the zoologists Peter Emschermann and Claus Nielsen. Their continuous studies of entoprocts are so profound to the history of entoproct research, that this section is dedicated to their work.

Emschermann's work encompasses general research and reviews of the phylum (Emschermann 1985, 1995), descriptions of new species (Emschermann 1972), peculiar organs (Emschermann 1969), while the main part of his research was dedicated to *Barentsia* (Emschermann 1961) and the freshwater *Urnatella*. Two main studies focused on the description of protonephridia in *Urnatella gracilis* and the sexual reproduction and larval stage of the same species (Emschermann 1965a, b). Other merits of his work lie in providing information about the laboratory culture of entoprocts and to clarify general issues regarding entoproct musculature and anatomy of the body wall. It was not the focus of Emschermann to hypothesise about the unresolved entoproct phylogeny within Spiralia. Instead, he was concerned with the internal evolution of the phylum. Based on thoughtful interpretations of what was known about the taxon, he presented an important concept of entoproct evolution in 1972.

The entoproct studies of Nielsen are nearly impossible to review here, because they have resulted from a life-long study. Indeed the reference list on entoproct and ectoproct research by this scientist would take more space than the entire article. Every future researcher who wants to work on Entoprocta, will undoubtedly study his work in detail. Nielsen's major works include comprehensive studies of the Atlantic entoproct fauna which contain a thorough key of the Danish and British Entoprocta (Nielsen 1964, 1989). Nielsen's work comprises research on the life cycle, metamorphosis and development of a notably large number of entoproct species (Nielsen 1966, 1971). Noteworthy is the focus by Nielsen on all life cycle stages and the comprehensive description of the metamorphosis

and the larval stage of many species. Several of his studies include ecological aspects, adding to the comprehensive approach of this researcher. Nielsen's work is to a great extent based on live and electron microscopical investigations. Major findings were made regarding entoproct anatomy (Nielsen and Jespersen 1997), structure of entoproct larval stages and metamorphosis (Nielsen 1967), as well as ecology (Nielsen 1964, figure 6). Major merits of Nielsen's research lie, in my opinion, in the multi-faceted approach to try to study various aspects of the phylum Entoprocta, in accentuating the importance of the larval stage, in summarizing previous studies in a comprehensive way, in new species descriptions, systematic reviews and revisions (Nielsen 1996, 2010). The phylogenetic relationships of Entoprocta and Ectoprocta are a major interest of Nielsen. His opinion conforms to that of some early researchers in treating entoprocts and ectoprocts as two classes of the phylum Bryozoa. His main arguments are the similarity of the larval stage and their metamorphosis, of the budding process, and of the formation of resting bodies. Regarding the difference of the coelomic condition, Nielsen believes that the narrow spaces between mesodermal cells in entoprocts have evolved into the ectoproct coelom. The arguments for a united Bryozoa are clearly put forward in Nielsen's earlier works. However, in his later works, the arguments for a close relationship of entoprocts and ectoprocts are reconsidered and Nielsen writes "the weak arguments for regarding ectoprocts and entoprocts as sister groups come from their ontogeny", and he concludes that ectoprocts are a very specialised taxon with uncertain origin, however possible sister-group relationship with entoprocts (Nielsen 2001).

8. Fossils and Australian entoprocts

An important discovery of the first entoproct fossil was made by Todd and Taylor in 1992. This Jurassic fossil was that of a colonial *Barentsia*. It was long considered that colonial entoprocts are more derived as solitary forms and if this was true, the ancestral entoproct evolved (much) earlier than 150 million years ago.

From the late 20th century onwards few scientists focused their research on Entoprocta. However, one exception is Kerstin Wasson who contributed to entoproct research by publishing papers about Australian entoprocts (Wasson 1995), clarifying the systematics of North American colonial entoprocts (Wasson 1997), provided several investigations concerning the sexual reproduction of colonial entoprocts (Wasson 1998), as well as reporting on entoprocts as invasive organisms (Wasson *et al.* 2000). Wasson's work also includes a remarkable review of the phylum (Wasson 2002).

9. Turning point – a new phylum, molecular tools and more morphology

The transition from the 20th to the 21st century was an important turning point in entoproct research. The discovery of the new phylum Cycliophora, the invention of molecular tools, and advances in tools investigating morphology enabled reconsiderations of the phylogeny of Entoprocta.

The description of the new animal phylum Cycliophora was groundbreaking also, because morphological characteristics were found, which suggested a close relationship to Entoprocta (Funch and Kristensen 1995, 1997). In the original description, the authors also considered Cycliophora to have a close relationship with ectoprocts. However, a main argument for this hypothesis was the disappearance of the larval apical ganglion at metamorphosis and this feature is probably uninformative, since it is common in many eumetazoans (see Wanninger and Haszprunar 2003).

The “era of molecular research” was initiated for Entoprocta in 1996. The molecular study included 18S rRNA data from two entoprocts and indicated that Entoprocta and Ectoprocta are not sister taxa and that Entoprocta are more closely related to other Spiralia than to ectoprocts (Mackey *et al.* 1996). The position of Entoprocta within Lophotrochozoa was later corroborated by large-scale studies of ribosomal and phylogenomic data (Paps *et al.* 2009a, Dunn *et al.* 2008, Giribet *et al.* 2000).

The close relationship of Entoprocta and Cycliophora was confirmed by mitochondrial and ribosomal DNA and RNA data, as well as phylogenomic data (Mallat *et al.* 2010, Fuchs *et al.* 2010, Hejnol *et al.* 2009, Paps *et al.* 2009b, Passamanek and Halanych 2006). Cladistic analyses of morphological data came to the same result (Obst 2003, Sørensen *et al.* 2000, Zrzavy *et al.* 1998).

However, some molecular studies showed a different result. An EST (expressed sequence tag) based study suggested high support for an entoproct-ectoproct clade (Hausdorf *et al.* 2007), however, important outgroups such as Cycliophora or Brachiozoa were missing in the study. Another EST study showed a sister group relationship of Entoprocta and Platyhelminthes (Hausdorf *et al.* 2010). The complete mitochondrial genomes of two entoproct species were determined and the gene organization was similar to that of molluscs, and analyses of protein-coding mitochondrial genes suggested a close relationship of Entoprocta with Phoronida instead (Yokobori *et al.* 2008).

A close relationship of Mollusca and Entoprocta was not revealed by other molecular studies, however, cladistic analyses of anatomical data showed a close relation of the two taxa (Haszprunar 1996). In 2007, the observation of the serotonergic nervous system of an entoproct creeping-type larva revealed an elaborate apical organ comprising about 14 bipolar neurons, four nerve cords and additional nerves (Wanninger *et al.* 2007). This tetraneural condition, the complex apical organ, and characteristics as a chitinous cuticle, a sinus circulatory system, and the structure of the ciliated creeping foot are shared by entoprocts and basal mollusks (Haszprunar and Wanninger 2008). Thus, the authors suggested a sistergroup relationship of entoprocts and molluscs, for which the name Tetraneuralia was proposed (Wanninger 2009).

The beginning of 21st century also brought further important insights concerning entoproct morphology and systematics. In Japan, new entoproct findings led to the recognition of a new entoproct genus and revision of entoproct systematics (Iseto 2001, 2002).

Incomplete knowledge of the muscular bodyplan and the nervous system was a large gap in entoproct research. However, F-actin staining and confocal laser scanning

microscopy revealed the myo-anatomy of some entoproct species, providing new characters for systematic inferences (Wanninger 2004, Fuchs and Wanninger 2008, Schwaha *et al.* 2010). The nervous system was studied by immunocytochemical investigations, revealing morphological characters useful in systematics and taxonomy (Fuchs *et al.* 2006).

In 2005, the second freshwater entoproct species was described from rivers in Thailand (Wood 2005). Its internal morphology revealed a highly complex nephridial system as adaptation to the life in freshwater and the study provides several characters for taxonomic and phylogenetic inferences (Schwaha *et al.* 2010). Comparison of the fine structure of attachment structures of solitary entoprocts provides new characters useful for entoproct taxonomy (Iseto and Hirose 2010). The internal relationships of Entoprocta were studied using mitochondrial and nuclear gene sequences of 18 species (Fuchs *et al.* 2010). The results confirmed the monophyly of Entoprocta and the two main clades Coloniales and Solitaria. The results suggested that the ancestral entoproct was a solitary, marine, epizoic organism.

10. Conclusion

Entoprocta are spiralian lophotrochozoans, and both morphological and molecular data suggest a sister group relationship of Entoprocta and Cycliophora. Further lophotrochozoan affinities of these clades are less clear.

The 21st century has shown that molecular data are crucial to resolve entoproct relationships and are required to provide a stable framework for phylogenetic inferences. Knowledge about fossils, morphology and ecology of all life cycle stages is needed to make inferences about the evolution of entoprocts while considering their molecular position. Listing morphological characters as “pros and cons” for inference of entoproct phylogenetic relationships gives little resolution and scientific methods as cladistic analyses, implementing these characters, can be used instead.

Clearly, much information about entoprocts is still lacking. Some of the aspects that could be addressed in future studies with modern methods are: cell-lineage and embryology of entoprocts, the development of the nervous system, the musculature and other organ systems, and higher entoproct taxon sampling in molecular studies with inclusion of basal species.

Combining all data should be the aim. Definitely, things are progressing as 250 years of entoproct research has demonstrated.

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