SPIRALIA/LOPHOTROCHOZOA

Torsten H. Struck
University of Oslo, Department of Research and Collections, Natural History Museum, PO Box 1172 Blindern, N-0318 Oslo, Norway
Zoological Research Museum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany

Keywords: Phylogeny, Spiralia, Lophotrochozoa, Annelida, Mollusca, Nemertea, Entoprocta, Cycliophora, Ectoprocta, Brachiopoda, Phoronida, Gastrotricha, Playthelminthes, Syndermata, Micrognathozoa, Gnathostomulida, Dicyemida, Chaetognatha

Contents

Summary

The names Spiralia and Lophotrochozoa have been used for a similar group of taxa in the last years. More specifically, inclusion of Gnathostomulida, Micrognathozoa, Syndermata, Gastrotricha, Playthelminthes, Brachiopoda, Phoronida, Ectoprocta, Nemertea, Entoprocta, Cycliophora, Mollusca and Annelida in this taxon is strongly supported. Although the names have been used interchangeably, their definition and delimitation differs between authors and, hence, a clear understanding of what we mean by these names is provided first. This clarification is useful for the discussion of the phylogeny of Spiralia and its history, which based on recent findings, to suggest that Lophotrochozoa is a subtaxon of Spiralia. In addition to the phylogeny of Spiralia, each of the 13 taxa will be reviewed with respect to its general biology, morphology, natural history, life strategies and internal phylogeny. The phylogenetic position of Dicyemida, Orthonectida and Chaetognatha with respect to Spiralia is also briefly discussed. The impact of the spiralian taxa on human life and welfare is summarized at the end.

1. Introduction to Spiralia and Lophotrochozoa

Spiralia has long been recognized within metazoans. The characteristic feature of the group is spiral cleavage in early development. Spiral cleavage is a stereotypic cleavage pattern in which the orientation of the mitotic spindle is orientated obliquely to the main egg axis. As a result, successive tiers of blastomeres arrange themselves radially to the polar axis and the blastomeres in an upper group rest in the furrow between two blastomeres in the next lower tier. The oblique orientation of the spindle to the axis alternates in strict clockwise-counterclockwise patterns. Moreover, the fate of blastomeres is strongly determined by the stereotypic cleavage. For example, the 4d blastomer gives rise to all cells of the endomesoderm. Typical spiral cleavage is observed in Annelida, Mollusca, Nemertea, Platyhelminthes and Entoprocta (also known as Kamptozoa). It has also been described for Gnathostomulida in one instance, but this needs further corroboration. All these taxa have been grouped together as Spiralia. Given the Articulata hypothesis grouping Annelida and Arthropoda together, Arthropoda were originally included although they do not exhibit spiral cleavage.

Based on molecular phylogenetic analyses, this traditional group of Spiralia is not supported as Arthropoda is placed within Ecdysozoa and in turn the lophophorate taxa Ectoprocta (also known as Bryozoa *sensu strictu*), Brachiopoda and Phoronida were regarded as more closely related to Mollusca and Annelida. Lophophorata were traditionally seen as taxa with deuterostome affinities. To reflect these changes the name Lophotrochozoa was coined and is well established in recent literature. The name reflects that most of these taxa possessed either a lophophore filtering apparatus or a trochophore larva. However, others still herald the name Spiralia by incorporating these changes. Hence, the two names are used interchangeably for the same set of taxa by different authors.

Recent studies based on genome-scale data, so-called phylogenomic studies, strongly supported a clade of 13 taxa comprising the spiralian taxa Annelida, Mollusca, Nemertea, Entoprocta, Playthelminthes and Gnathostomulida, the lophophorate taxa Ectoprocta, Brachiopoda and Phoronida and additionally Cycliophora, Gastrotricha, Syndermata ("Rotifera" and Acanthocephala) and Micrognathozoa. So far signatures of cleavage patterns similar to spiral one could only be shown for Phoronida. Moreover, support for a clade of these 13 taxa stems only from molecular data. No morphological apomorphic character is known that is common to all or the vast majority of these taxa, but not to any other animal taxon.

Although the two names Lophotrochozoa and Spiralia have been used interchangeably, their definition does not necessarily cover the same set of taxa. Spiralia has always been used for the clade that comprises all taxa with spiral cleavage and a few more for which descent from an ancestor with spiral cleavage has been assumed. Hence, Spiralia could be defined by the last common ancestor of taxa with spiral cleavage, plus all the descendants of that ancestor. Lophotrochozoa is defined by the last common ancestor of Annelida, Mollusca and the three lophophorate taxa Ectoprocta, Phoronida and Ectoprocta, plus all the descendants of that ancestor. Hence, Lophotrochozoa might be more or less inconclusive than Spiralia. For example, if Platyhelminthes were sister to all other taxa mentioned here, Lophotrochozoa would be less inclusive. Therefore, to avoid confusion, the two definitions will be strictly applied herein as they should be in future publications.

2. Phylogeny of Spiralia

The composition of Spiralia as encompassing 13 taxa has been confirmed by several studies, but their phylogenetic relationships still remain controversial. Some progress was supposedly achieved by the first phylogenomic studies consistently recovering three clades within Spiralia, which rendered Spiralia redundant with Lophotrochozoa (Figure 1A). The first clade was Polyzoa consisting of Ectoprocta, Entoprocta and Cycliophora, with the latter two constituting a clade within this clade. While no synapomorphic morphological character supports Polyzoa, the sister-group relationship of Entoprocta and Cycliophora is supported by the presence of mushroom-shaped extensions of the basal membrane into the ectodermal cells, among other characters. The second clade was Platyzoa, comprising Platyhelminthes, Gastrotricha, Gnathostomulida, Micrognathozoa and Syndermata. Again morphological synapomorphies are lacking for this clade. Within this clade, the taxon Gnathifera, comprising Gnathostomulida, Micrognathozoa and Syndermata, is strongly supported by both the presence of a complicated jaw apparatus and phylogenomic data. The remaining taxa Annelida, Mollusca, Nemertea, Brachiopoda and Phoronida were grouped together as Trochozoa in these studies. Although the name refers to the trochophore larva, the larva is not a character uniting these taxa. Within this clade, Nemertea, Brachiopoda and Phoronida were grouped together as Kryptrochozoa, but again without substantial support from either morphology or molecular data. Moreover, further analyses of the data indicated that artifacts like long-branch attraction and compositional heterogeneity might hamper the reconstruction of spiralian relationships.

Therefore, recent phylogenomic analyses not only increased the number of species and genes analyzed, but also conducted more thorough analyses to ameliorate misleading effects of artifacts. These analyses revealed that systematic biases had an impact on the previous results and found strong support for especially the more basal relationships within Spiralia (Figure 1B). None of the three previous clades mentioned above were recovered. Instead Platyzoa was paraphyletic with respect to all other spiralian taxa and support for the previous monophyly could be attributed to long-branch attraction. Gnathifera was monophyletic and branched off first from the spiralian stem lineage. Platyhelminthes was sister to Gastrotricha. Rouphozoa (= Platyhelminthes + Gastrotricha) was sister to a clade comprising Lophophorata (i.e., Phoronida, Brachiopoda, Ectoprocta), Mollusca, Annelida as well as Nemertea, Entoprocta and Cycliophora. Hence, this clade fulfilled the above-mentioned definition of Lophotrochozoa and Lophotrochozoa as originally defined is not synonymous with Spiralia. Within Lophotrochozoa monophyly of Lophophorata was also recovered with strong support after addressing compositional biases in the dataset. Hence, support for Kryptrochozoa and Polyzoa could be attributed to artificial signal in the dataset. Morphological characters like the lophophore filter-feeding apparatus also support monophyly of Lophophorata. Additionally, the close relationship of Entoprocta and Cycliophora was further substantiated by these studies. Additionally, based on morphological data a closer relationship of Mollusca and Entoprocta/Cycliophora is known as the Lacunifera or Tetraneuralia hypothesis. In the organization of this chapter the author followed the more recent and comprehensive analyses about spiralian relationships. However, it should be noted that these hypotheses are still in need for additional support.

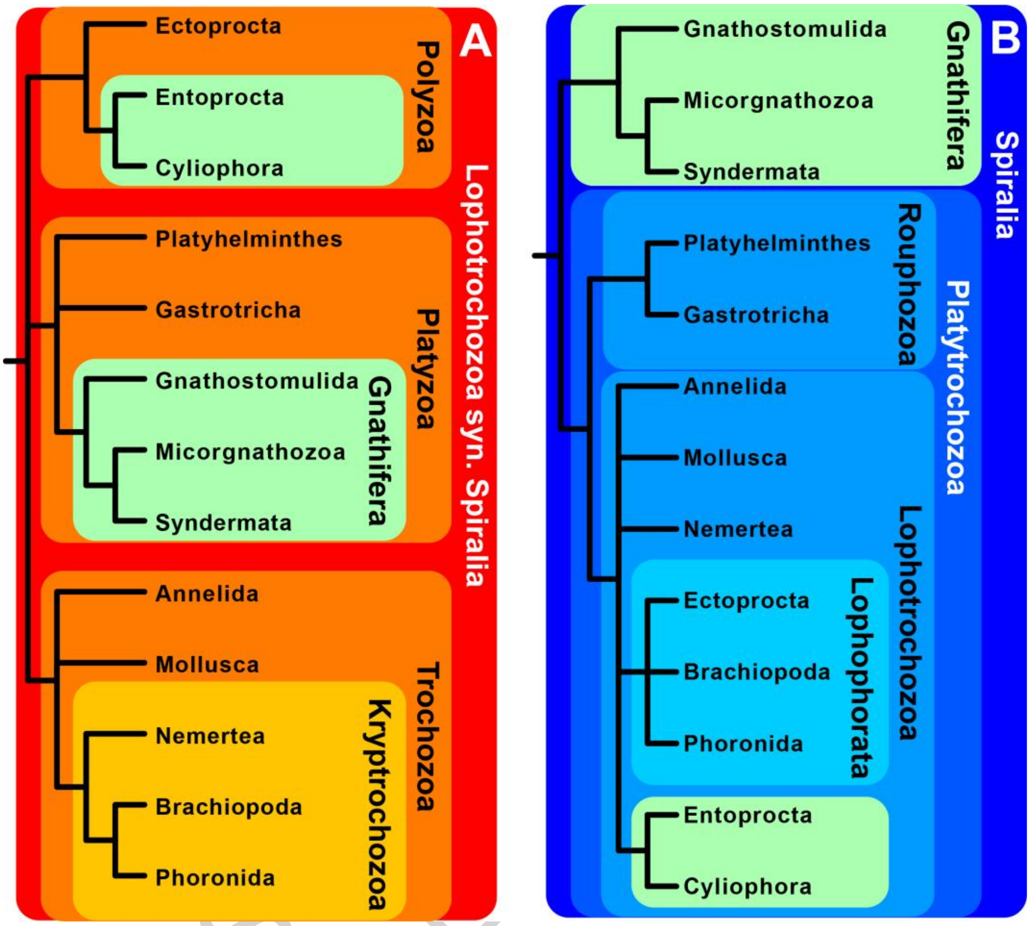

Figure 1. The two alternative phylogenies of Spiralia/Lophotrochozoa and their nomenclatural consequences. Groups that are in common between both are highlighted in green.

3. Gnathifera

Strong support for Gnathifera comprising Gnathostomulida, Micrognathozoa and Syndermata was gathered from both morphological and molecular data. All three taxa are characterized by the presence of a complicated jaw apparatus (Figure 2). Data from transcriptomic libraries and mitochondrial genomes substantiate this clade. Within Gnathifera an apical intracytoplasmic lamina in the cellular integument point to a closer relationship of Micrognathozoa and Syndermata.

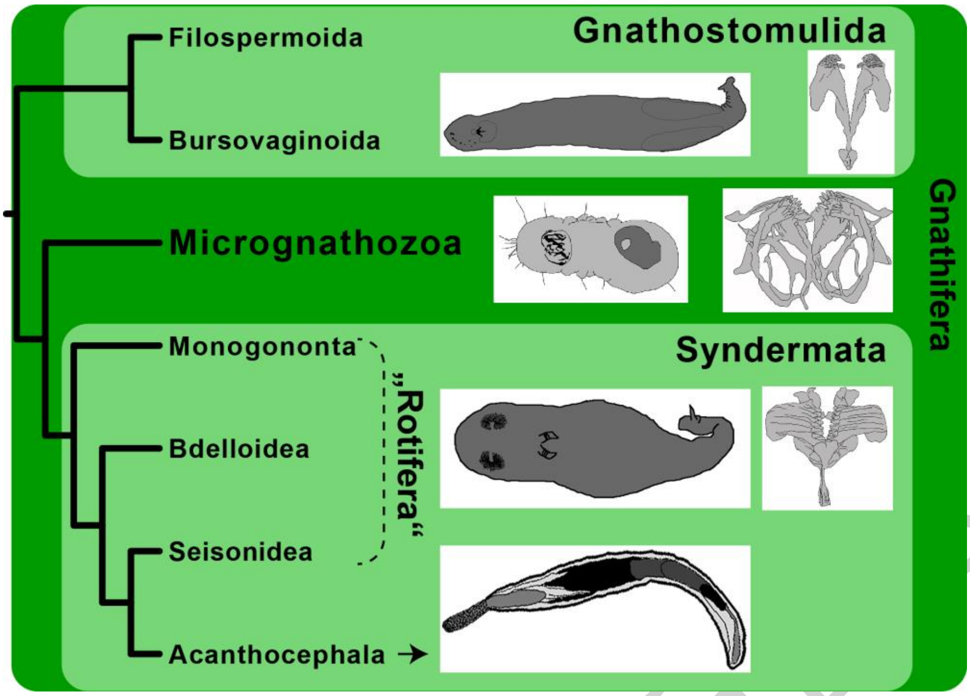

Figure 2. The phylogeny of Gnathifera and its subgroups Gnathostomulida and Syndermata. Graphical depictions of representatives of Gnathostomulida, Micrognathozoa, rotiferan Syndermata and Acanthocephala as well as of their jaw apparatuses are shown.

3.1. Gnathostomulida

Gnathostomulida (jaw worms) comprises about 100 species, which inhabit the marine interstitium. Gnathostomulids are especially abundant in sulfide- and detritus-rich sediments. Gnathostomulids are small animals with a length of usually 1 mm, rarely up to 4 mm and diameters of 40-100 µm. Both the “head” before the mouth and the posterior end can either be elongated and pointed or rounded. The mouth bears the ventral pharyngeal jaw apparatus. As a permanent anus is lacking, the gut is seemingly not straight and a one-way gut. However, a posterior region of the epidermis lacks a basal membrane and is in direct contact with the hindgut, so that this region might function as a temporary anus. The epithelial cells are monociliated and facilitate locomotion through the interstitium. The musculature is not involved in locomotion. The nervous system is simple, consisting of an unpaired frontal ganglion, an unpaired buccal ganglion and paired buccal and longitudinal nerves. Osmoregulation is achieved by protonephridia, and coelomic cavities are lacking.

Gnathostomulids are hermaphrodites with testis occurring behind the ovary. Sperm transfer is achieved either by hypodermal injection or copulation via a vagina or a bursa system. Egg cases are attached to sand grains, and the development is direct. The hatching juvenile stage is ciliated and bears a jaw apparatus.

Within Gnathostomulida two major groups are recognized: Filospermoida and Bursovaginoida (Figure 2). The body shape of Filospermoida is thread-like and the flagellate, mobile sperms are filiform. On the contrary, Bursovaginoida are stout with a rounded head. Sperm are immobile due to lack of a flagellum. A bursa system and/or a vagina are present.

3.2. Micrognathozoa

Micrognathozoa is represented by a single described species, *Limnognathia maerski*. It has, for example, been reported from a locality in Greenland, where it inhabits pads of mosses. *L. maerski* is small with a length of 100 to 150 µm, and locomotion is achieved by epidermal cilia. The anterior and posterior ends are rounded and caudal gland cells allow a strong, but reversible attachment to the substrate. Despite its small size, *Limnognathia* bears a large and complex jaw apparatus consisting of unpaired and several paired pieces. Hindgut and permanent anus are lacking, but the midgut might temporarily open at the posterior end and hence function as an anus. The nervous system consists of an unpaired frontal ganglion and paired longitudinal nerves. Osmoregulation is achieved by protonephridia and coelomic cavities are absent. Up to now only females are known; they possess paired ovaries. Development is direct.

3.3. Syndermata

Syndermata comprises the free-living rotifers (wheel animals) with more than 2,000 species and the parasitic acanthocephalans (thorny-headed worms) with 1,100 species (Figure 2). Rotifers and acanthocephalans have the syncytial epidermis in common. Due to their different life histories, rotifers and acanthocephalans differ not only in their habitats, but also have very different body organizations. Rotifers generally occur in limnetic habitats as well as in pads of mosses or wet soil. Some rotifers are also known from the marine interstitium and pelagic zone. Acanthocephalans are gut parasites in aquatic or terrestrial vertebrates like fishes, birds and mammals, with an obligate host change. The intermediate host is usually a crustacean or insect.

Rotifers are small, usually less than 1 mm in length. Some dwarf males are only 40 µm long. The body of rotifers is divided into three regions: head, trunk and foot. The head bears the wheel organ, which is a field of cilia involved in food collection and swimming. The foot ends in toes with adhesive glands for temporary or permanent attachment to substrates. Rotifers possess a complex jaw apparatus consisting of several pieces and an anus (Figure 2). Photoreceptors of different types like pigment-cup eyes or phaosomes occur. Three pairs of ganglia connected by a pair of lateroventral nerves constitute the nervous system. Osmoregulation is achieved by protonephridia and coelomic cavities are absent.

Acanthocephalans range from 2 mm to 70 cm. The gut is entirely lacking and food from the host, usually in the form of lipids, is taken up via the skin. Protonephridia are only present in acanthocephalans parasitizing terrestrial hosts. The nervous system comprises a cerebral ganglion with only a few cells and a pair of longitudinal nerves. The epidermis together with longitudinal and circular muscles envelops the large body cavity. The most prominent feature is the rostrum, a protrusible, hollow proboscis, which is retracted into a cavity (proboscis sac) by muscles. The cavity is separated from remaining body cavity by a sheath. A contraction of the circular body wall muscles and the pressure of the fluid-filled body cavity protrude the rostrum. The rostrum is covered with hooks to anchor acanthocephalans in the host. In some acanthocephalans hooks occur also on the trunk.

Syndermata exhibit very different reproductive strategies. Syndermata are generally gonochoristic, but Bdelloida lack males throughout their life and Monogononta have only occasionally dwarf males. In Seisonida and Acanthocephala the sexes are of equal size and only bisexual reproduction occurs. While Acanthocephala transfer sperm by copulation, Seisonida deposit the sperms in spermatophores. Bdelloida reproduce strictly unisexual by parthenogenesis. Monogononta show heterogony. Generally reproduction is achieved by parthenogenesis. However, upon worsening environmental conditions dwarf males are produced and the fertilized eggs become resting eggs till conditions improve. In rotifers development is direct and fast. In acanthocephalans development takes place within the mother till the acanthor larva, which is then enveloped by several layers and released with the feces of the host. This egg infests the intermediate host, where the acanthor hatches. It develops via the acanthella to the resting, infectious cystacanthus. When preying upon the intermediate host, the definitive host is infected and the adult stage develops.

Rotifers have been grouped into Seisonida, Monogononta and Bdelloida (Figure 2). Seisonida are epibiotic on crustaceans. Bdelloida are characterized by the presence of a rostrum, which is homolog to the one of Acanthocephala, but without hooks. Together with the foot, the rostrum is used for leech-like locomotion. Monogononta possess only one gonad. Molecular studies have shown that Acanthocephala are a subgroup of the rotifers as sister to Seisonida. They have in common a life history associated with an arthropod host and the reduction of the wheel organ. Bdelloida is the sister group of this clade and they share the presence of a retractable anterior end.

4. Rouphozoa

A clade Rouphozoa comprising Gastrotricha and Platyhelminthes has only recently been proposed based on phylogenomic analyses. However, no morphological character is known to date supporting this clade. The name Rouphozoa refers to the fact that both taxa in their ground pattern collect food only by the aid of their sucking pharynx. In all other spiralian taxa reconstructions of the ground patterns indicated that additional structures for food gathering like jaws in Gnathifera, palps in Annelida or a lophophore in Brachiopoda are present in their ancestor state.

-

-

-

Bibliography

Aguinaldo, A., Turbeville, J., Linford, L., Rivera, M., Garey, J., Raff, R., Lake, J. (1997). Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature*, 387, 489 - 493. [This is the classical article on Ecdysozoa]

Andrade, S.C.S., Montenegro, H., Strand, M., Schwartz, M., Kajihara, H., Norenburg, J.L., Turbeville, J.M., Sundberg, P., Giribet, G. (2014). A transcriptomic approach to ribbon worm systematics (Nemertea): resolving the Pilidiophora problem. *Mol. Biol. Evol.*, 31, 3206-3215. [This article provides a discussion of the phylogeny and evolution of Nemertea]

Edgecombe, G., Giribet, G., Dunn, C., Hejnol, A., Kristensen, R., Neves, R., Rouse, G., Worsaae, K., Sørensen, M. (2011). Higher-level metazoan relationships: recent progress and remaining questions. *Org. Divers. Evol.*, 11, 151-172. [This article provides a review of the phylogeny of Spiralia and Lophotrochozoa]

Egger, B., Lapraz, F., Tomiczek, B., Müller, S., Dessimoz, C., Girstmair, J., Škunca, N., Rawlinson, Kate A., Cameron, Christopher B., Beli, E., Todaro, M.A., Gammoudi, M., Noreña, C., Telford, M.J. (2015). A Transcriptomic-Phylogenomic Analysis of the Evolutionary Relationships of Flatworms. *Curr. Biol.*, 25, 1347–1353. [This article provides a discussion of the phylogeny and evolution of Platyhelminthes]

Fuchs, J., Iseto, T., Hirose, M., Sundberg, P., Obst, M. (2010). The first internal molecular phylogeny of the animal phylum Entoprocta (Kamptozoa). *Mol. Phylogenet. Evol.*, 56, 370-379. [This article provides a discussion of the phylogeny of Entoprocta]

Golombek, A., Tobergte, S., Struck, T.H. (2015). Elucidating the phylogenetic position of Gnathostomulida and first mitochondrial genomes of Gnathostomulida, Gastrotricha and Polycladida (Platyhelminthes). *Mol. Phylogenet. Evol.*, 86, 49–63. [This article provides a discussion of the phylogeny and evolution of Gnathifera]

Halanych, K.M. (2004). The new view of animal phylogeny. *Annu. Rev. Ecol. Evol. Syst.*, 35, 229-256. [This article provides a review of the phylogeny of Spiralia and Lophotrochozoa]

Halanych, K.M., Bacheller, J.D., Aguinaldo, A.M.A., Liva, S.M., Hillis, D.M., Lake, J.A. (1995). Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science*, 267, 1641-1643. [This is the classical article on Lophotrochozoa]

Hausdorf, B., Helmkampf, M., Nesnidal, M.P., Bruchhaus, I. (2010). Phylogenetic relationships within the lophophorate lineages (Ectoprocta, Brachiopoda and Phoronida). *Mol. Phylogenet. Evol.*, 55, 1121-1127. [This article provides a discussion of the phylogeny and evolution of Lophophorata]

Kieneke, A., Riemann, O., Ahlrichs, W.H. (2008). Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters. *Zool. Scr.*, 37, 429-460. [This article provides a discussion of the phylogeny and evolution of Gastrotricha]

Kocot, K.M., Cannon, J.T., Todt, C., Citarella, M.R., Kohn, A.B., Meyer, A., Santos, S.R., Schander, C., Moroz, L.L., Lieb, B., Halanych, K.M. (2011). Phylogenomics reveals deep molluscan relationships. *Nature*, 477, 452–456. [This article provides a discussion of the phylogeny and evolution of Mollusca]

Laumer, C.E., Hejnol, A., Giribet, G. (2015). Nuclear genomic signals of the 'microturbellarian' roots of platyhelminth evolutionary innovation. *eLife*, 4, e05503. [This article provides a discussion of the phylogeny and evolution of Platyhelminthes]

Laumer, C.E., Bekkouche, N., Kerbl, A., Goetz, F., Neves, Ricardo C., Sørensen, Martin V., Kristensen, Reinhardt M., Hejnol, A., Dunn, Casey W., Giribet, G., Worsaae, K. (2015). Spiralian Phylogeny Informs the Evolution of Microscopic Lineages. *Curr. Biol.*, 25, 2000-2006. [This article provides a discussion of the phylogenetic position of Micrognathozoa]

Nesnidal, M., Helmkampf, M., Meyer, A., Witek, A., Bruchhaus, I., Ebersberger, I., Hankeln, T., Lieb, B., Struck, T., Hausdorf, B. (2013). New phylogenomic data support the monophyly of Lophophorata and an Ectoproct-Phoronid clade and indicate that Polyzoa and Kryptrochozoa are caused by systematic bias. *BMC Evol. Biol.*, 13, 253. [This article provides a discussion of the phylogenetic position of Lophophorata]

Nielsen, C. (2012). *Animal Evolution - Interrelationships of the Living Phyla*, 402 pp. Oxford University Press Inc., New York. [This book provides a comprehensive review of the animal kingdom]

Petrov, N.B., Aleshin, V.V., Pegova, A.N., Ofitserov, M.V., Slyusarev, G.S. (2010). New insight into the phylogeny of Mesozoa: Evidence from the 18S and 28S rRNA genes. *Moscow Univ. Biol.Sci. Bull.*, 65, 167-169. [This article provides a discussion of the phylogeny and evolution of Dicyemida and Orthonectida]

Smith, S.A., Wilson, N.G., Goetz, F.E., Feehery, C., Andrade, S.C.S., Rouse, G.W., Giribet, G., Dunn, C.W. (2011). Resolving the evolutionary relationships of molluscs with phylogenomic tools. *Nature*, 480, 364–367. [This article provides a discussion of the phylogeny and evolution of Mollusca]

Struck, T.H., Paul, C., Hill, N., Hartmann, S., Hösel, C., Kube, M., Lieb, B., Meyer, A., Tiedemann, R., Purschke, G., Bleidorn, C. (2011). Phylogenomic analyses unravel annelid evolution. *Nature*, 471, 95–98. [This article provides a discussion of the phylogeny and evolution of Annelida]

Struck, T.H., Wey-Fabrizius, A.R., Golombek, A., Hering, L., Weigert, A., Bleidorn, C., Klebow, S., Iakovenko, N., Hausdorf, B., Petersen, M., Kück, P., Herlyn, H., Hankeln, T. (2014). Platyzoan paraphyly based on phylogenomic data supports a non-coelomate ancestry of Spiralia. *Mol. Biol. Evol.*, 31, 1833-1849. [This article provides a discussion of the phylogenetic position of Platyhelminthes, Gastrotricha and Gnathifera]

Struck, T.H., Golombek, A., Weigert, A., Franke, Franziska A., Westheide, W., Purschke, G., Bleidorn, C., Halanych, Kenneth M. (2015). The Evolution of Annelids Reveals Two Adaptive Routes to the Interstitial Realm. *Curr. Biol.*, 25, 1993-1999. [This article provides a discussion of the phylogeny and evolution of small annelid taxa.]

Wägele, J.W., Bartolomaeus, T. (Eds.) (2014) *Deep Metazoan Phylogeny: The backbone of the Tree of Life - New insights from analyses of molecules, morphology, and theory of data analysis*, 736 pp. De Gruyter, Berlin. [This book provides a comprehensive review of recent findings on the evolution of Metazoa using morphological and molecular data]

Weigert, A., Helm, C., Meyer, M., Nickel, B., Arendt, D., Hausdorf, B., Santos, S.R., Halanych, K.M., Purschke, G., Bleidorn, C., Struck, T.H. (2014). Illuminating the base of the annelid tree using transcriptomics. *Mol. Biol. Evol.*, 31, 1391-1401. [This article provides a discussion of the phylogeny and evolution of Annelida]

Wey-Fabrizius, A.R., Herlyn, H., Rieger, B., Rosenkranz, D., Witek, A., Welch, D.B.M., Ebersberger, I., Hankeln, T. (2014). Transcriptome data reveal syndermatan relationships and suggest the evolution of endoparasitism in Acanthocephala via an epizoic stage. *PLoS ONE*, 9, e88618. [This article provides a discussion of the phylogeny and evolution of Syndermata]

Biographical sketch

Torsten H. Struck received his PhD at the University of Osnabrück in Germany in 2003, which was followed by a postdoctoral position in the laboratory of Kenneth M. Halanych at Auburn University in Auburn, AL. He then participated in the special priority program "Deep Metazoan Phylogeny" with his own projects and therefore moved back to Osnabrück, Germany, in 2005. In 2011 he was awarded a Heisenberg fellowship of the German Science Foundation to work at the Zoological Research Museum Alexander Koenig in Bonn, Germany. Since fall 2015 he has been appointed as professor at the Natural History Museum of the University of Oslo, Norway. His research interests are in the evolution of worms, including Annelida, Platyhelminthes, Nemertea, Gastrotricha and Gnathostomulida, using molecular tools such as next generation sequencing of transcriptomes and genomes. Additionally, he addresses methodological topics to improve the reconstruction of phylogenies including the developmental of new bioinformatics tools. He has published many scientific papers, among others in highly ranked journal. He is a member of several scientific societies and is in the editorial board of *Marine Biology Research*.