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Careful amendment of morphological datasets improves phylogenetic frameworks: re-evaluating placement of the fossil *Amiskiwia sagittiformis*

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Abstract

The Cambrian fossil *Amiskwia sagittiformis* has puzzled palaeontologists for more than a century, but recent reinvestigation of its morphology suggested a close relationship with the bilaterian clade Gnathifera, comprising Rotifera, Gnathostomulida and Micrognathozoa. Since *Amiskwia* has already been considered closely related to Chaetognatha, this new interpretation of its morphology supports recent molecular and developmental studies finding a close phylogenetic relationship between Chaetognatha and Gnathifera. The recent redescription of the jaw apparatus of *Amiskwia* with the first phylogenetic analysis by Vinther and Parry (2019) found a rather surprising topology with *Amiskwia* and Chaetognatha being sister groups and nested inside Gnathifera. Furthermore, a following paper from Caron and Cheung (2019) redescribed the jaws of *Amiskwia* as very similar to the configuration found in Gnathostomulida, but did not carry any phylogenetic analysis. Here we test the topology of Vinther and Parry with various parsimony and Bayesian analyses, taking into account the new description of Caron and Cheung with careful re-amendment of the matrix of Vinther and Parry. According to our results and the recent findings on the molecular phylogeny of their extant members, we suggest a new systematization of these taxa. We recovered *Amiskwia* as a stem group chaetognath within a clade Cucullophora nov., rejecting the Caron and Cheung hypothesis of its close affinity to Gnathostomulida, and found a more consensual topology with monophyletic Gnathifera sister group to Cucullophora, altogether forming the clade Chaetognathifera. Furthermore the clade Rotifera + Micrognathozoa form a clade named Gynognathifera nov.. We discuss the characters supporting each clade and the reasons that account for the topology found by Vinther and Parry. Finally, we hope that this carefully amended matrix focused on Gnathifera, *Amiskwia* and Chaetognatha, together with a formal classification and robust phylogeny, will be of use for future studies on the palaeontology and morphology of these clades.

Keywords

morphological matrix, parsimony, Bayesian analysis, Spiralia, Cladistics, Gnathifera

Introduction

Gnathifera is a clade of predominantly (and ancestrally) microscopic animals, which is composed of three subclades - Gnathostomulida, Micrognathozoa and Rotifera (the latter including also parasitic, macroscopic Acanthocephala). The name Gnathifera and close relation between Gnathostomulida and Rotifera was proposed in 1995 based on the presence of similar cuticular jaw apparatus in those two groups of animals (Ahlrichs 1995). In the same year, the exact ultrastructural arrangement of those jaws (as composed of rod-shaped elements with the electron-dense core and electron-translucent halo) was proposed as an important synapomorphy of the clade (Rieger & Tyler 1995). In the 1990s the reports of another, officially yet undescribed animal with similar jaw apparatus were already circulating in the zoological community and in several articles from that period the animal is mentioned (as “New group A”, or “New group 1”) as a possible member of the gnathiferan clade with the position sister to rotifers (Ahlrichs 1997; Herlyn & Ehlers 1997; Kristensen 1995; Kristensen & Funch 1995). In 2000, this newly described animal, *Limnognathia maerski* Kristensen and Funch, 2000, the sole member of the clade Micrognathozoa, officially joined Gnathostomulida and Rotifera as a third major gnathiferan lineage (Kristensen & Funch 2000).

The clade Gnathifera was established solely on morphological data and initially its monophyly was not supported by the early molecular analyses of few genetic loci (Giribet *et al.* 2004; Littlewood *et al.* 1998; Sørensen *et al.* 2006). However, later transcriptome- and genome-based molecular phylogenies confidently supported not only the monophyly of the clade but also a sister relation between Rotifera and Micrognathozoa and showed that Gnathifera belongs to Spiralia, one of the principal super-clades of Metazoa, which also includes such protostome lineages as annelids, brachiopods and molluscs (Drábková *et al.* 2022, Laumer *et al.* 2015; Laumer *et al.* 2019; Witek *et al.* 2009; Zverkov *et al.* 2019). Interestingly, the recent molecular phylogenies also indicated that Gnathifera might additionally include Chaetognatha (Marlétaz *et al.* 2019) or represent their sister group (Kocot *et al.* 2017; Laumer *et al.* 2019), resolving a long-standing discussion about phylogenetic position of the latter group (summarised in e.g., Bleidorn 2019; Perez *et al.* 2014). Although those findings contradicted most of the previous molecular phylogenies, they provided an elegant explanation for certain similarities observed in the neuroanatomy (Gąsiorowski *et al.* 2017b) and Hox gene complement (Fröbius & Funch 2017) between chaetognaths and Gnathifera *sensu stricto*.

There are virtually no known fossils of Gnathifera (*s.s.*), with a possible exception of bdelloid rotifers, for which the putative, poorly preserved, fossils have been reported from Permian deposits (Jha *et al.* 2011) and much more recent Dominican amber (Poinar & Ricci 1992; Waggoner & Poinar 1993). Chaetognaths, on the other hand, are better documented in the fossil record already from the Cambrian and are represented both by the fossilised hard mouthparts (the so called Protoconodonts, Szaniawski 2002) and specimens with well preserved soft bodied anatomy (Briggs & Caron 2017; Chen & Huang 2002; Hu *et al.* 2007; Shu *et al.* 2017; Vannier *et al.* 2007). Recently Vinther and Parry (2019) redescribed the fossil *Amiswkia sagittiformis* Walcott, 1911, a problematic soft bodied fossil known from the Cambrian deposits from the Burgess Shale in Canada (Walcott 1911) and Maotianshan Shales in China (Chen *et al.* 2002). Using phylogenetic analysis of morphological characters Vinther and Parry not only found a sister group relationship between Chaetognatha (extant and extinct) and *Amiskwia*, but also a sister relationship of this clade with rotifers. Since their analysis failed in recovering the morphologically and molecularly well supported clades (e.g., Rotifera+Micrognathozoa, Gnathifera *s.s.*), we inspected the morphological matrix used to construct that phylogeny and found that several characters crucial for the internal relation within Gnathifera+Chaetognatha clade have been oddly, or simply erroneously coded. Moreover, a following paper from Caron and Cheung (2019) redescribed the jaw elements of *Amiskwia* in more details, showing more similarities with Gnathifera, and suggested that *Amiskiwa* might represent a stem group gnathiferan or gnathostomulid (however the authors did not test this suggestion phylogenetically), even further questioning the accuracy of the original analysis by Vinther and Parry. Therefore, we aim to re-code the morphological matrix of Vinther and Parry putting more emphasis on the Gnathifera important characters and reanalyse it using both Vinther and Parry (2019) and Caron and Cheung (2019) interpretation of *Amiskwia* jaw elements. We aim to not only resolve the phylogenetic position of *Amiskwia* within Gnathifera, but also to provide the up-to-date morphological matrix, which in future can be used by palaeontologists to more confidently place putative gnathiferan and spiralian fossils.

Material and methods

Five different matrices have been analysed (Tab. 1) and the results compared. The first one is the original matrix from Vinther and Parry, 2019 (O-VP). The other sets are re-coded and modified matrices with characters redefined, added and/or removed from Vinther and Parry, 2019. Detailed discussion on those modified characters can be found in Supplementary Information I. Two of those versions consider Vinther and Parry’s interpretation of the jaw apparatus of Amiskwia (M-VP) and the other ones the interpretation of Caron and Cheung, 2019 (M-CC). Both matrices have been analysed with or without the parsimony uninformative characters (M-VP-PN, M-CC-PN and M-VP-PI, M-CC-PI, respectively) under parsimony, while only the matrices with Caron and Cheung, 2019 interpretation of *Amiswkwia*'s jaws (M-CC-PN and M-CC-PI) have been analysed with both parsimony and Bayesian inference. Moreover, two additional re-codings have been done for Caron and Cheung interpretation of the jaws - removing *Amiskwia* and fusing together the fin characters. The different matrices and analyses are summarized in the Tab. 1, and all the matrices used in this study can be found as nexus files in Supplementary Information II. The results of only two analyses following Caron and Cheung interpretation of the jaws, with only parsimony informative characters (M-CC-PI) with both parsimony and Bayesian inference are shown here (Fig. 1 and Fig. 2). The other results can be found in the Supplementary Information III (Figs. S1-S7).

The parsimony analyses have been run in Paup*4 (Swofford 2003), with the heuristic search with default parameters. Multistate characters have been considered as unordered. Strict consensus, consistency index (CI), retention index (RI), branch length (with ACCTRAN) as well as the bootstrap have been also determined with PAUP*4. When bootstrap has been calculated it was with 1000 replicates with a heuristic search, starting trees obtained via stepwise addition. Character optimization for Fig. 3 has been done in Mesquite (Maddison and Maddison 2021), using DELTRAN (in order to avoid artificial reconstruction of characters on deep nodes in the cases of massive missing data for fossil taxa that are sister groups to extant clades). Retention index (RI) of each character has been calculated on the consensus tree from the parsimony analysis of the M-CC-PI matrix using Mesquite and can be found in the Supplementary Information III (Tab. S1).

The Bayesian analyses have been computed following Vinther and Parry 2019 script in MrBayes (Ronquist & Huelsenbeck 2003), with a lset rates= gamma, a MCMC number of generation of 5,000,000, and a burnin of 10%.

Trees have been extracted from analysis in “.tre” format, read with the FigTree (Rambaut 2022) and exported into “.svg” format. Figures have been made with Adobe Illustrator and Affinity Designer.

Results

First of all, we analysed the original matrix of Vinther and Parry, 2019 (O-VP) under parsimony. There are 3150 final trees of 138 steps, and 75 parsimony informative characters for 140 characters in total, with a CI of 0.645 and a RI of 0.844. The topology is similar to the one that Vinther and Parry found in their paper with Bayesian analysis, although less resolved, probably due to the strict consensus (see Supplementary Information III, Fig. S1).

For the following analyses, the matrices used have been modified from Vinther and Parry (2019) and several characters have been recoded, removed or added (see Supplementary Information I for a detailed discussion on the modified characters). Analyses using parsimony non-informative or only parsimony-informative characters have been conducted: no topological differences have been found, and the bootstrap for parsimony and posterior probabilities for Bayesian analysis are similar with or without non-informative characters (compare Figs. 1 and 2 with Figs. S4 and S5 in Supplementary Information III). Coding according to the interpretation of the jaws of *Amiskwia* from Vinther and Parry (2019) and Caron and Cheung (2019) has been made and no significant topological differences have been found (Supplementary information III, Figs. S6 and S7). Since the Caron and

Cheung interpretation seems to be based on better-preserved material, we decided to keep and mainly discuss trees from this dataset, using only parsimony-informative characters in both parsimony and Bayesian analyses (Figs. 1 and 2). Trees have been rooted using Ecdysozoa as an outgroup, the bipartition Spiralia/Ecdysozoa being found in all analyses with rather high support as for a limited morphological dataset.

The result of the parsimony analysis using the Caron and Cheung interpretation of the jaws of *Amiskwia*, and only with informative characters from a matrix of 33 taxa and 74 characters (M-CC-PI) with heuristic search using default parameters, gave 540 trees of 145 steps, each with a consistency index of 0.566 and a retention index of 0.733. The strict consensus (Fig. 1) has a length of 148 steps, a CI of 0.554 and a RI of 0.761.

We consider Gnathifera as the clade [Gnathostomulida + [Micrognathozoa + Rotifera]], and consider Rotifera as a synonym to Syndermata. For commodity we reuse the name “Chaetognathifera” from Conway Morris *et al.* (2020): [Gnathifera + Chaetognatha] and introduce new clade names, namely Gynognathifera: [Micrognathozoa + Rotifera] and Cucullophora [*Amiskwia* + Chaetognatha] (see discussions for details). The topology between the parsimony and Bayesian trees are quite similar, apart from a lower resolution of the Bayesian tree, however both topologies are compatible (Figs. 1 and 2). Ecdysozoa as an outgroup is found monophyletic and consequently Spiralia as well (Figs. 1 and 2). Spiralia is divided into a trifurcation: Gastrotricha, Platytrochozoa and [Chaetognathifera + *Inquicus fellatus* Cong, et al. 2017]. From now on, we only describe the Chaetognathifera topology since this is the focus of the study. With parsimony, Chaetognathifera, Gnathifera, Gynognathifera, Cucullophora and Chaetognatha are monophyletic, and

Chaetognathifera is the sister group of *Inquicus fellatus* (Fig. 1). However, only Chaetognatha, Cucullophora and Rotifera have a bootstrap superior to 50% with values of respectively 65, 78 and 99% (Fig. 1). The Bayesian tree does not recover the monophyly of Gnathifera and is constituted of four lineages: Gnathostomulida, Gynognathifera, *Inquicus fellatus* and Cucullophora (Fig. 2). Chaetognathifera, Gynognathifera, Rotifera, Cucullophora and Chaetognatha have respectively a posterior probability of 0.62, 0.58, 1, 0.98, and 0.93 (Fig. 2). In all trees Chaetognatha are divided between the fossils *Ankalodous*, *Capinator* and an unresolved crown clade containing all modern chaetognaths (*Flacisaggita*, *Spadella* and *Eukrohnia*) as well as the fossil *Protosagitta* (Figs. 1 and 2, Supplementary information III, Figs. S1–S7).

The RI of each character for the parsimony analysis on the strict consensus tree have been estimated using Mesquite (see Supplementary Information III, Table S1). Over the 74 characters retained we found:

- 41 characters (~55%) with a RI of one (meaning strict synapomorphies),
- 21 characters (~28%) with a RI equal or superior to 0.5 and inferior to one,
- 6 characters (~8%) with a RI under 0.5 and superior to zero,
- 6 characters (~8%) with a RI of zero (meaning that they do no help grouping taxa together)

The optimization of the different characters varying within Chaetognathifera and representing apomorphies of particular clades is given in Fig. 3.

Discussion

Phylogeny and nomenclature of Chaetognathifera

In general, our analysis confirmed the sister relation of *Amiskwia* and Chetognatha, however, in contrast to the results of Vinther and Parry (2019), this clade was positioned as a sister group to the Gnathifera and not nested within the latter. Recent years have witnessed an increased interest in morphological and molecular evolution of Gnathifera and Chaetognatha with plenty of new data coming from the fossil record (Caron & Cheung 2019; Conway Morris *et al.* 2020; Shu *et al.* 2017; Vinther & Parry 2019), comparative morphology (Bekkouche *et al.* 2014; Bekkouche & Worsaae 2016; Gąsiorowski *et al.* 2017a; Gąsiorowski *et al.* 2017b; Herlyn 2017), molecular phylogeny (Laumer *et al.* 2015; Laumer *et al.* 2019; Marlétaz *et al.* 2019; Witek *et al.* 2009) and developmental biology (Fröbius & Funch 2017; Martin-Duran *et al.* 2018). In order to help systematisation of the discussion of Gnathiferan evolution here we aimed not only at resolving the position of *Amiskwia* but also at providing consistent definitions for characters and clade names used in the discussion of Gnathifera and their relatives.

Historically, Gnathifera was defined as a group composed of gnathostomulids and rotifers (Ahlrichs 1995) and unified by the presence of jaws with a particular ultrastructure (Rieger & Tyler 1995). Since the term was introduced long before demonstration of a close relation of chetognaths and gnathiferans *s.s.*, it is widely used in the literature in its more exclusive sense (Ahlrichs 1997; Bekkouche *et al.* 2014; Bekkouche & Worsaae 2016; Drábková *et al.* 2022, Gąsiorowski *et al.* 2017a; Gąsiorowski *et al.* 2017b; Gąsiorowski *et al.* 2019; Giribet *et al.* 2004; Hejnol 2015; Herlyn 2017; Herlyn & Ehlers 1997; Jenner 2004; Kristensen & Funch 2000; Littlewood *et al.* 1998; Sørensen 2000; 2003; Sørensen *et al.* 2000; Sørensen & Sterrer 2002; Witek *et al.* 2009). According to most of the recent molecular phylogenies (and also our morphological parsimony-based analysis) Gnathifera (s.s.) is a clade (e.g., Drábková *et al.* 2022, Laumer *et al.* 2015; Laumer *et al.* 2019; Zverkov *et al.* 2019), therefore we believe it is beneficial to keep using this name in its stricter, original sense in order to avoid confusion between older and newer literature. That is why we decided not to include Chaetognatha and *Amiskwia* within Gnathifera but within a clade coined by Conway Morris *et al.* (2020): Chaetognathifera, as a composite of Chaetognatha and Gnathifera. Here we re-define Chaetognathifera as the smallest clade including *Adineta vaga* (Rotifera) and *Parasagitta elegans* (Chaethognatha). We therefore use a stricter definition of Gnathifera as the smallest clade including *Adineta vaga* (Rotifera) and *Gnathostomula paradoxa* (Gnathostomulida), with the synapomorphy of possessing jaws composed of rod-like elements with electrondense core and electroluscent outer part under transmission electron microscopy, which is closer to the original definition of the clade. We also introduce two new names here: Gynognathifera and Cucullophora. The first one is the smallest clade including *Limnognathia maerski* and *Adineta vaga*, since a sister group relationship between Micrognathozoa and Rotifera has been well demonstrated (e.g., Drábková *et al.* 2022 Laumer *et al.* 2015; Laumer *et al.* 2019; Marlétaz *et al.* 2019; Zverkov *et al.* 2019; our analysis). The etymology of the prefix comes from the Greek “*Gyno*” for female, referring to the tendency of having a majority of females in most of its sub-clades (only females are known in Micrognathozoa and Bdelloidea and females are predominant in Monogononta). Cucullophora, on the other hand, is the smallest clade comprising *Amiskwia sagittiformis* and *Parasagita elegans*, which has been recovered as monophyletic in most of our analyses (Figs. 1 and 2, Supplementary Information III, Figs. S4-S7), with the exception of the matrix modification, in which fin characters have been merged (Supplementary Information III, Figs. S2). The etymology of this clade comes from the Latin prefix “*cucullus*”, hood and the Latin suffix “*feros*”, bear, meaning “bearing a hood” and referring to the presence of the head hood as morphological synapomorphy of this group (Fig. 3A).

Another nomenclature was proposed by Tokioka (1965), and followed recently by Müller *et al.* (2019). In this system, *Amiskwia sagittiformis* is included within Chaetognatha, representing the class Archisagittoidea, while extant Chaetognatha form class Sagittoidea (which therefore is synonymous with the crown group Chaetognatha). According to our topology, as well as the one in Vinther and Parry, *Amiskwia* is so far the only known member of the proposed Archisagittoidea. No precision has been made on whether other fossil Chaetognatha: *Capinator praetermissus* (Briggs & Caron 2017), *Ankalodous sericus* (Shu et al. 2017), and *Eognathacantha ercainella* (Chen & Huang 2002) syn. with *Protosagitta spinosa* (Hu *et al.* 2007) should be placed inside Sagittoidea or not. As for *Protosagitta*, it falls into a clade of extant Chaetognatha, and therefore formally inside Sagittoidea, while *Amiswkia*, *Capinator* and *Ankalodous* represent a stem group of Chaetognatha. In order to provide consistent nomenclature and integrate both systems we propose the following systematic division of the clade Chaetognathifera:

- Clade: **Chaetognathifera**:
  - Clade: **Gnathifera**:
    - Clade: **Gnathostomulida**
    - Clade: **Gynognathifera nov.**:
      - Clade: **Micrognathozoa**
      - Clade: **Rotifera** (syn. **Syndermata**)
  - Clade: **Cucullophora nov.**:
    - Clade: **Archisagittoidea**
      - Genus: ***Amiskwia***

Clade: **Chaetognatha**:
  Genus: ***Capinator***
  Genus: ***Ankalodous***
  Clade: **Sagittoidea** (crown group **Chaetognatha**, incl. ***Protosagitta***)

Morphological evolution of Chaetognathifera

Although our analysis was not focused on the ancestral state reconstruction, we were able to identify sets of apomorphic characters specific for each of chaetognathiferan clades (Fig. 3A). Some of those clades such as rotifers or Sagittoidea are supported by numerous morphological characters, while the others have only limited number of synapomorphies. Chaetognathifera themselves are unified by two well-defined morphological characters (C29 *“Stomatogastric ganglion(s) directly connected to the brain”* and C47 *“Jaws with symphysis”*), one vaguely defined morphological character (C28 *“Circumoral nerve ring”*, see discussion in Supplementary Information I) and one genomic character (C66 *“MedPost gene”*). Three additional morphological characters (C15 *“Body divided with distinct inflated head and neck region”*, C41 *“Sclerotized elements in the anterior digestive tract”* and C46 *“Internal jaws”*) that were reconstructed as present in the common chaetognathiferan ancestor are also shared with a jawed fossil *Inquicus fellatus* (Cong *et al.* 2017) and thus they probably represent synapomorphies of the clade Chaetognathifera+*Inquicus* and are not *bona fide* chaetognathiferan apomorphies.

The internal relations within Chaetognathifera have important consequences for the reconstruction of the evolution of their body sizes. Both Vinther and Parry (2019) and Caron and Cheung (2019) proposed complex evolutionary scenarios where miniaturisation occurred several times independently in different lineages of

Chaetognathifera. In case of Vinther and Parry (2019) it was due to the paraphyly of Gnathifera (s.s.), while in Caron and Cheung (2019) due to the position of *Amiskwia* as a stem gnathostomulid. In our analysis, despite using Caron and Cheung interpretation of jaw apparatus and despite correcting or excluding other characters which artificially increased affinity of *Amiskwia* to chaetognaths (e.g. position of anal pore or presence of several fin characters, see Supplementary Information I for detailed discussion), we still recover a sister position of *Amiskwia* and chaetognaths and (in most of the cases) monophyly of Gnathifera. Therefore, our analysis provides a more parsimonious explanation for the body size evolution: Chaetognathifera is divided into microscopic Gnathifera (s.s.) and Cucullophora that are characterised by much larger body sizes. Taking into account that Spiralia are probably ancestrally microscopic (Laumer *et al.* 2015), Gnathifera seem to retain plesiomorphic conditions, while Cucullophora secondarily evolved larger body size, likely as an adaptation to the active life style in the open water column (Hu *et al.* 2007, Szaniawski 2002, Vannier *et al.* 2007), as further supported by the shared presence of trunk and caudal fins in members of this group (Fig. 3).

Remarks on the other possible fossil chaetognathiferans

Due to the lack of detailed characters of *Inquicus fellatus* (Cong *et al.* 2017) we do not think it is relevant to discuss its phylogenetic position and characters in detail, but it seems to be related to Chaetognathifera. Additionally, we think that a very superficial resemblance with seisonid rotifers (class Seisonidea) should be pointed out, as seisonids also have a slender body with a well-delimited head, live as epibionts of Ecdysozoa (specifically, the leptostracan *Nebalia*), attach to their hosts by the posterior end and have a stiff and elongated jaw apparatus (e.g. Ahlrichs &

Riemann 2019; Fontaneto & De Smet 2015; Sørensen *et al.* 2005). On the other hand, similar body shape and lifestyle (ectocommensalism on large ecdysozoans) is found as well in the members of the annelid family Histriobdellidae, which also possess complex and elongated jaw apparatus in their pharynx (Jennings & Gelder 1976; Tzetlin *et al.* 2020). Histriobdellidae were traditionally considered as a member of Eunicida, which do not appear in the fossil records until the late Cambrian (Parry *et al.* 2015, 2019). However, since the recent transcriptome-based analyses failed at resolving exact position of Histriobdellidae on the annelid phylogeny (Tilic et *al.* 2022), the temporal discrepancy between age of Eunicida and *Inquicus* does not necessarily argue against affinity of *Inquicus* to histriobdellids.

Another recently described Cambrian fossil, *Dakorhachis thambus* Conway Morris et al. 2020, from the Weeks Formation (Miaolingian, Guzhangian), has been also suggested to be a gnathiferan (Conway Morris *et al.* 2020). However, the arrangement of its sclerotized mouth parts reminds more of scalids (circumoral arrangement), and the circular section of the animal as well as the trunk annulation point in the direction of an affinity with Scalidophora (Ricardo Neves, personal communication). Therefore, we decided to exclude *D. thambus* from our analysis, since it is not in the original matrix of Vinther and Parry (2019), and the reasons to include this animal in the Gnathifera seem, to that day, insufficient for us.

Homology of jaws among Chaetognathifera

We think that a close relationship between Chaetognatha and Gnathifera is not enough to assert a homology between their respective jaw apparatuses, and therefore we reject it (as has been also argued in Shu *et al.* 2017). Although both Chaetognatha and Gnathifera have paired skeletal elements in the anterior digestive tract, their position, morphology, and microstructure differ profoundly. The jaws of Gnathifera are intrapharyngeal, constituted of a pincer-like central element with a symphysis, and are composed of specific rod-like elements, while the jaw-like elements of Chaetognatha are external and do not form a pincer structure, but instead a grasping apparatus (Fig. 4). The ultrastructure of the chaetognath teeth and spines consists of an outer part of two concentric tubes separated by a layer of less electron-dense material (Bone *et al.* 1983). The outer part is composed of obliquely arranged lamellae with an inner electron-translucent part (Bone *et al.* 1983), quite opposite to what is found in Gnathifera (e.g. Herlyn & Ehlers 1997; Kristensen & Funch 2000; Rieger & Tyler 1995). The diameter of the rods of Gnathifera varies between 100 nm to 500 nm (Kristensen & Funch 2000; Rieger & Tyler 1995), while the outer part of the Chaetognatha spines and teeth is about 1µm wide (Bone *et al.* 1983) and although the dimensions are roughly comparable, the microstructure is quite different. As for the inner part of the spines and teeth of Chaetognatha, it is composed of a cellular pulp (Bone *et al.* 1983).

Although the microstructure of the jaws of *Amiskwia* is unknown, the interpretation of the Caron and Cheung (2019) suggests that its jaws have a similar position and morphology to that of Gnathifera (and especially Gnathostomulida considering the basal plate; Fig. 4), meaning that the Gnathifera-like jaw apparatus was either lost in Chaetognatha or evolved twice convergently in Gnathifera and *Amiskwia*. Taking into account branch lengths (much longer branch leading to chaetognaths than to *Amiskwia*; Fig. 2) it is more likely that the jaws of *Amiskwia* are indeed homologous with the jaw apparatus of Gnathifera, and hence presence of the jaws with symphysis represents one of the synapomoprhies of Chaetognathifera, lost in the chaetognath lineage (Fig. 3). It is widely accepted that the grasping spines represent an adaptation to the active predatory lifestyle, characteristic for both recent and fossil chaetognaths (Hu et al. 2007, Szaniawski 2002, Vannier et al. 2007), and, according to our scenario, they evolved in the chaetognath lineage as new mouth apparatus replacing the Gnathifera-like jaws (Fig. 3).

These objections to the homology of jaws of Gnathifera and Chaetognatha do not mean that further studies on, *e.g.* the development or biochemistry, will not refute this interpretation. For example, Martin Vinther Sørensen (personal communication) points out that a high chitin content could be a characteristic of the jaws of Chaetognathifera. However these studies are quite rare or not yet published. For example, we did not find publications confirming the presence of chitin in the jaws of Gnathostomulida and Micrognathozoa (even though it is reasonable to think that it is present). Furthermore, these studies are quite scarce on Rotifera (Kleinow 1993 and Klusemann *et al.* 1990, showing a chitin content of approximativelly 64% in *Brachionus plicatilis* Müller, 1786), and Chaetognatha (being mostly focussed on *Sagitta*; Atkins et al. 1979, Bone et al 1983, Saito and Okano 1995). If a similar biochemical signature is shown between jaws of Chaetognathifera, this would add a structural argument for a primary homology between these different jaw elements.

We also would like to point out that jaw-like elements are very common in Bilateria and the homology hypothesis should be asserted with care when considering such homoplastic structures. Horizontally-oriented, pincer-like structures with the superficial structural similarity to the gnathiferan jaws can be found as autapomorphies of some ingroups of platyhelminths (proboscis hooks of Kalyptorhynchia, e.g. Smith III *et al.* 2015), molluscs (pincer-shaped radula of Caudofoveata, e.g. Señarís *et al.* 2017; Señarís *et al.* 2014) or annelids (jaws of

Eunicida, e.g. Paxton 2009; Tzetlin *et al.* 2020), indicating that complex jaw-like elements evolved many times independently among Bilateria.

Methodological remarks on cladistic analysis

Although the classical support indexes in our analysis are relatively low (most bootstrap values are under 50% for Gnathifera in parsimony and relatively low posterior probabilities for the Bayesian tree), we think that our results are quite robust since they are supported by an array of data and alternative analyses. Indeed, the Retention Index of 0,73 is rather good and more than half of the characters have a RI of one, meaning these characters have no homoplasy. Furthermore, despite the number of trees obtained in parsimony (540), the strict consensus is well resolved, meaning most of the 540 topologies are congruent. Furthermore, it is worth noting that low bootstrap values were expected (as well as low posterior probabilities values), considering the low number of characters (e.g., Soltis & Soltis 2003).

It could have been expected from the jaws interpretation of Caron and Cheung (2019) that *Amiskwia* falls into Gnathifera, possibly as the sister group to Gnathostomulida, but the position as sister to Chaetognatha is well supported. To test this position, we decided to “merge” the three characters concerning the fins into one (C51, C52, C53), as they may be dependent. This only gave a trichotomy between Gnathifera, Chaetognatha, and *Amiskwia* (see Supplementary Information III, Fig. S2), showing that the position of *Amiskwia* outside Gnathifera *s.s.* is well supported. To test the effect of *Amiskwia* on the topology, an analysis of the M-CC-PI matrix without *Amiskwia* has been run, leading to only one minor change of

Inquicus fellatus found sister group to Gnathifera (see Supplementary Information III, Fig. S3).

Detailed comparison with the matrix from Vinther and Parry (2019)

Although only the trees resulting from analysis with parsimony-informative characters are shown here, trees resulting from matrices including parsimony uninformative were not differing in topology (Supplementary Information III, Figs. S4-S7) and showed similar branch lengths in case of Bayesian analyses (compare Fig. 2 and Fig S5 in Supplementary Information III). We consider that adding parsimony uninformative characters artificially increases the number of characters, and hence the chance to introduce coding mistakes in the matrix. Therefore, we believe that inflation of such characters should be avoided when constructing or re-using a morphological matrix.

We believe that several miscodings and character interpretations of Vinther and Parry (2019) constrained the position of Cucullophora inside Gnathifera in their analysis. Among them the character 102/63 from O-VP matrix, “Major nerve plexus in the trunk” (character 31 in the emended matrix), that we decided to code as absent for Rotifera (see Supplementary Information I for detailed discussion), was duplicated. This happens as well for the character 62/101 (29 in the emended matrix) “stomatogastric nerve plexi” that we decided to rename “Stomatogastric ganglion(s) directly connected to the brain” and also recode, following recent literature on the topic (see Supplementary Information I for detailed discussion). Several other characters were miscoded, e.g., the position of the anus of Chaetognatha coded as dorsal in O-VP matrix (character 39, now 13), or disputable characters such as character 88 (jaw forming clusters of homonomous elements) that was coded as present in Rotifera and Chaetognatha, although we think this does not concern homologous structures and has been removed (see Supplementary Information I for detailed discussion). We think these various characters explain the surprising topology found in Vinther and Parry (i.e., grouping of rotifers with Cucullophora and non-monphyly of Gynognathifera), while our corrections and re-coding resulted in a topology with monophyletic Gnathifera that is more congruent with previous analyses, both morphological (e.g., Shu *et al.* 2017; Sørensen *et al.* 2000) and molecular (e.g., Laumer *et al.* 2015; Laumer *et al.* 2019; Marlétaz *et al.* 2019).

We gave special attention to characters concerning Chaetognathifera as it was the focus of the study, and it is not a review of morphological characters of all Metazoa and hence the discussion has stayed quite brief for other characters, or they have not been debated at all. However, there are likely additional characters wrongfully coded for some taxa due to the reproduction of the errors in the older matrices on which O-VP matrix had been built upon. Problems with mistakes being passed from matrices to matrices in metazoan phylogeny has been extensively discussed by Jenner (2001) and we do not think it is realistic to ask phylogenetists and palaeontologists to entirely correct large datasets, however, specific part of the matrices related to specific topic can be more carefully handled, as we did here with Chaetognathifera and associated characters.

Conclusions

Based on our Chaetognathifera-focused matrix, we can conclude that *Amiskwia* and chaetognaths (here unified into the clade Cucullophora) are not nested within Gnathifera, as found by Vinther and Parry (2019), but instead, Cucullophora and Gnathifera are sister groups. The previous topology, incongruent with most of the previous molecular and morphological analyses, was an effect of several coding errors in the matrix used by Vinther and Parry. One of the major sources for those problems was the fact that originally the matrix used by Vinther and Parry was developed for resolving the phylogeny of Metazoa and hence it lacked several characters important for the internal relations within Chaetognathifera. Here, we provide a detailed and up-to-date morphological matrix that can be directly used for future analyses of chaetognathiferan fossils and, after slight modifications, for analysing other spiralian taxa. Our work demonstrates that morphological matrices focused on specific clades and enriched in the supposedly relevant characters can perform better than general matrices in the pursuit of placing fossil taxa on the phylogenetic tree.

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Figures:

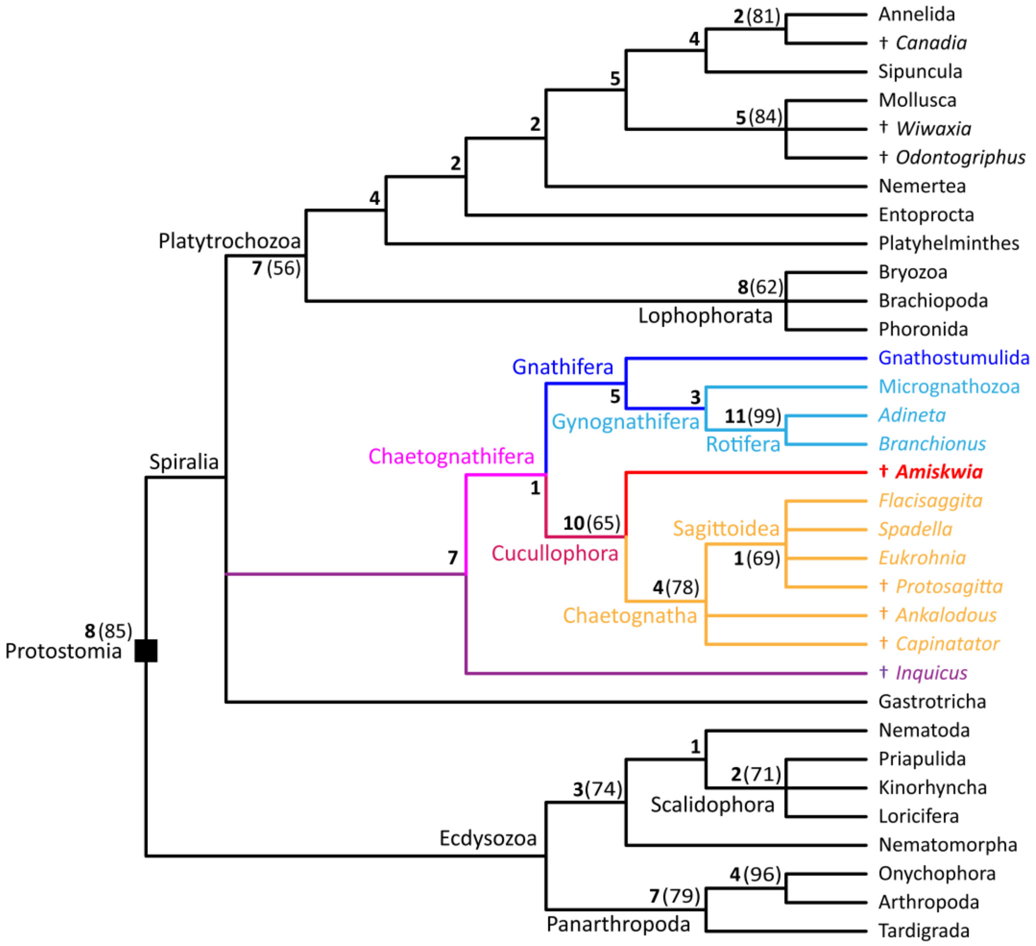


Figure 1. Strict consensus of the parsimony analysis of the modified matrix, following the Caron and Cheung interpretation of the jaws of *Amiswkia* (M-CC-PI). For 33 taxa and 74 characters, 540 trees of 145 steps were found, each with a consistency index of 0.566 and a retention index of 0.733. The strict consensus has a length of 148 steps, a CI of 0.554 and a RI of 0.761. The numbers in bold are the numbers of steps (branch length) for each internal node determined with ACCTRAN, and the numbers in parenthesis are the bootstraps. Nodes without bootstraps have a value under 50%.

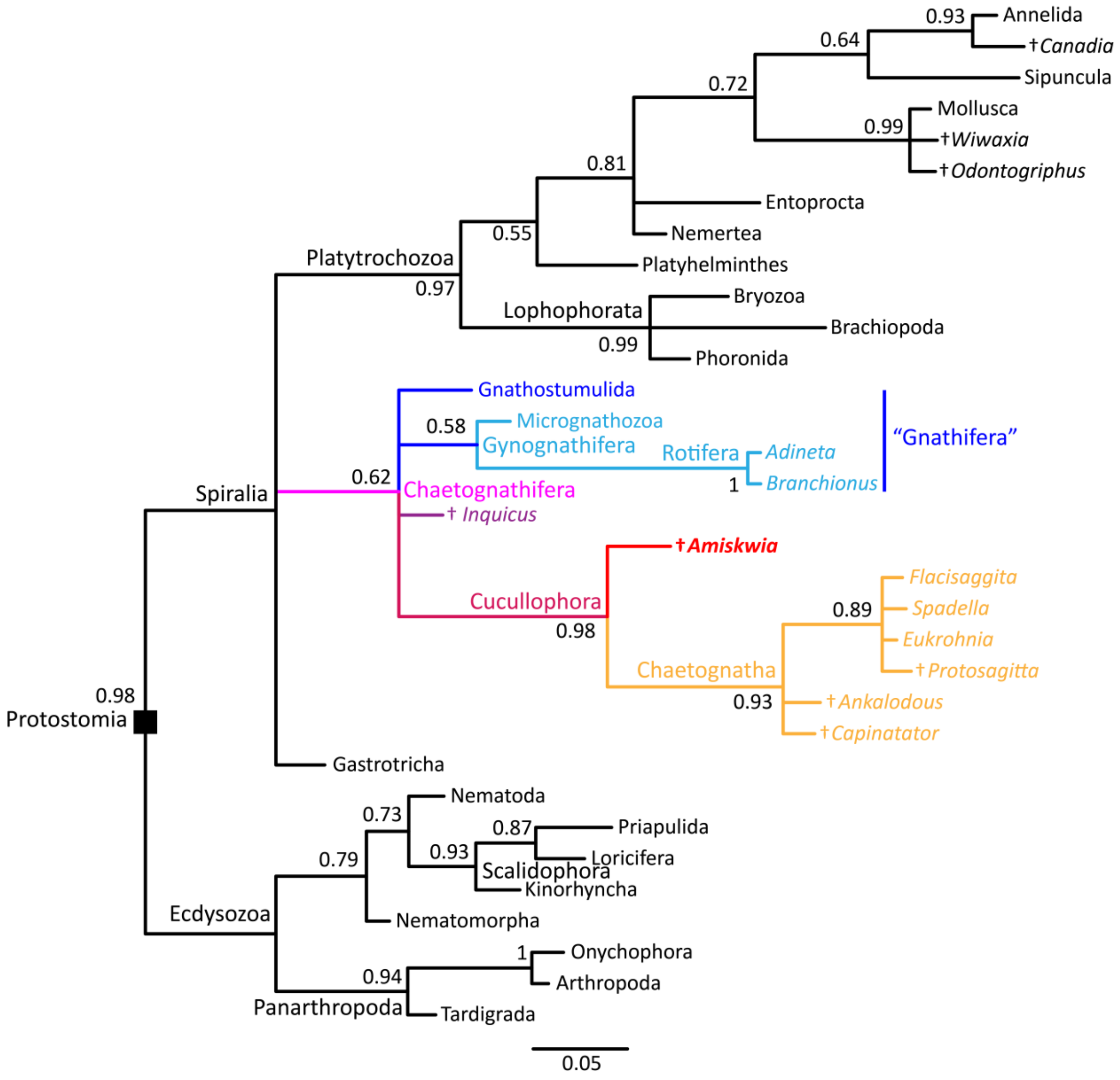
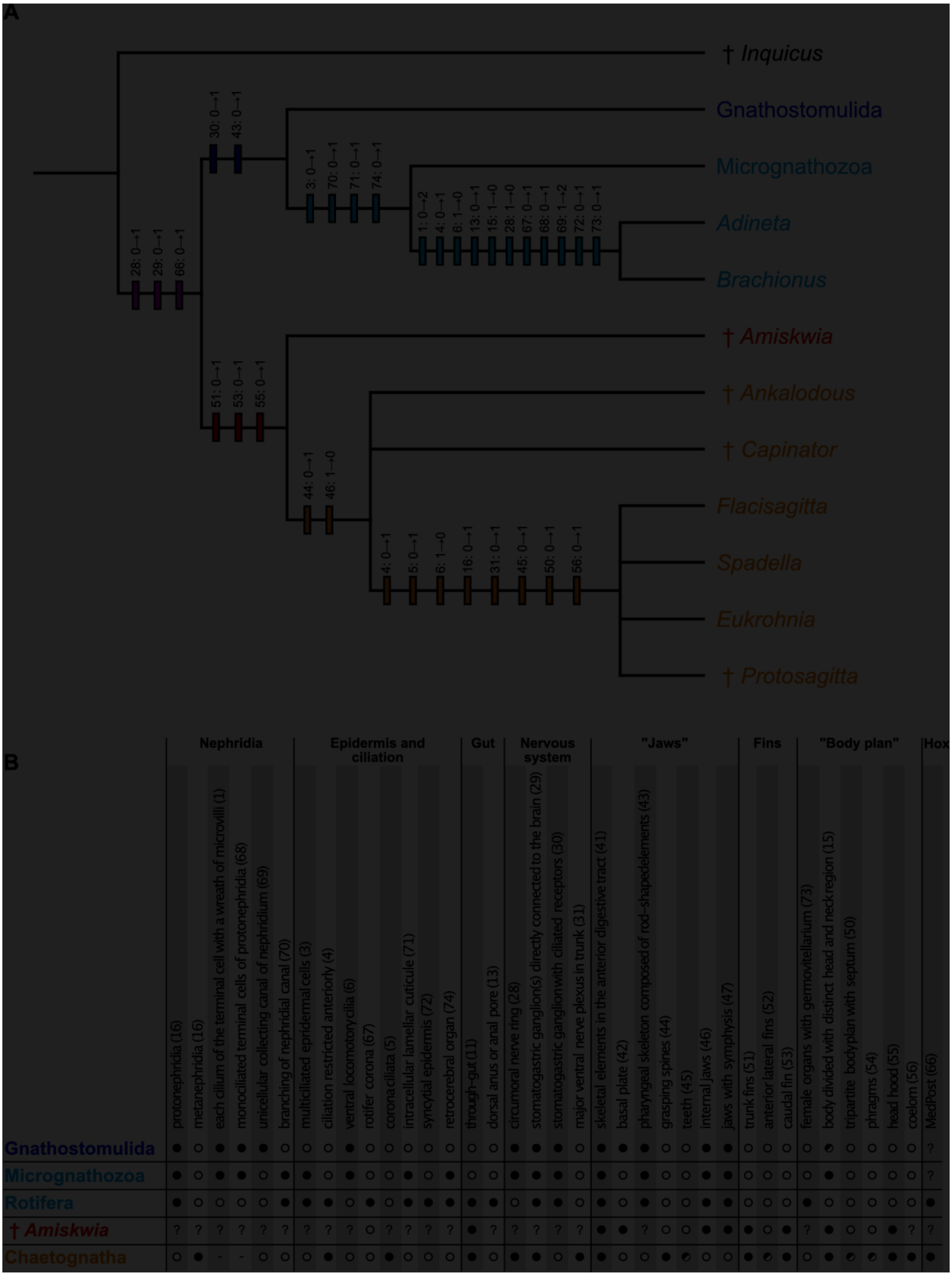


Figure 2. Results of the Bayesian analysis of the modified matrix, following the Caron and Cheung interpretation of the jaws of *Amiswkia* with parsimony-informative characters only (M-CC-PI). The branch numbers are the posterior probabilities. The result including the parsimony uninformative characters is found in the supplementary information III, Figure S5.



	Nephridia						Epidermis and ciliation								Gut		Nervous system				"Jaws"							Fins			"Body plan"						Hox
	protonephridia (16)	metanephridia (16)	each cilium of the terminal cell with a wreath of microvilli (1)	monociliated terminal cells of protonephridia (68)	unicellular collecting canal of nephridium (69)	branching of nephridial canal (70)	multiciliated epridermal cells (3)	ciliation restricted anteriorly (4)	ventral locomotory cilia (6)	rotifer corona (67)	corona ciliata (5)	intracellular lamellar cuticule (71)	syncytial epidermis (72)	retrocerebral organ (74)	through-gut (11)	dorsal anus or anal pore (13)	circumoral nerve ring (28)	stomatogastric ganglion(s) directly connected to the brain (29)	stomatogastric ganglionwith ciliated receptors (30)	major ventral nerve plexus in trunk (31)	skeletal elements in the anterior digestive tract (41)	basal plate (42)	pharyngeal skeleton composed of rod-shapedelements (43)	grasping spines (44)	teeth (45)	internal jaws (46)	jaws with symphysis (47)	trunk fins (51)	anterior lateral fins (52)	caudal fin (53)	female organs with germovitellarium (73)	body divided with distinct head and neck region (15)	tripartite bodyplan with septum (50)	phragms (54)	head hood (55)	coelom (56)	MedPost (66)
Gnathostomulida	●	○	●	●	●	○	○	○	●	○	○	○	○	○	○	○	●	●	●	○	●	●	●	○	○	●	●	○	○	○	○	◐	○	○	○	○	?
Micrognathozoa	●	○	●	●	○	●	●	○	●	○	○	●	○	●	○	○	●	●	●	○	●	○	●	○	○	●	●	○	○	○	○	●	○	○	○	○	?
Rotifera	●	○	○	○	○	●	●	●	○	●	○	●	●	●	●	●	○	●	●	○	●	○	●	○	○	●	●	○	○	○	●	○	○	○	○	○	●
† *Amiskwia*	?	?	?	?	?	?	?	?	?	○	?	?	?	?	●	?	?	?	?	?	●	●	?	○	○	●	●	●	○	●	?	●	○	○	●	?	?
Chaetognatha	○	●	-	-	○	○	○	●	○	○	●	○	○	○	●	○	●	●	○	●	●	○	○	●	◐	○	○	●	◐	●	○	●	◐	◐	●	●	●

Figure 3. A, Parsimony optimization (DELTRAN) of characters varying within Chaetognathifera using a strict consensus tree from our parsimony analysis. Synapomorphic characters supporting each clade are marked on the respective

branches with the character number (according to our matrix) and the direction of state transition indicated above the branch. Autapomorphic character transitions are removed from the tree for clarity. **B,** Table summarising states of the same characters in all major clades of Chaetognathifera. Characters are divided into different biological categories while their numbers in the matrix are provided in brackets. Black dot indicates presence, white dot absence, and black/white dot variation between both states within the clade. “-” stands for inapplicable and “?” for missing data.

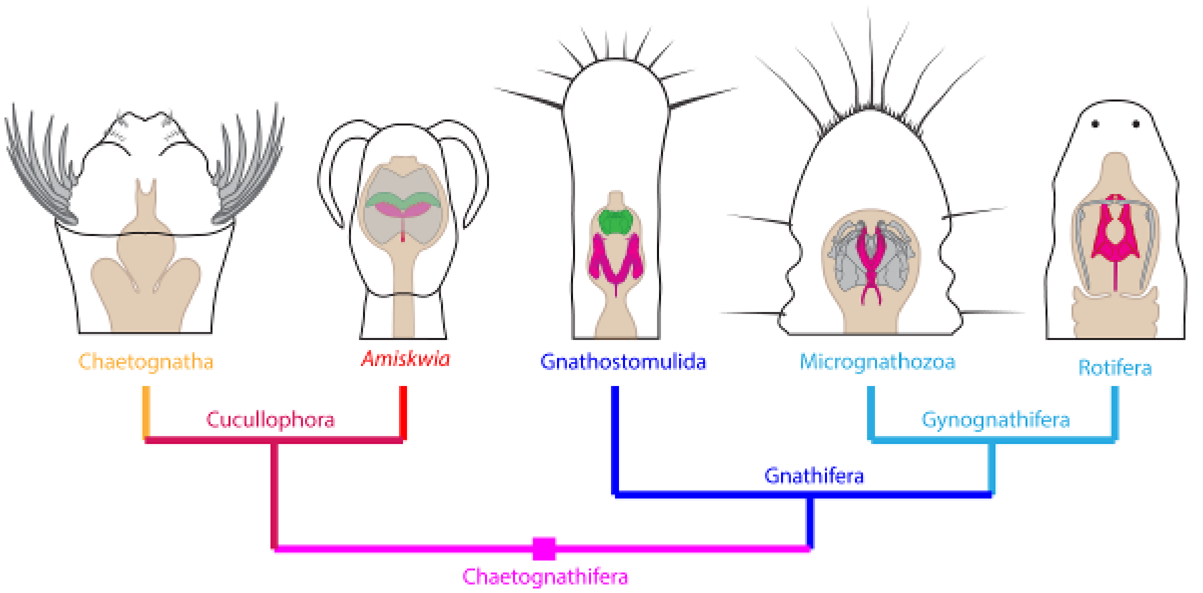


Figure 4. Schematic comparison of the jaws of the different Chaetognathifera. The magenta elements are the “pincer-like” elements of the jaws with symphysis. The green elements are the basal plates. The grey elements are part of the jaws with debated homology. The brown part is the mouth cavity and gut. The underlying tree is the topology found in our analysis. Chaetognatha: *Parasagitta elegans*, Gnathostomulida: *Gnathostomaria sp.*, Micrognathozoa: *Limnognathia maerski*, Rotifera: *Dicranophorus forcipatus*. *Amiskwia sagittiformis* according to Caron and Cheung (2019) jaw interpretation.

Supplementary Information I

Careful amendment of morphological datasets improves phylogenetic frameworks: re-evaluating placement of the fossil *Amiskiwia sagittiformis*

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Removal of uninformative characters

Several of the characters in the original matrix (O-VP) were uninformative due to the same state for all of the coded taxa (characters 1-14, 21-24, 28-36, 71, 74-76, 97, 99, 100, 107, 108, 112, 115-130, 132) or as autapomorphic characters differing only in a single terminal (43, 52-55, 75, 79, 91). These also include deuterostome-specific characters (115-126), which were absent or inapplicable for all protostome groups. After testing that presence of uninformative characters does not influence results of the phylogeny (see Supplementary Information III), we remove those characters from the final matrix.

Recoding of the original matrix

Following characters from the original matrix have been redefined and re-coded. Character numbers follow the original numbering in the Vinther and Parry matrix (O-VP). In brackets, we provided new numbers as used in our final morphological matrix (M-CC-PI). For the characters, in which we changed character states, the exact coding is also provided.

16. Multiciliate epidermal cells (3)

The character has been coded as "?" for Micrognathozoa, however, *Limnognathia maerski* possesses numerous ventral, multiciliated locomotory cells, the so-called ciliophores (Bekkouche & Worsaae 2016; Kristensen & Funch 2000). Therefore, we re-coded this character as present in Micrognathozoa.

17. Ciliation restricted anteriorly (4)

Coding of this character for several spiralian taxa was incorrect. For Micrognathozoa it had been coded as "?", while *L. maerski* clearly possesses ciliation in the posterior part of the body (Bekkouche & Worsaae 2016; Kristensen & Funch 2000). The character had been coded as "present" in Chaetognatha, even though chaetognaths possess multiple fence organs composed of ciliated sensory epidermal cells and distributed throughout their entire bodies (Bone & Pulsford 1978; Müller *et al.* 2014). Furthermore, the character had been coded as "absent" for taxa, in which motile cilia are actually restricted only to the anterior epidermis - i.e. bryozoa (e.g. Schwaha *et al.* 2020) and Sipuncula (e.g. Hausen 2005; Rice 1993). The coding was corrected accordingly.

18. Ciliated corona with paired nerves (5 and 67)

The original coding of this character explicitly assumed homology of rotifer corona and corona ciliata of chaetognaths. Even though both organs are located on the head and composed of multiple cells of motile cilia their homology seems unlikely. The corona of rotifers is composed of multiciliated, cushion shaped cells, which surround the mouth opening, often forming two concentric circles - trochus and cingulum (e.g. Fontaneto & De Smet 2015; Melone & Ricci 1995). The corona of rotifers serves for locomotion and food capture and its ciliated cells are directly innervated from the brain by the anterior coronal nerves (e.g. Gąsiorowski *et al.* 2019; Hochberg 2006; Hochberg & Lilley 2010; Kotikova *et al.* 2005; Leasi *et al.* 2009). In contrast, the corona ciliata present in chaetognaths is located on the dorsal head and neck area, serves sensory, excretory and respiratory functions (Bleich *et al.* 2017; Müller *et al.* 2019) and its monociliated cells are not directly innervated from the central nervous system (Müller *et al.* 2014). In our opinion, the differences in position, connectivity, ultrastructure and function of both organs preclude the primary statement on their homology. We therefore split this character into two new characters: **"Corona ciliata"** (char. 5 in the modified matrix), which refers to the organ present in chaetognaths, and **"Rotifer corona"** (char. 67 in the modified matrix; scored as present only in rotifers). Both new characters are dependent on the character "ciliated epidermis".

25. Spiral or radial cleavage (8)

We merged the original character 25 (Spiral cleavage) with the character 110 (Radial cleavage). Since we do not want to homologize the different types of alternative modes of cleavages, this is a dual state character: Spiral, Radial or inapplicable.
Character states:
0="radial"; 1="spiral"

26. Appical cross (9)

The character is dependent on the character "spiral cleavage" and should be scored as "-" for all species lacking spiral cleavage. Second, the apical cross was reported in Gnathostomulida (Riedl 1969).

37. Through-gut (11)

In the original matrix this character was scored as "absent" only for Platyhelminthes. The proper anus (and hence, through-gut) is however also missing in Micrognathozoa, Gnathostomulida and in rhynchonelliformean brachiopods (Hejnol & Martin-Duran 2015).

39. Dorsal anus or anal pore (13)

The anus is ventral in Chaetognatha (e.g. Arnaud *et al.* 1996; Müller *et al.* 2019; Schmidt-Rhaesa 2007). Additionally, several taxa for which the character had been scored as "absent" in fact possess dorsal anus - i.e., Bryozoa (e.g. Schwaha *et al.* 2020), Phoronida (e.g. Hermann 1997) and Sipuncula (e.g. Rice 1993). Moreover, in Gnathostomulida and Micrognathozoa, the anus, or more precisely an anal pore, is hypothetical and only suspected due to ultrastructural observations (Hejnol & Martin-Duran 2015; Knauss 1979). This possible pore is therefore not considered as primarily homologous to an anus, and we consider the anus as absent in these two groups, therefore it is inapplicable.

40. Ventral mouth and **41. Terminal mouth (14)**
Since the mouth cannot be both ventral and terminal at the same time, we believe that those two characters should be merged into a single character “mouth” with two possible states “ventral” and “terminal”. Additionally, we re-coded the character as “ventral” for lophophorates (where the mouth opens ventrally to the epistome, which itself represents a terminal structure) and “terminal” for Gastrotricha, where the mouth is the most anterior structure.
Character states:
0=”ventral”; 1=”terminal”

42. Body divided with distinct inflated head and neck region (15)
Division of the body into the head and neck region is also present in bursovaginoid Gnathostomulids (while absent in filospermoids; Sterrer & Sørensen 2015). Therefore, we re-coded the character as both “present” and “absent” for Gnathostomulida.

44. Nephridia (16)
This character had been vaguely defined and incorrectly coded for several taxa. We re-defined nephridia as ultrafiltration-based excretory organs (see e.g. Andrikou *et al.* 2021), which include proto- and metanephridia but neither H-cells of nematodes nor Malpighian tubules of tardigrades. Homology of proto- and metanephridia is well established (e.g. Gąsiorowski *et al.* 2021; Ruppert 1994; Ruppert & Smith 1988), yet the presence of one or the other type might bear phylogenetic signal. Therefore, we coded this character as multi-state with the four possible character states “absent”, “protonephridia”, “metanephridia” and “ontogenetic transition from proto- to metanephridia”. In general, our coding for particular taxa followed that from Schmidt-Rhaesa (2007), however, we coded the character as “metanephridia” for chaetognaths, following recent report of metanephridia-like organs in this taxon (Müller *et al.* 2019). Furthermore, we added two new characters focused on the nephridial canal (“cellularity of the nephridium collecting canal” and “shape of nephridial canal”) as well as additional two characters on the fine structure of protonephridia (“terminal cells of protonephridia” and “cilia-microvilli arrangement in protonephridia”) – see below for more details (section **New characters**).
Character states:
0=”protonephridia”; 1=”metanephridia”; 2=”transition from proto to metanephridia”; 3=”absent”

45. Fate of blastopore (17)
This character had been coded incorrectly for several taxa. First of all, amphistomy has never been clearly demonstrated and probably represents either observational artifact or autapomorphic conditions of a handful of taxa (Martindale & Hejnol 2009). Second, the character had been scored as “?” or “uniqe” for some taxa for which protostomy has been reported (e.g., Sipuncula, Rotifera, Gastrotricha, Platyhelminthes). We re-coded this character following Martindale and Hejnol (2009) and Hejnol and Martin-Duran (2015).

46. Annelid type cuticle (18)

The annelid-type cuticle as defined by Ax (2000) is: “The cuticle is interspersed by a multilayered network of collagen fibers. The layers cross one another at right angles. The fibers are embedded in a matrix which peripherally forms an epicuticle”. This character is described in detail in Hausen (2005). However, it should be noted that this character is probably not a synapomorphy of annelids, but a synapomorphy of the clade [Amphinomidae + Sipunculida +Pleistoannelida] (Purschke et al 2014), excluding Palaeoannelida ([Oweniidae + Magelonidae] and Chaetopteriformia ([Apistobranchidae + Psammodrilidae + Chaetopteridae]).

59. Longitudinal ventral nerve cords (26)

Paired ventral nerve cords are also present in larval phoronids (Temereva & Wanninger 2012; Temereva 2012) and Brachiopods (Martin-Duran *et al.* 2018; Santagata 2011).

61. Circumoral nerve ring (28)

This character is originally described in Rouse and Fauchald (1995) as following: “Circumoesophageal nerve ring. The nerve ring connects the dorsal cephalic ganglion (or brain) at or near the anterior end to the rest of the nervous system, which is developed as a few (one to four) longitudinal nerve trunks. This is found in all taxa in the analysis except the Platyhelminthes and Nemertea (see Brusca & Brusca 1990). In the latter group, a nerve ring is present, but it surrounds the rhynchocoel (Turbeville 1991)“. We did not re-code this character, but we think that it would be worth reconsidering according to this definition for future studies.

62. Stomatogastric nerve plexi (29)

The character is vaguely defined. The stomatogastric nerves as such are present virtually in all analyzed taxa and therefore do not bear any phylogenetic signal. Since the stomatogastric nervous system of chaetognathiferans has a specific architecture with a pharynx-related ganglion(s) connecting directly to the brain, we re-defined this character as “**Stomatogastric ganglion(s) directly connected to the brain**”, which refers to the mass of stomatogastric perikarya that connects directly to the brain and not to the nerve cords. With such definition this character is scored as present in Mollusca (buccal ganglia; Sigwart & Sumner-Rooney 2016), Gnathostomulida (buccal ganglion; Gąsiorowski *et al.* 2017), Chaetognaths (vestibular and subesophageal ganglia; Rieger *et al.* 2010) and Rotifers (mastax ganglion; Gąsiorowski *et al.* 2019; Hochberg 2007). Although comparable structure is present in Micrognathozoa (pharyngeal ganglion; Bekkouche & Worsaae 2016; Kristensen & Funch 2000), its connection to the brain remains unknown (Bekkouche & Worsaae 2016).

63. Major ventral nerve plexus in trunk (31)

We do not understand which part of the Rotifera nervous system consists of a plexus, at least in the terms of Richter *et al.* (2010). In contrast, all detailed studies of the rotifer nervous system showed presence of well-defined nerve cords and lack of structures fulfilling the definition of nerve plexus (summarized in e.g. Fontaneto & De Smet 2015). In the absence of more information, we find it more reasonable to code this nerve plexus as “absent” in Rotifera.

67. Immunoreactivity of horseradish peroxidase (HRP) (removed)

This character has been removed since the study it is based on (Haase *et al.* 2001) uses a very limited sample of Protostomata for their study. This character has been re-coded by Vinther and Parry (2019) from Ou *et al.* (2017) (character 85 therein) as being generally present in all Ecdysozoa and absent in all other Metazoa even for taxa where it has never been tested. We therefore removed this character, although it could be more precisely re-coded according to Haase *et al.* (2001) with an exemplar approach.

68. Trochophores (35)

The undisputable trochophores are only present in Annelids (including sipunculids), molluscs and entoprocts (Wanninger 2015 and citation therein). Accordingly, we scored the character as absent for platyhelminthes and rotifers (which both seems to be ancestrally direct developers) and as "?" for nemerteans (the homology of nemertean larva and trochophore is ambiguous, see discussion in von Döhren 2015).

69. Prototroch (removed)

The prototroch is a defining feature of trochophore larvae and therefore each taxon that has a trochophore has to be scored present for prototroch and vice versa. Since two characters are linked and reciprocally dependent, we removed the character "prototroch" from our matrix.

73. Chaetae (38)

The chaetae have been scored as present in Brachiopoda, Annelida, Mollusca and Bryozoa. Homology of chaetae in the three former clades is well justified. However, the structures sometimes interpreted as homologues in bryozoans (the so-called gizzard teeth; Gordon 1975), are present only in some Stenolemata and Gymnolemata and cannot be reconstructed as ancestral bryozoan character (Schwaha *et al.* 2020). Therefore, we scored the character as "absent" for Bryozoa.

74. Chaetae in bundles (removed)

The character is scored as "absent" only for Mollusca. However, in the fossil mollusc *Pelagiella exigua* the chaetae are also organized in bundles (Thomas *et al.* 2020). Because of that the character is strictly linked and repetitive with the character "chaetae" and therefore uninformative. We removed the character from the matrix.

78. Radula (40)

We re-coded this character as depending on the character 80: "sclerotized elements in the anterior digestive tract.".

80. Chitinous pharyngeal structure (41)

The character is described in Vinther and Parry (2019) as: "We score the jaws of gnathiferans and chaetognaths as homologs, following the proposal of Fröbius and Funch 2017". However, sclerotized pharyngeal, buccal or peribuccal structures are found in many groups. Strictly speaking the radula is one (as coded here). Placoids, the perforating stylet of tardigrades, and the heavily cuticulated sucking pharynx of gastrotrichs or nematodes can also be considered as such. We consider the character as coded by Vinther and Parry (2019) as misleading/too vague and therefore recoded it as present in many Protostomata in order to allow for more strict definitions. This implies that “jaws” characters can only be coded in animals with a present state for this character, and are non-applicable in animals with an absent state. We also renamed the character as “**Sclerotized elements in the anterior digestive tract**” as chemical composition of several of those jaw-like elements remains unknown (e.g., jaws of gnathostomulids) and their position is not always pharyngeal (e.g., grasping spines of chaetognaths).

81. Pharyngeal skeleton composed of rod-shaped elements (43)

The character is described as: “tube like support rods in jaw: Rotifers, gnathostumulids and micrognathozoans share the presence of tubes with an electron dense core (Kristensen and Funch, 2000)” in Vinther and Parry (2019) where it just has been mistakenly coded as “?” for Micrognathozoa, and has been consequently re-coded as present in Micrognathozoa. We also rephrased the name of the character as these structures are not only support elements but constitutive parts of the Gnathifera jaw apparatus (Sterrer & Sørensen 2015).

82. Grasping spines (44)

Following our coding grasping spines have been coded as present in all Chaetognatha (extant and extinct) and absent in all the other taxa with the present state for the character 80, while it was considered as inapplicable for non-Chaetognatha in Vinther and Parry (2019).

83. Teeth (45)

Following our coding, teeth have been coded as present in all extant Chaetognatha and *Protosagitta* and absent in all the other taxa with the present state for the character 80, while it was considered as inapplicable for Spiralia (except Chaetognatha) and unknown for Ecdysozoa in Vinther and Parry (2019).

84. Anterior teeth and **85. Posterior teeth (removed)**

Since only one set of possible teeth is described in *Protosagitta* (Vannier et al. 2007) with an imprecise position, their anterior or posterior nature cannot be asserted, therefore the characters anterior and posterior teeth are only applicable for the three extant Chaetognatha, constraining these two characters to be parsimony uninformative.

87. Jaw apparatus forming a ventral membrane (removed)

This character is not further described in Vinther and Parry (2019). We are not sure what this membrane stands for. Here we consider a jaw membrane as a part of the jaws (hence sclerotized element of the pharynx) forming a continuous surface. To our knowledge, there is no equivalent of a ventral membrane in gnathostomulids. Even though the rostral apophysis, the caudal apophysis or the postero-lateral part of the jaws can make up a surface (Sørensen & Sterrer 2002), these are all different structure with a wide variation between Gnathostomulida and are not specifically ventral. The basal plate could also be considered as such, but usually does not occupy a proportionally large surface (Lammert 1991; Sterrer & Sørensen 2015). The fibularium of Micrognathoza indeed forms a membrane ventral to the rest of the jaws, also extending laterally (De Smet 2002; Kristensen & Funch 2000; Sørensen 2003), however, the homology of this structure with the different parts of the trophi of Rotifera is unsure (Bekkouche *et al.* 2014). In *Brachionus* (e.g. Guerrero-Jiménez *et* *al.* 2015) with a maleate trophi, the manubrium, the unci with connected teeth, and the ramus form some sort of surface (Fontaneto & De Smet 2015). In *Adineta*, with a ramate trophi (Fontaneto & De Smet 2015), there is no such membrane, although uncus teeth are quite numerous and can give an impression of membrane, this last structure is not continuous (e.g. Melone *et al.* 1998). All in all, these multiple "membranous" structures are derived from different parts of the jaw apparatus of Gnathifera s.s., and cannot be considered as homologous in our state of knowledge. Considering *Amiskwia*, while Vinther and Parry (2019) described the jaws as two bilateral membranous elements: a ventral and a dorsal pair, only a dorsal unpaired membrane sclerotized element is described by Caron and Cheung (2019). However, the ventral or dorsal sclerotized membranes cannot be homologized with the one of Gnathifera s.s., as they cannot even be homologized within Gnathifera. For all these reasons we decided to remove this character.

88. Jaw forming clusters of homonomous elements (removed)

The character is described in Vinther and Parry (2019) as "In chaetognaths the jaws are formed of clusters of self-similar elements such as teeth or grasping spines. In some fossil chaetognaths this includes four clusters of grasping spines (Shu et al. 2017 and Briggs and Caron 2017).". While repeated teeth can be considered as homologous between Chaetognatha, this is coded as present as well in Rotifera. However, this can be only stated for the uncus teeth of the maleate trophi of *Brachionus* and the ramate trophi of *Adineta* (also found in malleoramate trophi) (Fontaneto & De Smet 2015). Although repeated uncus teeth homology between these two tophi arrangements is still disputed (Sørensen 2002), it is probably not homologous with the teeth of Chaetognatha since the structure, position and connection of these repeated jaw elements differ radically between Rotifera and Chaetognatha. Furthermore, repeated jaw elements can be found in Gnathostomulida (e.g. in *Labidognathia*) or in Micrognathozoa with the pharyngeal lamellae (Sørensen 2003), without obvious relation of homology between all these structures in Gnathifera. For all these reasons we decided to remove this character.

90. Mantle cavity (49)

A mantle cavity is also present in brachiopods (e.g. Reed 1987).

92. Subterminal anus (removed)

We decided to remove this character because it is redundant to the character 39 (13)

94. Trunk fins (51)

We coded them as absent Nemertea as it is a derived state of only some pelagic nemerteans (Maslakova & Norenburg 2001).

101. Stomatogastric nerve plexi (removed)

The character is repetitive with character 62.

102. Major ventral nerve plexus in trunk (removed)

The character is repetitive with character 63.

103. Lateral sensory antennae (removed)

This character has been coded from Fröbius and Funch (2017), with very few elements to support the comparison between the so-called antennae of Rotifera and

Chaetognatha. We assume that in Chaetognatha they refer to the ciliary fence receptors (Shinn 1997). On the other hand, among Rotifers, only in Monogonont there is a pair of lateral antennae (Fontaneto & De Smet 2015), while other antennae can be found in different position in Bdelloidea, Seisonidea and Monogononta (Ahlrichs & Riemann 2019; Fontaneto & De Smet 2015). However, there is no specific argument to homologize these sensory organs to the one found in Chaetognatha. Moreover, sensory organs scattered through the body are found in many animals. Therefore, we decided to remove this character as ambiguous and vaguely defined.

104. Posterior adhesion structure (removed)

The homology of this character is discussed in Vinther and Parry (2019) and we agree with the objections they emit. However, we think that this character is too homoplastic to be scored. It has also been miscoded in some taxa in their matrix as absent in *Spadella* where they are present (also they are specific to the genera) or absent in gastrotricha. All in all, there is the wide presence and variation of posterior adhesive structure in Metazoa and their homology remain dubious (Fontaneto & De Smet 2015; Kristensen & Funch 2000; Martin 1978; Müller *et al.* 2019; Tyler & Rieger 1980). Therefore, we decided to remove this character.

105. Head tentacles (removed)

As discussed in Vinther and Parry (2019), antennae of *Spadella* are quite different from the antennae of *Amiswkia*, furthermore we found no studies describing in detail the tentacles of *Spadella*. In the light of the general state of knowledge, and considering that other Chaetognatha do not seem to have tentacles, we think that it is dubious to homologize the tentacles of *Spadella* and *Amiskwia* and decided to remove this character.

110. Radial cleavage (removed)

See character 25 (8).

113. Serially repeated paired coelomic cavities (57)

Coelomic cavities of phoronids are unpaired (e.g. Temereva & Malakhov 2015), the character has been re-coded accordingly.

114. Trimeric coelom (58)

Trimeric coelom is present in all lophophorates (Wanninger 2015 and citation therein) and in chaetognaths (Müller *et al.* 2019).

137. Duplication of Ubx/Abd-A into lox4 and lox2 (64)

According to the newer data on spiralian Hox gene complement, *lox2* has been demonstrated thus far only in annelids, molluscs, entoprocts and nemerteans (Gąsiorowski & Hejnol 2020). The character has been re-coded accordingly.

New characters

In addition to the original set of characters we added 10 new characters, which focus on the morphological diversity within Gnathifera and Spiralia. In the following list we provide the number of each character according to our final matrix (M-CC-PI), along with a short description of the character. For each character the exact coding is also given.

1. Cilia-microvilli arrangement in protonephridia

In protonephridial excretory organs microvilli and cilia of terminal cells can occur in three basic arrangements: irregularly distributed, with a wreath of microvilli surrounding each cilium or with a wreath of microvilli surrounding all cilia of the terminal cell. The coding of the character follows revisions on the nephridial morphology (Andrikou *et al.* 2021; Schmidt-Rhaesa 2007) and citations therein. The character depends on the character 16 (“Nephridia”) and is scored only for those terminals that have protonephridia.

Character states:

0=”each cilium with single wreath of microvilli”; 1=”all cilia surrounded by common wreath of microvilli”; 2=”irregular”

30. Stomatogastric ganglion with ciliated receptors

Stomatogastric ganglion(s) are present in Gnathifera (s.s.) and Chaetognatha, however only in gnathiferans they bear ciliated pharyngeal receptors that protrude sensory cilia into the pharyngeal cavity. For further discussion of this character see Gąsiorowski *et al.* (2017).

Character states:

0=”absent”; 1=”present”

42. Basal plate

The basal plate is an unpaired ventral and sclerified element of the jaw apparatus found in most (but not all) Gnathostomulida at the posterior rim of the mouth opening (Lammert 1991; Sterrer & Sørensen 2015), and recently described also in the fossil *Amiskwia sagittiformis* (Caron & Cheung 2019).

Character states:

0=”absent”; 1=”present”

67. Rotifer corona

According Fontaneto and De Smet (2015) “The ground plan of the corona comprises a ciliated area, the buccal field, surrounding the usually ventrally located mouth opening. The buccal field is evenly ciliated with short cilia. It extends upward around the head and forms a circumapical band, delimiting an unciliated apical field. The cilia of the ciliary ring at the anterior margin of the circumapical band are strong and form the preoral trochus - a ciliary row of usually finer cilia at the posterior margin forms the pastoral cingulum. Between the trochus and the cingulum runs a finely ciliated groove. The apical field bears numerous sensory receptors and is often provided with setae and tufts or rows of cilia (styli, cirri, membranelles).” According to this description, and although there is a range of variation, this only applies to the two rotifers included in our matrix, *Adineta* and *Branchionus.* This also excludes Micrognathozoa in which the head ciliation is organized differently (the mouth is surrounded by the cilia of the posterior ciliated field of the rostrum, and the head and the median anterior ciliophores), without a homogeneously ciliated buccal field (Bekkouche & Worsaae 2016).

Character states:

0=”absent”; 1=”present”

68. Terminal cells of protonephridia

Terminal cells of protonephridia can bear single, double or multiple cilia. In the latter case the cilia can be irregular or arranged in the unison flame. The character is multistate with the following possible states: monociliated, biciliated, multiciliated (irregular), multiciliated (flame). The coding of the character follows revisions on the nephridial morphology (Andrikou *et al.* 2021; Schmidt-Rhaesa 2007) and citations therein. The character depends on the character 16 (“Nephridia”) and is scored only for those terminals that have protonephridia.
Character states:
0=”monociliated”; 1=”biciliated”; 2=”multiciliated (irregular)”; 3=”multiciliated (flame)”

69. Cellularity of the nephridium collecting canal

The collecting canal of the nephridium can be built by several adjacent cells with the extracellular lumen or by a single cell with the intracellular canal. The coding of the character follows revisions on the nephridial morphology (Andrikou *et al.* 2021; Schmidt-Rhaesa 2007) and citations therein. The character depends on the character 16 (“Nephridia”) and is scored only for those terminals that have nephridia (either proto- or metanephridia).
Character states:
0=”unicellular”; 1=”multicellular”; 2=”syncytial”

70. Shape of nephridial canal

The collecting canal of the nephridium can be straight (continuous lumen without any branching pattern) or have two or more branches, with independent filtering units opening to each branch of the same collecting canal. The coding of the character follows revisions on the nephridial morphology (Andrikou *et al.* 2021; Schmidt-Rhaesa 2007) and citations therein. The character depends on the character 16 (“Nephridia”) and is scored only for those terminals that have nephridia (either proto- or metanephridia).
Character states:
0=”straight”; 1=”branching”

71. Intracellular lamellar cuticule

In rotifers, the internal lamella, or intracytoplasmic lamina, is intracellular, and composed of two filamentous keratin-like proteins (Fontaneto & De Smet 2015). According to Sørensen and Kristensen (2015), “In Micrognathozoa the epidermal cells are supported internally by an intracellular lamina. The intracellular lamina is considered homologous with the corresponding lamina found in species of the syndermate taxa Seisonacea, Acanthocephala, and Eurotatoria”; see also Kristensen and Funch (2000) and Ahlrichs (1995) for further discussion. Marlétaz *et al.* (2019) mention the possibility of an intracellular mode of cuticle formation in Chaetognatha, citing Van Der Land and Nørrevang (1985) “In Rotifera, Acanthocephala, and Chaetognatha (Nørrevang unpublished) there is an “intracellular” cuticle, which mean there is no extracellular layer on the body surface”. This is, however, not confirmed by various sources never mentioning an intracellular cuticle neither in the text, the figures nor the TEM photography (Ahnelt 1984; Müller *et al.* 2019; Shinn 1997). Therefore, we coded character as absent in Chaetognatha.
Character states:
0=”absent”; 1=”present”

72. Epidermis structure

In rotifers (Syndermata), the epidermis is syncytial (Fontaneto & De Smet 2015). This is not the case in Micrognathozoa (Kristensen & Funch 2000).
Character states:
0=”cellular”; 1=”syncytial”

73. Female organs with germovitellarium

Here we use the character 13 from Sørensen and Giribet (2006): “A syncytial germovitellarium is present in all rotifers except Seisonidea. The germovitellarium present in some platyhelminths is not considered homologous with the rotifer germovitellarium.”. Apart from the oocyte, further structures of the ovary are not described in Gnathostomulida and Micrognathozoa.
Character states:
0=”absent”; 1=”present”

74. Retrocerebral organ

According Fontaneto and De Smet (2015), the retrocerebral organ of Monogononta and Bdelloidea is an organ “located dorsal to the brain and mastax and consists of the unpaired median retrocerebral sac and 2 lateral subcerebral glands. From the anterior part of the sac, an unpaired duct runs toward the corona and bifurcates anteriorly into 2 ducts opening on the apical field (...). The subcerebral glands have ducts alongside those of the sac”. Furthermore, the retrocerebral organ seems to mostly secrete mucus lubricating the cilia of the corona and can also be involved in adhesive attachment of the rostrum. In Seisonidae, glands considered homologous are found as well, but the retrocerebral sac is paired (Ahlrichs & Riemann 2019). In Micrognathozoa, “The tripartite anterior gland of *Limnognathia maerski*, consisting of one unpaired median and a pair of medio-lateral glands opening dorso-apically is very similar in position and size to the retrocerebral organ found in most Rotifera” (Bekkouche & Worsaae 2016), the unpaired median gland being possibly homologous to the retrocerebral sas, and the bilateral glands being possibly homologous to the subcerebral glands. Due to the similar position and arrangement of these glands to the retrocerebral organ found in Rotifera, we code it as homologous. While no similar organ is described in Gnathostomulida, a retrocerebral organ is described in Chaetognatha. However, the retrocerebral organ of Chaetognatha has a different ultrastructure and arrangement. It is constituted of only two sacs arranged bilaterally tapered into narrow canals (“retrocerebral canals”) converging to a common canal ending into the retrocerebral pore. These organs contain multiple modified cilia and is considered to be a sensory organ (while in Rotifera the function is secretive), more similar to what can be found in Annelida or Nemertea instead of than what is found Rotifera (Müller *et al.* 2019). Therefore, in the current state of knowledge, we do not homologize the retrocerebral organ of Chaetognatha to any secretive or sensory organs of other Metazoa.
Character states:
0=”absent”; 1=”present”

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Supplementary Information II

Careful amendment of morphological datasets improves phylogenetic frameworks: re-evaluating placement of the fossil *Amiskiwia sagittiformis*

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M-CC-PI

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Odontogriphus Canadia Entoprocta Platyhelminthes Gastrotricha Gnathostumulida
Micrognathozoa 'Inquicus-fellatus' Amiskwia Flacisaggita Spadella Eukrohnia
Protosagitta Ankalodous Capinatator Adineta Branchionus Nemertea Onychophora
Arthropoda Tardigrada Nematoda Nematomorpha Priapulida Kinorhyncha Loricifera
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each_cilium_with_single_wreath_of_microvilli
all_cilia_surrounded_by_common_wreath_of_microvilli irregular,
                2 ciliated_epidermis_15 /  absent present,
                3 multiciliate_epridermal_cells_16 /  absent present,
                4 ciliation_restricted_anteriorly_17 /  absent present,
                5 corona_ciliata /  absent present,
                6 ventral_locomotory_cilia_19 /  absent present,
                7 ventral_surface_modified_into_foot_20 /  absent present,
                8 Spiral_or_radial_cleavage_25 /  radial spiral,
                9 apical_cross_26 /  absent present,
                10 4d_mesentoblast_27 /  absent present,
                11 'Through-gut 37' /  absent present,
                12 'U-shaped gut 38' /  absent present,
                13 Dorsal_anus_or_anal_pore_39 /  absent present,
                14 Mouth_40 /  ventral terminal,
                15 body_divided_with_distinct_inflated_head_and_neck_region_42 /
absent present,
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16 Nephridia_44 / protonephridia metanephridia transition_from_proto_to_metanephridia absent,

17 Fate_of_blastopore_45 / protostomy deuterostomy amphistomy unique,

18 annelid_type_cuticle_46 / absent present,

19 Body_cuticle_with_chitin_47 / absent present,

20 'Body cuticle with ?-chitin 48' / absent present,

21 Body_cuticle_molted_49 / absent present,

22 lorica_50 / absent present,

23 lobopods_or_segmented_limbs_51 / absent present,

24 'Mixocoel (haemocoel) surrounded by segmented mesoderm 57' / absent present,

25 Teloblastic_segmentation_58 / absent present,

26 'Longitudinal ventral nerve cord(s) 59' / absent present,

27 paired_vnc_60 / absent present,

28 circumoral_nerve_ring_61 / absent present,

29 'Stomatogastric ganglion(s) directly connected to the brain' / absent present,

30 stomatogastric_ganglion_with_ciliated_receptors / absent present,

31 Major_ventral_nerve_plexus_in_trunk_63 / absent present,

32 'Circum-pharyngeal, collar-shaped brain with anterior rings of perikary 64' / absent present,

33 Introvert_with_scalid_rings_65 / absent present,

34 Flosculi_66 / absent present,

35 Trochophores_68 / absent present,

36 apical_organ_70 / absent present,

37 Segmental_metanephridia_sacculus_72 / absent present,

38 chaetae_73 / absent present,

39 Parapodia_with_dorsal_and_ventral_branches_terminated_by_chaetae_77 / absent present,

40 radula_78 / absent present,

41 sceletal_elements_in_the_anterior_digestive_tract_80 / absent present,

42 basal_plate / absent present,

43 'pharyngeal sceleton composed of rod-shaped elements' / absent present,

44 grasping_spines_82 / absent present,

45 teeth_83 / absent present,

46 internal_jaws / absent present,

47 jaws_with_symphysis / absent present,

48 Ctenidia_89 / absent present,

49 Mantle_cavity_90 / absent present,

50 tripartite_body_plan_with_septum_93 / absent present,

51 trunk_fins_94 / absent present,

52 anterior_lateral_fins_95 / absent present,

53 caudal_fin_96 / absent present,

54 Phragms_98 / absent present,

55 head_hood_106 / absent present,

56 coelom_111 / absent present,

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57 serially_repeated_paired_coelomic_cavities_113 /  absent present,
58 Trimeric_coelom_114 /  absent present,
59 Lophophore_131 /  absent present,
60 ftz_133 /  absent present,
61 'Ubx/abd-A 134' /  absent_ present,
62 AbdB_135 /  absent present,
63 lox5_136 /  absent present,
64 duplication_of_UbdA_into_lox4_and_lox2_137 /  absent present,
65 Post2_138 /  absent present,
66 MedPost_140 /  absent present,
67 rotifer_corona /  absent present,
68 terminal_cells_of_protonephridia /  monociliated biciliated 'multiciliated (irregular)' 'multiciliated (flame)',
69 cellularity_of_the_nephridium_collecting_canal /  unicellular multicellular syncytial,
70 shape_of_nephridial_canal /  straight branching,
71 Intracellular_lamellar_cuticule /  absent present,
72 Epidermis_structure /  cellular syncytial,
73 Female_organs_with_germovitellarium /  absent present,
74 Retrocerebral_organ /  absent present ;
MATRIX
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Brachiopoda     -1010000-0(0 1)10001200-000001100-000001010-0------0100-00011110?010100-100000
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Micrognathozoa 0110010???0--010?00-000-0111?100000?000010100110000-0000--0???????00111001

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Spadella -101100--0100011100-000001111010000000000100110-0011011110100000010-100000

Eukrohnia -101100--0100011100-000001111010000000000100110-001111111010??0????0-100000

Protosagitta ??????????100?1???????0?0????????????0?010?110-???????1???0???????0???????

Ankalodous ??????????????1????????????????????????010?100-???????1???0???????0???????

Capinatator ??????????10??1???????0?0???????0????00010?100-???????1???0???????0???????

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Branchionus 1111000--01?1000??0-00000110110000000000010100110000-0000--0000000113211111

Nemertea 21100101011000000000-000001100-0000?1000-0------0000-00010-000011100211-000

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        Tardigrada        -0----0--010010300111011?1100-0000000000100000-0000-
00010-0???????0---0000
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M-CC-PI-Bayesian

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MATRIX
Bryozoa          -11100000?111003000-000000-00-000001000-0------0000-
00010110?010100---0000
Brachiopoda      -1010000-0(0 1)10001200-000001100-000001010-0------
0100-00011110?010100-100000
Phoronida        21000000-0111002000-000001100-000001000-0------0000-
0001011????01?00110000
Annelida         2110000111100002210-000011110-000011011-0------0000-
00011000001110001000000
Sipuncula        -1110001111110011?10-000?01010-000011000-0------0000-
0001100???????0-100000
Mollusca         2110011111110000220(0 1)00000011110?000110(0
1)0110000001100-0001000000111002100000
Wiwaxia          ??????1???10000???0-?00?0???????0????10110?000-
?1?0-0?0???0???????0???????
Odontogriphus    ??????1???10000???0-?00?0???????0?????0110?000-
1100-0?0???0???????0???????
Canadia          ?????0????10000???0-?00?11110-000????11-0------1000-
0?0???0???????0???????
Entoprocta       211?011111110?000010000-00-00-000011000-0------0000-
0000--0????11?02??0000
```

Platyhelminthes 11100101110--000300-000-01100-000001000-0------0000-0000--000010100311-000

Gastrotricha 0110010--01001?0?00-000-01100-000000000-0------0000-0000--0???????00000000

Gnathostumulida 010001011?0--0(0 1)0?00-000-011111000000000011100110000-0000--0???????0000-000

Micrognathozoa 0110010???0--010?00-000-0111?100000?000010100110000-0000--0???????00111001

Inquicus ??????0???10??1???????0?0???????0????00010?0010?000-0?0???0???????0???????

Amiskwia ??????0???10?01???????0?0???????0????00011?0011000101?1???0???????0???????

Flacisaggita -101100--0100011100-0000011110100000000100110-00111101101000000010-100000

Spadella -101100--0100011100-0000011110100000000100110-00110111101000000010-100000

Eukrohnia -101100--0100011100-0000011110100000000100110-001111111010??0????0-100000

Protosagitta ??????????100?1???????0?0????????????0?010?110-???????1???0???????0???????

Ankalodous ??????????????1????????????????????????010?100-???????1???0???????0???????

Capinatator ??????????10??1???????0?0???????0????00010?100-???????1???0???????0???????

Adineta 1111000--01?1000??0-00000110110000000000010100110000-0000--0000000113211111

Branchionus 1111000--01?1000??0-00000110110000000000010100110000-0000--0000000113211111

Nemertea 211001010110000000-000001100-0000?1000-0------0000-00010-000011100211-000

Onychophora -0----0--010010120111011111110-000000100010000100000-00010-011100000-100000

Arthropoda -0----0--010010120111011111110-000000100010000100000-0000-00010-011100000-100000

Tardigrada -0----0--010010300111011?1100-000000000100000-0000-00010-0???????0---0000

Nematoda -0----0--01001032010100-01000-0110000000100000-0000-0000--0???????0---0000

Nematomorpha -0----0--01001031010100-01000-0010000000100000-0000-0000--0???????0---0000

Priapulida 20----0--01001001011110-01000-0111000000100000-0000-0000--0101000000110000

Kinorhyncha 10----0--0100100??10100-?1000-0111000000100000-0000-0000--0???????01100000

Loricifera 00----0--0100100??10110-01100-0111000000100000-0000-0000--0???????00100000

```
;
END;


Begin MrBayes;
lset rates=gamma;


mcmc ngen = 5000000 append = n;
sumt burninfrac=0.1;
sump burninfrac=0.1;
end;
```

M-CC-PI-Fins characters fused

```
#NEXUS

BEGIN TAXA;
	TITLE Taxa;
	DIMENSIONS NTAX=33;
	TAXLABELS
		Bryozoa Brachiopoda Phoronida Annelida Sipuncula Mollusca Wiwaxia
Odontogriphus Canadia Entoprocta Platyhelminthes Gastrotricha Gnathostumulida
Micrognathozoa 'Inquicus-fellatus' Amiskwia Flacisaggita Spadella Eukrohnia
Protosagitta Ankalodous Capinatator Adineta Branchionus Nemertea Onychophora
Arthropoda Tardigrada Nematoda Nematomorpha Priapulida Kinorhyncha Loricifera
```

```
        ;

END;


BEGIN CHARACTERS;
        TITLE  'Matrix in file "character_matrix"';
        DIMENSIONS  NCHAR=72;
        FORMAT DATATYPE = STANDARD RESPECTCASE GAP = - MISSING = ?
SYMBOLS = "  0 1 2 3 4";
        CHARSTATELABELS
                1 'cilia-microvilli arrangement in protonephridia' /
each_cilium_with_single_wreath_of_microvilli
all_cilia_surrounded_by_common_wreath_of_microvilli irregular,
                2 ciliated_epidermis_15 /  absent present,
                3 multiciliate_epridermal_cells_16 /  absent present,
                4 ciliation_restricted_anteriorly_17 /  absent present,
                5 corona_ciliata /  absent present,
                6 ventral_locomotory_cilia_19 /  absent present,
                7 ventral_surface_modified_into_foot_20 /  absent present,
                8 Spiral_or_radial_cleavage_25 /  radial spiral,
                9 apical_cross_26 /  absent present,
                10 4d_mesentoblast_27 /  absent present,
                11 'Through-gut 37' /  absent present,
                12 'U-shaped gut 38' /  absent present,
```

13 Dorsal_anus_or_anal_pore_39 / absent present,

14 Mouth_40 / ventral terminal,

15 body_divided_with_distinct_inflated_head_and_neck_region_42 / absent present,

16 Nephridia_44 / protonephridia metanephridia transition_from_proto_to_metanephridia absent,

17 Fate_of_blastopore_45 / protostomy deuterostomy amphistomy unique,

18 annelid_type_cuticle_46 / absent present,

19 Body_cuticle_with_chitin_47 / absent present,

20 'Body cuticle with ?-chitin 48' / absent present,

21 Body_cuticle_molted_49 / absent present,

22 lorica_50 / absent present,

23 lobopods_or_segmented_limbs_51 / absent present,

24 'Mixocoel (haemocoel) surrounded by segmented mesoderm 57' / absent present,

25 Teloblastic_segmentation_58 / absent present,

26 'Longitudinal ventral nerve cord(s) 59' / absent present,

27 paired_vnc_60 / absent present,

28 circumoral_nerve_ring_61 / absent present,

29 'Stomatogastric ganglion(s) directly connected to the brain' / absent present,

30 stomatogastric_ganglion_with_ciliated_receptors / absent present,

31 Major_ventral_nerve_plexus_in_trunk_63 / absent present,

32 'Circum-pharyngeal, collar-shaped brain with anterior rings of perikary 64' / absent present,

33 Introvert_with_scalid_rings_65 / absent present,

34 Flosculi_66 / absent present,

35 Trochophores_68 / absent present,

36 apical_organ_70 / absent present,

37 Segmental_metanephridia_sacculus_72 / absent present,

38 chaetae_73 / absent present,

39 Parapodia_with_dorsal_and_ventral_branches_terminated_by_chaetae_77 / absent present,

40 radula_78 / absent present,

41 sceletal_elements_in_the_anterior_digestive_tract_80 / absent present,

42 basal_plate / absent present,

43 'pharyngeal sceleton composed of rod-shaped elements' / absent present,

44 grasping_spines_82 / absent present,

45 teeth_83 / absent present,

46 internal_jaws / absent present,

47 jaws_with_symphysis / absent present,

48 Ctenidia_89 / absent present,

49 Mantle_cavity_90 / absent present,

50 tripartite_body_plan_with_septum_93 / absent present,

51 Chaetognath_like_fins_94 / absent present,

52 Phragms_98 / absent present,

53 head_hood_106 / absent present,

54 coelom_111 / absent present,

55 serially_repeated_paired_coelomic_cavities_113 / absent present,

56 Trimeric_coelom_114 / absent present,

57 Lophophore_131 / absent present,

58 ftz_133 / absent present,

59 'Ubx/abd-A 134' / absent_ present,

60 AbdB_135 / absent present,

61 lox5_136 / absent present,

62 duplication_of_UbdA_into_lox4_and_lox2_137 / absent present,

63 Post2_138 / absent present,

64 MedPost_140 / absent present,

65 rotifer_corona / absent present,

66 terminal_cells_of_protonephridia / monociliated biciliated 'multiciliated (irregular)' 'multiciliated (flame)',

67 cellularity_of_the_nephridium_collecting_canal / unicellular multicellular syncytial,

68 shape_of_nephridial_canal / straight branching,

69 Intracellular_lamellar_cuticule / absent present,

70 Epidermis_structure / cellular syncytial,

71 Female_organs_with_germovitellarium / absent present,

72 Retrocerebral_organ / absent present ;

MATRIX

Bryozoa -11100000?111003000-000000-00-000001000-0------00000010110?010100---0000

Brachiopoda -1010000-0(0 1)10001200-000001100-000001010-0------01000011110?010100-100000

Phoronida 21000000-0111002000-000001100-000001000-0------0000001011????01?00110000

Annelida 2110000111100002210-000011110-000011011-0------0000001100000111000100000

Sipuncula -111000111111001?10-000?01010-000011000-0------0000001100???????0-100000

Mollusca 211001111110000220(0 1)00000011110?000110(0 1)0110000001100001000000111002100000

Wiwaxia ??????1???10000???0-?00?0???????0????10110?000-?1?0?0???0???????0???????

Odontogriphus ??????1???10000???0-?00?0???????0?????0110?000-1100?0???0???????0???????

Canadia ?????0????10000???0-?00?11110-000????11-0------1000?0???0???????0???????

Entoprocta 211?011111110?000010000-00-00-000011000-0------0000000--0????11?02??0000

Platyhelminthes 11100101110--000300-000-01100-000001000-0------0000000--000010100311-000

Gastrotricha 0110010--01001?0?00-000-01100-000000000-0------0000000--0???????00000000

Gnathostumulida 010001011?0--0(0 1)0?00-000-0111110000000000011100110000000--0???????0000-000

Micrognathozoa 0110010???0--010?00-000-0111?100000?00001010011000000--0???????00111001

'Inquicus-fellatus'??????0???10??1???????0?0???????0????00010?0010?000?0???0???????0???????

Amiskwia ??????0???10?01???????0?0???????0????00011?00110001?1???0???????0???????

Flacisaggita -101100--0100011100-00000111010000000000100110-001101101000000010-100000

Spadella -101100--0100011100-00000111010000000000100110-001111101000000010-100000

Eukrohnia -101100--0100011100-00000111010000000000100110-0011111010??0????0-100000

Protosagitta ??????????100?1???????0?0????????????0?010?110-?????1???0???????0???????

Ankalodous ??????????????1????????????????????????010?100-?????1???0???????0???????

Capinatator ??????????10??1???????0?0???????0????00010?100-?????1???0???????0???????

Adineta 1111000--01?1000??0-0000011011000000000010100110000000--0000000113211111

```
        Branchionus       1111000--01?1000??0-
00000110110000000000010100110000000--0000000113211111
        Nemertea          21100101011000000000-000001100-0000?1000-0------
00000010-000011100211-000
        Onychophora       -0----0--01001012011101111110-
00000010001000010000000010-011100000-100000
        Arthropoda        -0----0--01001012011101111110-0000001000100000-
00000010-011100000-100000
        Tardigrada        -0----0--010010300111011?1100-0000000000100000-
00000010-0???????0---0000
        Nematoda          -0----0--01001032010100-01000-0110000000100000-
0000000--0???????0---0000
        Nematomorpha      -0----0--01001031010100-01000-0010000000100000-
0000000--0???????0---0000
        Priapulida        20----0--01001001011110-01000-0111000000100000-
0000000--0101000000110000
        Kinorhyncha       10----0--0100100??10100-?1000-0111000000100000-
0000000--0???????01100000
        Loricifera        00----0--0100100??10110-01100-0111000000100000-
0000000--0???????00100000


;


END;
```

M-CC-PI without *Amiskwia*

```
#NEXUS


BEGIN TAXA;
	TITLE Taxa;
	DIMENSIONS NTAX=32;
	TAXLABELS
		Bryozoa Brachiopoda Phoronida Annelida Sipuncula Mollusca Wiwaxia
Odontogriphus Canadia Entoprocta Platyhelminthes Gastrotricha Gnathostumulida
Micrognathozoa 'Inquicus-fellatus' Flacisaggita Spadella Eukrohnia Protosagitta
Ankalodous Capinatator Adineta Branchionus Nemertea Onychophora Arthropoda
Tardigrada Nematoda Nematomorpha Priapulida Kinorhyncha Loricifera
	;

END;



BEGIN CHARACTERS;
	TITLE  'Matrix in file "character_matrix"';
	DIMENSIONS  NCHAR=74;
	FORMAT DATATYPE = STANDARD RESPECTCASE GAP = - MISSING = ?
SYMBOLS = "  0 1 2 3 4";
	CHARSTATELABELS
```

1 'cilia-microvilli arrangement in protonephridia' / each_cilium_with_single_wreath_of_microvilli all_cilia_surrounded_by_common_wreath_of_microvilli irregular,

2 ciliated_epidermis_15 / absent present,

3 multiciliate_epridermal_cells_16 / absent present,

4 ciliation_restricted_anteriorly_17 / absent present,

5 corona_ciliata / absent present,

6 ventral_locomotory_cilia_19 / absent present,

7 ventral_surface_modified_into_foot_20 / absent present,

8 Spiral_or_radial_cleavage_25 / radial spiral,

9 apical_cross_26 / absent present,

10 4d_mesentoblast_27 / absent present,

11 'Through-gut 37' / absent present,

12 'U-shaped gut 38' / absent present,

13 Dorsal_anus_or_anal_pore_39 / absent present,

14 Mouth_40 / ventral terminal,

15 body_divided_with_distinct_inflated_head_and_neck_region_42 / absent present,

16 Nephridia_44 / protonephridia metanephridia transition_from_proto_to_metanephridia absent,

17 Fate_of_blastopore_45 / protostomy deuterostomy amphistomy unique,

18 annelid_type_cuticle_46 / absent present,

19 Body_cuticle_with_chitin_47 / absent present,

20 'Body cuticle with ?-chitin 48' / absent present,

21 Body_cuticle_molted_49 / absent present,

22 lorica_50 / absent present,

23 lobopods_or_segmented_limbs_51 / absent present,

24 'Mixocoel (haemocoel) surrounded by segmented mesoderm 57' / absent present,

25 Teloblastic_segmentation_58 / absent present,

26 'Longitudinal ventral nerve cord(s) 59' / absent present,

27 paired_vnc_60 / absent present,

28 circumoral_nerve_ring_61 / absent present,

29 'Stomatogastric ganglion(s) directly connected to the brain' / absent present,

30 stomatogastric_ganglion_with_ciliated_receptors / absent present,

31 Major_ventral_nerve_plexus_in_trunk_63 / absent present,

32 'Circum-pharyngeal, collar-shaped brain with anterior rings of perikary 64' / absent present,

33 Introvert_with_scalid_rings_65 / absent present,

34 Flosculi_66 / absent present,

35 Trochophores_68 / absent present,

36 apical_organ_70 / absent present,

37 Segmental_metanephridia_sacculus_72 / absent present,

38 chaetae_73 / absent present,

39 Parapodia_with_dorsal_and_ventral_branches_terminated_by_chaetae_77 / absent present,

40 radula_78 / absent present,

41 sceletal_elements_in_the_anterior_digestive_tract_80 / absent present,

42 basal_plate / absent present,

43 'pharyngeal sceleton composed of rod-shaped elements' / absent present,

44 grasping_spines_82 / absent present,

45 teeth_83 / absent present,

46 internal_jaws / absent present,

47 jaws_with_symphysis / absent present,

48 Ctenidia_89 / absent present,

49 Mantle_cavity_90 / absent present,

50 tripartite_body_plan_with_septum_93 / absent present,

51 trunk_fins_94 / absent present,

52 anterior_lateral_fins_95 / absent present,

53 caudal_fin_96 / absent present,

54 Phragms_98 / absent present,

55 head_hood_106 / absent present,

56 coelom_111 / absent present,

57 serially_repeated_paired_coelomic_cavities_113 / absent present,

58 Trimeric_coelom_114 / absent present,

59 Lophophore_131 / absent present,

60 ftz_133 / absent present,

61 'Ubx/abd-A 134' / absent_ present,

62 AbdB_135 / absent present,

63 lox5_136 / absent present,

64 duplication_of_UbdA_into_lox4_and_lox2_137 / absent present,

65 Post2_138 / absent present,

66 MedPost_140 / absent present,

67 rotifer_corona / absent present,

68 terminal_cells_of_protonephridia / monociliated biciliated 'multiciliated (irregular)' 'multiciliated (flame)',

69 cellularity_of_the_nephridium_collecting_canal / unicellular multicellular syncytial,

70 shape_of_nephridial_canal / straight branching,

71 Intracellular_lamellar_cuticule / absent present,

72 Epidermis_structure / cellular syncytial,

73 Female_organs_with_germovitellarium / absent present,

74 Retrocerebral_organ / absent present ;

MATRIX

Bryozoa -11100000?111003000-000000-00-000001000-0------0000-00010110?010100---0000

Brachiopoda -1010000-0(0 1)10001200-000001100-000001010-0------0100-00011110?010100-100000

Phoronida 21000000-0111002000-000001100-000001000-0------0000-0001011????01?00110000

Annelida 2110000111100002210-000011110-000011011-0------0000-0001100000111000100000

Sipuncula -111000111111001?10-000?01010-000011000-0------0000-0001100???????0-100000

Mollusca 211001111110000220(0 1)00000011110?000110(0 1)0110000001100-00010000000111002100000

Wiwaxia ??????1???10000???0-?00?0???????0????10110?000-?1?0-0?0???0???????0???????

Odontogriphus ??????1???10000???0-?00?0???????0?????0110?000-1100-0?0???0???????0???????

Canadia ?????0????10000???0-?00?11110-000???11-0------1000-0?0???0???????0???????

Entoprocta 211?011111110?000010000-00-00-000011000-0------0000-0000--0???11?02??0000

Platyhelminthes 11100101110--000300-000-01100-000001000-0------0000-0000--000010100311-000

Gastrotricha 0110010--01001?0?00-000-01100-000000000-0------0000-0000--0???????00000000

Gnathostumulida 010001011?0--0(0 1)0?00-000-0111110000000000011100110000-0000--0???????0000-000

Micrognathozoa 0110010???0--010?00-000-0111?100000?000010100110000-0000--0???????00111001

'Inquicus-fellatus'??????0???10??1???????0?0???????0????00010?0010?000-0?0???0???????0???????

Flacisaggita -101100--0100011100-000001111010000000000100110-001111011010000000010-100000

Spadella -101100--0100011100-000001111010000000000100110-001101111010000000010-100000

Eukrohnia -101100--0100011100-00000111101000000000100110-001111111010??0????0-100000

Protosagitta ??????????100?1???????0?0????????????0?010?110-???????1???0???????0???????

Ankalodous ??????????????1????????????????????????010?100-???????1???0???????0???????

Capinatator ??????????10??1???????0?0???????0????00010?100-???????1???0???????0???????

Adineta 1111000--01?1000??0-0000011011000000000010100110000-0000--0000000113211111

Branchionus 1111000--01?1000??0-0000011011000000000010100110000-0000--0000000113211111

Nemertea 2110010101100000000-000001100-0000?1000-0------0000-00010-000011100211-000

Onychophora -0----0--01001012011101111110-000000100010000100000-00010-011100000-100000

Arthropoda -0----0--01001012011101111110-0000001000100000-0000-00010-011100000-100000

Tardigrada -0----0--010010300111011?1100-0000000000100000-0000-00010-0???????0---0000

Nematoda -0----0--01001032010100-01000-0110000000100000-0000-0000--0???????0---0000

Nematomorpha -0----0--01001031010100-01000-0010000000100000-0000-0000--0???????0---0000

```
	Priapulida	20----0--01001001011110-01000-0111000000100000-0000-0000--0101000000110000
	Kinorhyncha	10----0--0100100??10100-?1000-0111000000100000-0000-0000--0???????01100000
	Loricifera	00----0--0100100??10110-01100-0111000000100000-0000-0000--0???????00100000

;

END;
```

M-CC-PN

```
#NEXUS

BEGIN TAXA;
	TITLE Taxa;
	DIMENSIONS NTAX=33;
	TAXLABELS
		Bryozoa Brachiopoda Phoronida Annelida Sipuncula Mollusca Wiwaxia Odontogriphus Canadia Entoprocta Platyhelminthes Gastrotricha Gnathostumulida Micrognathozoa 'Inquicus-fellatus' Amiskwia Flacisaggita Spadella Eukrohnia Protosagitta Ankalodous Capinatator Adineta Branchionus Nemertea Onychophora Arthropoda Tardigrada Nematoda Nematomorpha Priapulida Kinorhyncha Loricifera
	;
```

```
END;



BEGIN CHARACTERS;

        TITLE  'Matrix in file "character_matrix"';

        DIMENSIONS  NCHAR=138;

        FORMAT DATATYPE = STANDARD RESPECTCASE GAP = - MISSING = ?

SYMBOLS = "  0 1 2 3 4";

        CHARSTATELABELS

                1 'cilia-microvilli arrangement in protonephridia' /

each_cilium_with_single_wreath_of_microvilli

all_cilia_surrounded_by_common_wreath_of_microvilli irregular,

                2 Collar_complex_1 /  absent present,

                3 Multicellularity_with_extracellular_matrix_2 /  absent present,

                4 'Septate junctions (SJs) 3' /  absent present,

                5 'Tight junctions (TJs) 4' /  absent present,

                6 'Gap junctions (GJs) 5' /  absent present,

                7 'Adherens junctions (AJs) 6' /  absent present,

                8 Hemidesmosomes_7 /  absent present,

                9 Epithelia_8 /  absent present,

                10 Basal_laminae_9 /  absent present,

                11 Collagen_10 /  absent present,

                12 Nerve_cells_11 /  absent present,

                13 Acetylcholine_used_as_a_neurotransmitter_12 /  absent present,

                14 Diffuse_nervous_system_13 /  absent present,
```

15 Epidermis_with_pulsatite_bodies_14 / absent present,

16 ciliated_epidermis_15 / absent present,

17 multiciliate_epridermal_cells_16 / absent present,

18 ciliation_restricted_anteriorly_17 / absent present,

19 corona_ciliata / absent present,

20 ventral_locomotory_cilia_19 / absent present,

21 ventral_surface_modified_into_foot_20 / absent present,

22 Xenacoelomorph_cilia_21 / absent present,

23 Striated_ciliary_rootlets_22 / absent present,

24 Diploblasts_built_of_two_germ_layers_23 / absent present,

25 Triploblasts_built_of_three_germ_layers_24 / absent present,

26 Spiral_or_radial_cleavage_25 / radial spiral,

27 apical_cross_26 / absent present,

28 4d_mesentoblast_27 / absent present,

29 Colloblasts_28 / absent present,

30 'Coelenteron (gastrovascular cavity) 29' / absent present,

31 Cnidae_30 / absent present,

32 Structure_of_mitochondrial_DNA_31 / circular linear,

33 Actinopharynx_32 / absent present,

34 'Siphonoglyph (sulcus) 33' / absent present,

35 Planulae_34 / absent present,

36 Polyp_stage_35 / absent present,

37 Medusoid_stage_36 / absent present,

38 'Through-gut 37' / absent present,

39 'U-shaped gut 38' / absent present,

40 Dorsal_anus_or_anal_pore_39 / absent present,

41 Mouth_40 / ventral terminal,

42 body_divided_with_distinct_inflated_head_and_neck_region_42 / absent present,

43 bipartite_gut_with_cuticular_pharynx_43 / absent_ present,

44 Nephridia_44 / protonephridia metanephridia transition_from_proto_to_metanephridia absent,

45 Fate_of_blastopore_45 / protostomy deuterostomy amphistomy unique,

46 annelid_type_cuticle_46 / absent present,

47 Body_cuticle_with_chitin_47 / absent present,

48 'Body cuticle with ?-chitin 48' / absent present,

49 Body_cuticle_molted_49 / absent present,

50 lorica_50 / absent present,

51 lobopods_or_segmented_limbs_51 / absent present,

52 limb_type_52 / lobopods arthrodized_limbs,

53 anteriorly_facing_last_pair_of_limbs_53 / absent present,

54 Slime_papillae_54 / absent present,

55 Telescoping_mouth_cone_with_protrudable_stylets_55 / absent present,

56 Respiration_via_metameric_tracheae_and_spiracles_56 / absent present,

57 'Mixocoel (haemocoel) surrounded by segmented mesoderm 57' / absent present,

58 Teloblastic_segmentation_58 / absent present,

59 'Longitudinal ventral nerve cord(s) 59' / absent present,

60 paired_vnc_60 / absent present,

61 circumoral_nerve_ring_61 / absent present,

62 'Stomatogastric ganglion(s) directly connected to the brain' / absent present,

63 stomatogastric_ganglion_with_ciliated_receptors / absent present,

64 Major_ventral_nerve_plexus_in_trunk_63 / absent present,

65 'Circum-pharyngeal, collar-shaped brain with anterior rings of perikary 64' / absent present,

66 Introvert_with_scalid_rings_65 / absent present,

67 Flosculi_66 / absent present,

68 Trochophores_68 / absent present,

69 apical_organ_70 / absent present,

70 Apical_organ_with_muscles_extending_to_the_hyposphere_71 / absent present,

71 Segmental_metanephridia_sacculus_72 / absent present,

72 chaetae_73 / absent present,

73 chaetae_in_bundles_74 / absent present,

74 mineralised_chaetae_75 / absent present,

75 serially_repeated_chaetal_bundles_76 / absent present,

76 Parapodia_with_dorsal_and_ventral_branches_terminated_by_?_77 / chaetae ' / absent present,' 78 Radula '/' absent present,

77 radula_78 / absent present,

78 Radula_tooth_rows_79 / few_rows many_rows,

79 sceletal_elements_in_the_anterior_digestive_tract_80 / absent present,

80 basal_plate / absent present,

81 'pharyngeal sceleton composed of rod-shaped elements' / absent present,

82 grasping_spines_82 / absent present,

83 teeth_83 / absent present,

84 anterior_teeth_84 / absent present,

85 posterior_teeth_85 / absent present,

86 internal_jaws / absent present,

87 jaws_with_symphysis / absent present,

88 Ctenidia_89 / absent present,

89 Mantle_cavity_90 / absent present,

90 Eversible_proboscis_surrounded_by_rhynchocoel_91 / absent present,

91 tripartite_body_plan_with_septum_93 / absent present,

92 trunk_fins_94 / absent present,

93 anterior_lateral_fins_95 / absent present,

94 caudal_fin_96 / absent present,

95 fins_supported_by_rays_97 / absent present,

96 Phragms_98 / absent present,

97 phragms_in_trunk_99 / absent present,

98 phragms_in_tail_100 / absent present,

99 head_hood_106 / absent present,

100 AP_axis_107 / absent present,

101 dorsoventral_axis_108 / absent present,

102 coelom_111 / absent present,

103 Coelom_formation_112 / schizocoely enterocoely,

104 serially_repeated_paired_coelomic_cavities_113 / absent present,

105 Trimeric_coelom_114 / absent present,

106 Pharyngeal_slits_115 / absent present,

107 'Endostyle (or homologues) 116' / absent present,

108 Notochord_117 / absent present,

109 Stomochord_118 / absent present,

110 Haemal_system_with_axial_complex_119 / absent present,

111 Calcareous_endoskeleton_composed_of_separate_ossicles_120 / absent present,

112 Tornaria_type_larva_121 / absent present,

113 Longitudinal_dorsal_nerve_cord_122 / absent present,

114 Zig_zag_myomeres_123 / absent present,

115 Endothelium_that_lines_the_inner_wall_of_blood_vessels_124 / absent present,

116 Neural_crest_125 / absent present,

117 Neurogenic_placodes_126 / absent present,

118 Body_symmetry_127 / radial bilateral biradial,

119 Mesoglea_128 / absent present,

120 Cydippid_larvae_129 / absent present,

121 Ciliary_rosettes_130 / absent present,

122 Lophophore_131 / absent present,

123 'Hox/ParaHox genes 132' / absent present,

124 ftz_133 / absent present,

125 'Ubx/abd-A 134',

126 AbdB_135 / absent present,

127 lox5_136 / absent present,

128 duplication_of_UbdA_into_lox4_and_lox2_137 / absent present,

129 Post2_138 / absent present,

130 MedPost_140 / absent present,

131 rotifer_corona / absent present,

132 terminal_cells_of_protonephridia / monociliated biciliated 'multiciliated (irregular)' 'multiciliated (flame)',

133 cellularity_of_the_nephridium_collecting_canal / unicellular multicellular syncytial,

134 shape_of_nephridial_canal / straight branching,

135 Intracellular_lamellar_cuticule / absent present,

136 Epidermis_structure / cellular syncytial,

137 Female_organs_with_germovitellarium / absent present,

138 Retrocerebral_organ / absent present ;

MATRIX

Bryozoa -011011111111001110000010100?0000000001110003000-
000--000000-00-000001-00-0-0--0--------00000-0-0--
01110010000000000001000110?010100---0000

Brachiopoda -011011111111001010000101 0-0000000000(0
1)100001200-000--000001100-000001-0110(0 1)0--0--------01000-0-0--
01110110000000000001000110?010100-100000

Phoronida 2011011111111001000000101 0-0000000000111000 2000-000--000001100-000001-00---0--0--------00000-0-0--0111001000000000000100011????01?00110000

Annelida 2011011111111001100000101111000000000100002210-000--000011110-0000111011011--0--------00000-0-0--0111010000000000000100001000111000100000

Sipuncula -0110111111110011100001011110000000001110001?10-000--000?01010-000011100---0--0--------00000-0-0--01110100000000000001000 01???????0-100000

Mollusca 2011011111111001100110101111000000000100000220(0 1)0000--0000011110?0001100(0 1)1(0 1)001110000--0011000-0-0--0111000000000000000100001000111002100000

Wiwaxia ????????????????????1????????0???????100000???0-?00--00??0???????0?????110101010?00--0-?10?0-0-???011????0????0??????1???0????????0???????

Odontogriphus ????????????????????1????????0???????100000???0-?00--00??0???????0?????????01010?00--0-11000-0-???011????0????0??????1???0????????0???????

Canadia ???????????????????0?????????0???????100000???0-?00--000?11110-000?????11011--0-------?10000-0-???011????0????0?0????1???0????????0???????

Entoprocta 2011011111111110011?0110101111000000000110?0000010000--000-00-00-000011100---0--0-------?00000-0-0--0110---000000000000100001???11?02??0000

Platyhelminthes 10110111111110011001001011110000000000--0000300-000--000-01100-000001-00---0--0-------?00000-0-0--0110---00000000000010000100010100311-000

Gastrotricha 0011011111111001100100101--0000000001001?10?00-000--000-01100-000000-00---0--0-------?00000-0-0--0110---000000000000100001???????00000000

Gnathostumulida 001101111111100100010010111?0000000000--0(0 1)00?00-000--000-011111000000-00---00-11100--1100000-0-0--0110---000000000000100001???????0000-000

Micrognathozoa 001?????1?11?001100100?01???0000000000--0100?00-000--000-0111?100000??00---00-10100--1100000-0-0--0110---00000000000010000????????00111001

'Inquicus-fellatus'????????????????????0????????0??0????10??1????????0--?0??0???????0?????0--?00-10?00--10?0?00-0-???011????0????0??0???1???0????????0???????

Amiskwia ????????????????????0????????0??0????10?010???????0--?0??0???????0?????0---00-11?00--1100?01010???111????0????0??0???1???0????????0???????

Flacisaggita -011011111111001011000101--00000000001000101100-000--0000011110100000-00---00-10011110-000111110--1111001000000000000010000100000010-100000

Spadella -01101111111001011000101--0000000001000101100-

000--0000011110100000-00---00-10011110-

0001101111011110010000000000001000010000001 0-100000

Eukrohnia -01101111111001011000101--0000000001000101100-

000--0000011110100000-00---00-10011010-

00011111101111001000000000000100001??0????0-100000

Protosagitta

????????????????????????????0???????100?1????????0?-

?0??0??????????????0---?0-10?11??0-

???????????111?????????0??0???1???0????????0???????

Ankalodous

??1?????????????????????????

?????????0-10?10??0-

???????????111?????????0??????1???0????????0???????

Capinatator

????????????????????????????0???????10??1????????0--

?0??0???????0?????0---00-10?10??0-

???????????111?????????0??0???1???0????????0???????

Adineta 10110111111110011100001 01--00000000001?10000??0-

000--0000011011000000-00---00-10100--1100000-0-0--0110---

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Branchionus 10110111111110011100001 01--00000000001?10000??0-

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000000000000100001000000113211111

Nemertea 2011011111111001100100101101000000000001000000000-000--000001100-0000?1?00---0--0--------00100-0-0--011100-00000000000010000100011100211-000

Onychophora -011011111111000----00101--00000000001001001201110100101111110-000000-10---00-10000--1000000-0-0--011100-000000000000100001111000000-100000

Arthropoda -011011111111000----00101--0000000000100100120111011000(0 1)111110-000000-10---00-10000--0-00000-0-0--011100-000000000000100001111000000-100000

Tardigrada -011011111111000----00101--00000000001001003001110101010 1?1100-000000-00---00-10000--0-00000-0-0--011100-000000000000100001???????0---0000

Nematoda -011011111111000----00101--0000000000100100320101 00--000-01000-011000-00---00-10000--0-00000-0-0--0110---000000000000100001???????0---0000

Nematomorpha -011011111111000----00101--000000000010010031010100--000-01000-001000-00---00-10000--0-00000-0-0--0110---000000000000100001???????0---0000

Priapulida 2011011111111000----00101--000000000010010001011110--000-01000-011100-00---00-10000--0-00000-0-0--0110---000000000000100001101000000110000

Kinorhyncha 1011011111111000----00101--00000000001001000??10100--000-?1000-011100-00---00-10000--0-00000-0-0--0110---000000000000100001???????01100000

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        Loricifera      0011011111111000----00101--00000000001001000??10110--
000-01100-011100-00---00-10000--0-00000-0-0--0110---
000000000000100001???????00100000

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END;
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M-CC-PN-Bayesian

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        FORMAT DATATYPE = STANDARD GAP = - MISSING = ?;

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000--000000-00-000001-00-0-0--0--------00000-0-0--
01110010000000000001000110?010100---0000
        Brachiopoda     -0110111111110010100001010-0000000000(0
1)100001200-000--000001100-000001-0110(0 1)0--0--------01000-0-0--
01110110000000000001000110?010100-100000
        Phoronida       20110111111110010000001010-00000000001110002000-
000--000001100-000001-00---0--0--------00000-0-0--
0111001000000000000100011????01?00110000
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Annelida 20110111111110011000001011110000000001000002210-000--000011110-0000111011011--0--------00000-0-0--0111010000000000000100001000111000100000

Sipuncula -0110111111110011100001011110000000001110001?10-000--000?01010-000011100---0--0--------00000-0-0--011101000000000000100001???????0-100000

Mollusca 20110111111110011001101011110000000000100000220(0 1)0000--0000011110?0001100(0 1)1(0 1)001110000--0011000-0-0--0111000000000000000100001000111002100000

Wiwaxia ????????????????????1????????0???????100000???0-?00--00??0???????0?????110101010?00--0-?10?0-0-???011????0????0??????1???0????????0???????

Odontogriphus ????????????????????1????????0???????100000???0-?00--00??0???????0?????????01010?00--0-11000-0-???011????0????0??????1???0????????0???????

Canadia ???????????????????0?????????0???????100000???0-?00--000?11110-000????11011--0-------?10000-0-???011????0????0?0????1???0????????0???????

Entoprocta 20110111111110011?0110101111000000000110?0000010000--000-00-00-000011100---0--0-------?00000-0-0--0110---000000000000100001???11?02??0000

Platyhelminthes 10110111111110011001001011110000000000--0000300-000--000-01100-000001-00---0--0-------?00000-0-0--0110---00000000000010000100010100311-000

Gastrotricha 0011011111111001100100101--0000000000001001?10?00-000--000-01100-000000-00---0--0-------?00000-0-0--0110---000000000000100001???????00000000

Gnathostumulida 0011011111111001000100101111?0000000000--0(0 1)00?00-000--000-011111000000-00---00-11100--1100000-0-0--0110---000000000000100001???????0000-000

Micrognathozoa 001?????1?11?001100100?01???0000000000--0100?00-000--000-0111?100000??00---00-10100--1100000-0-0--0110---000000000000010000????????00111001

Inquicus ????????????????????0????????0??0????10??1????????0--?0??0???????0?????0--?00-10?00--10?0?00-0-???011????0????0??0???1???0????????0???????

Amiskwia ????????????????????0????????0??0????10?010???????0--?0??0???????0?????0---00-11?00--1100?01010???111????0????0??0???1???0????????0???????

Flacisaggita -011011111111001011000101--00000000001000101100-000--0000011110100000-00---00-10011110-000111110--11110010000000000000100001000000010-100000

Spadella -011011111111001011000101--00000000001000101100-000--0000011110100000-00---00-10011110-000110111101111001000000000000010000100000010-100000

Eukrohnia -011011111111001011000101--00000000001000101100-000--0000011110100000-00---00-10011010-000111111101111001000000000000100001??0????0-100000

Protosagitta ?????????????????????????????0???????100?1????????0?-?0??0?????????????0---?0-10?11??0-???????????111?????????0??0???1???0????????0???????

Ankalodous ???1??????????????????????????????????0-10?10??0-???????????111?????????0??????1???0????????0???????

Capinatator ?????????????????????????????0???????10??1????????0--?0??0???????0?????0---00-10?10??0-???????????111?????????0??0???1???0????????0???????

Adineta 1011011111111001110000101--00000000001?10000??0-000--0000011011000000-00---00-10100--1100000-0-0--0110---000000000000100001000000113211111

Branchionus 1011011111111001110000101--00000000001?10000??0-000--0000011011000000-00---00-10100--1100000-0-0--0110---000000000000100001000000113211111

Nemertea 2011011111111001100100101101000000001000000000-000--000001100-0000?1?00---0--0--------00100-0-0--011100-00000000000010000100011100211-000

Onychophora -011011111111000----00101--000000000010010012011101001011111 10-000000-10---00-10000--1000000-0-0--011100-000000000000100001111 00000-100000

Arthropoda -011011111111000----00101--0000000000100100120111011000(0 1)111110-000000-10---00-10000--0-00000-0-0--011100-0000000000001000011110 0000-100000

Tardigrada -011011111111000----00101--0000000000100100300111010101 01?1100-000000-00---00-10000--0-00000-0-0--011100-000000000000100001???????0---0000

Nematoda -011011111111000----00101--00000000001001003201010 0--000-01000-011000-00---00-10000--0-00000-0-0--0110---000000000000100001???????0---0000

Nematomorpha -011011111111000----00101--0000000000100100310101 00--000-01000-001000-00---00-10000--0-00000-0-0--0110---000000000000100001???????0---0000

Priapulida 2011011111111000----00101--0000000000100100010111 10--000-01000-011100-00---00-10000--0-00000-0-0--0110---000000000000100001101000000110000

Kinorhyncha 1011011111111000----00101--00000000001001000??10100--000-?1000-011100-00---00-10000--0-00000-0-0--0110---000000000000100001???????01100000

Loricifera 0011011111111000----00101--00000000001001000??10110--000-01100-011100-00---00-10000--0-00000-0-0--0110---000000000000100001???????00100000

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;
END;


Begin MrBayes;
lset rates=gamma;


mcmc ngen = 5000000 append = n;
sumt burninfrac=0.1;
sump burninfrac=0.1;
end;
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M-VP-PI

```
#NEXUS

BEGIN TAXA;
	TITLE Taxa;
	DIMENSIONS NTAX=33;
	TAXLABELS
		Bryozoa Brachiopoda Phoronida Annelida Sipuncula Mollusca Wiwaxia Odontogriphus Canadia Entoprocta Platyhelminthes Gastrotricha Gnathostumulida Micrognathozoa 'Inquicus-fellatus' Amiskwia Flacisaggita Spadella Eukrohnia Protosagitta Ankalodous Capinatator Adineta Branchionus Nemertea Onychophora Arthropoda Tardigrada Nematoda Nematomorpha Priapulida Kinorhyncha Loricifera
```

```
	;

END;



BEGIN CHARACTERS;
	TITLE  'Matrix in file "character_matrix"';
	DIMENSIONS  NCHAR=74;
	FORMAT DATATYPE = STANDARD RESPECTCASE GAP = - MISSING = ?
SYMBOLS = "  0 1 2 3 4";
	CHARSTATELABELS
		1 'cilia-microvilli arrangement in protonephridia' /
each_cilium_with_single_wreath_of_microvilli
all_cilia_surrounded_by_common_wreath_of_microvilli irregular,
		2 ciliated_epidermis_15 /  absent present,
		3 multiciliate_epridermal_cells_16 /  absent present,
		4 ciliation_restricted_anteriorly_17 /  absent present,
		5 corona_ciliata /  absent present,
		6 ventral_locomotory_cilia_19 /  absent present,
		7 ventral_surface_modified_into_foot_20 /  absent present,
		8 Spiral_or_radial_cleavage_25 /  radial spiral,
		9 apical_cross_26 /  absent present,
		10 4d_mesentoblast_27 /  absent present,
		11 'Through-gut 37' /  absent present,
		12 'U-shaped gut 38' /  absent present,
```

13 Dorsal_anus_or_anal_pore_39 / absent present,

14 Mouth_40 / ventral terminal,

15 body_divided_with_distinct_inflated_head_and_neck_region_42 / absent present,

16 Nephridia_44 / protonephridia metanephridia transition_from_proto_to_metanephridia absent,

17 Fate_of_blastopore_45 / protostomy deuterostomy amphistomy unique,

18 annelid_type_cuticle_46 / absent present,

19 Body_cuticle_with_chitin_47 / absent present,

20 'Body cuticle with ?-chitin 48' / absent present,

21 Body_cuticle_molted_49 / absent present,

22 lorica_50 / absent present,

23 lobopods_or_segmented_limbs_51 / absent present,

24 'Mixocoel (haemocoel) surrounded by segmented mesoderm 57' / absent present,

25 Teloblastic_segmentation_58 / absent present,

26 'Longitudinal ventral nerve cord(s) 59' / absent present,

27 paired_vnc_60 / absent present,

28 circumoral_nerve_ring_61 / absent present,

29 'Stomatogastric ganglion(s) directly connected to the brain' / absent present,

30 stomatogastric_ganglion_with_ciliated_receptors / absent present,

31 Major_ventral_nerve_plexus_in_trunk_63 / absent present,

32 'Circum-pharyngeal, collar-shaped brain with anterior rings of perikary 64' / absent present,

33 Introvert_with_scalid_rings_65 / absent present,

34 Flosculi_66 / absent present,

35 Trochophores_68 / absent present,

36 apical_organ_70 / absent present,

37 Segmental_metanephridia_sacculus_72 / absent present,

38 chaetae_73 / absent present,

39 Parapodia_with_dorsal_and_ventral_branches_terminated_by_?_77 / absent present,

40 radula_78 / absent present,

41 sceletal_elements_in_the_anterior_digestive_tract_80 / absent present,

42 basal_plate / absent present,

43 'pharyngeal sceleton composed of rod-shaped elements' / absent present,

44 grasping_spines_82 / absent present,

45 teeth_83 / absent present,

46 internal_jaws / absent present,

47 jaws_with_symphysis / absent present,

48 Ctenidia_89 / absent present,

49 Mantle_cavity_90 / absent present,

50 tripartite_body_plan_with_septum_93 / absent present,

51 trunk_fins_94 / absent present,

52 anterior_lateral_fins_95 / absent present,

53 caudal_fin_96 / absent present,

54 Phragms_98 / absent present,

55 head_hood_106 / absent present,

56 coelom_111 / absent present,

57 serially_repeated_paired_coelomic_cavities_113 / absent present,

58 Trimeric_coelom_114 / absent present,

59 Lophophore_131 / absent present,

60 ftz_133 / absent present,

61 'Ubx/abd-A 134',

62 AbdB_135 / absent present,

63 lox5_136 / absent present,

64 duplication_of_UbdA_into_lox4_and_lox2_137 / absent present,

65 Post2_138 / absent present,

66 MedPost_140 / absent present,

67 rotifer_corona / absent present,

68 terminal_cells_of_protonephridia / monociliated biciliated 'multiciliated (irregular)' 'multiciliated (flame)',

69 cellularity_of_the_nephridium_collecting_canal / unicellular multicellular syncytial,

70 shape_of_nephridial_canal / straight branching,

71 Intracellular_lamellar_cuticule / absent present,

72 Epidermis_structure / cellular syncytial,

73 Female_organs_with_germovitellarium / absent present,

74 Retrocerebral_organ / absent present ;

MATRIX

Bryozoa -11100000?111003000-000000-00-000001000-0------0000-00010110?010100---0000

Brachiopoda -1010000-0(0 1)10001200-000001100-000001010-0------0100-00011110?010100-100000

Phoronida 21000000-0111002000-000001100-000001000-0------0000-0001011????01?00110000

Annelida 2110000111100002210-000011110-000011011-0------0000-0001100000111000100000

Sipuncula -1110001111111001?10-000?01010-000011000-0------0000-0001100???????0-100000

Mollusca 2110011111110000220(0 1)00000011110?000110(0 1)0110000001100-0001000000111002100000

Wiwaxia ??????1???10000???0-?00?0???????0????10110?000-?1?0-0?0???0???????0???????

Odontogriphus ??????1???10000???0-?00?0???????0?????0110?000-1100-0?0???0???????0???????

Canadia ?????0????10000???0-?00?11110-000???11-0-----?1000-0?0???0???????0???????

Entoprocta 211?011111110?000010000-00-00-000011000-0-----?0000-0000--0????11?02??0000

Platyhelminthes 11100101110--000300-000-01100-000001000-0-----?0000-0000--000010100311-000

Gastrotricha 0110010--01001?0?00-000-01100-000000000-0-----?0000-0000--0???????00000000

Gnathostumulida 010001011?0--0(0 1)0?00-000-0111110000000000011100110000-0000--0???????0000-000

Micrognathozoa 0110010???0--010?00-000-0111?100000?000010100110000-0000--0???????00111001

'Inquicus-fellatus'??????0???10??1???????0?0???????0????00010?0010?000-0?0???0???????0???????

Amiskwia ??????0???10?01???????0?0???????0????00010?0010000101?1???0???????0???????

Flacisaggita -101100--0100011100-000001111010000000000110110-0011110110100000010-100000

Spadella -101100--0100011100-000001111010000000000100110-0011011110100000010-100000

Eukrohnia -101100--0100011100-000001111010000000000100110-001111111010??0????0-100000

Protosagitta ??????????100?1???????0?0????????????0?010?110-???????1???0???????0???????

Ankalodous ??????????????1????????????????????????010?100-???????1???0???????0???????

Capinatator ??????????10??1???????0?0???????0????00010?100-???????1???0???????0???????

Adineta 1111000--01?1000??0-0000011011000000000010100110000-0000--0000000113211111

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00000110110000000000010100110000-0000--0000000113211111
        Nemertea        211001010110000000 0-000001100-0000?1000-0------0000-
00010-000011100211-000
        Onychophora     -0----0--01001012011101111110-
00000010001000010000 0-00010-011100000-100000
        Arthropoda      -0----0--01001012011101111110-0000001000100000-0000-
00010-011100000-100000
        Tardigrada      -0----0--010010300111011?1100-0000000000100000-0000-
00010-0???????0---0000
        Nematoda        -0----0--01001032010100-01000-0110000000100000-0000-
0000--0???????0---0000
        Nematomorpha    -0----0--01001031010100-01000-0010000000100000-
0000-0000--0???????0---0000
        Priapulida      20----0--01001001011110-01000-0111000000100000-0000-
0000--0101000000110000
        Kinorhyncha     10----0--0100100??10100-?1000-0111000000100000-0000-
0000--0???????01100000
        Loricifera      00----0--0100100??10110-01100-0111000000100000-0000-
0000--0???????00100000

;

END;
```

M-VP-PN

```
#NEXUS


BEGIN TAXA;
	TITLE Taxa;
	DIMENSIONS NTAX=33;
	TAXLABELS
		Bryozoa Brachiopoda Phoronida Annelida Sipuncula Mollusca Wiwaxia
Odontogriphus Canadia Entoprocta Platyhelminthes Gastrotricha Gnathostumulida
Micrognathozoa 'Inquicus-fellatus' Amiskwia Flacisaggita Spadella Eukrohnia
Protosagitta Ankalodous Capinatator Adineta Branchionus Nemertea Onychophora
Arthropoda Tardigrada Nematoda Nematomorpha Priapulida Kinorhyncha Loricifera
	;

END;



BEGIN CHARACTERS;
	TITLE  'Matrix in file "character_matrix"';
	DIMENSIONS  NCHAR=138;
	FORMAT DATATYPE = STANDARD RESPECTCASE GAP = - MISSING = ?
SYMBOLS = "  0 1 2 3 4";
	CHARSTATELABELS
```

1 'cilia-microvilli arrangement in protonephridia' /

each_cilium_with_single_wreath_of_microvilli

all_cilia_surrounded_by_common_wreath_of_microvilli irregular,

2 Collar_complex_1 / absent present,

3 Multicellularity_with_extracellular_matrix_2 / absent present,

4 'Septate junctions (SJs) 3' / absent present,

5 'Tight junctions (TJs) 4' / absent present,

6 'Gap junctions (GJs) 5' / absent present,

7 'Adherens junctions (AJs) 6' / absent present,

8 Hemidesmosomes_7 / absent present,

9 Epithelia_8 / absent present,

10 Basal_laminae_9 / absent present,

11 Collagen_10 / absent present,

12 Nerve_cells_11 / absent present,

13 Acetylcholine_used_as_a_neurotransmitter_12 / absent present,

14 Diffuse_nervous_system_13 / absent present,

15 Epidermis_with_pulsatite_bodies_14 / absent present,

16 ciliated_epidermis_15 / absent present,

17 multiciliate_epridermal_cells_16 / absent present,

18 ciliation_restricted_anteriorly_17 / absent present,

19 corona_ciliata / absent present,

20 ventral_locomotory_cilia_19 / absent present,

21 ventral_surface_modified_into_foot_20 / absent present,

22 Xenacoelomorph_cilia_21 / absent present,

23 Striated_ciliary_rootlets_22 / absent present,

24 Diploblasts_built_of_two_germ_layers_23 / absent present,

25 Triploblasts_built_of_three_germ_layers_24 / absent present,

26 Spiral_or_radial_cleavage_25 / radial spiral,

27 apical_cross_26 / absent present,

28 4d_mesentoblast_27 / absent present,

29 Colloblasts_28 / absent present,

30 'Coelenteron (gastrovascular cavity) 29' / absent present,

31 Cnidae_30 / absent present,

32 Structure_of_mitochondrial_DNA_31 / circular linear,

33 Actinopharynx_32 / absent present,

34 'Siphonoglyph (sulcus) 33' / absent present,

35 Planulae_34 / absent present,

36 Polyp_stage_35 / absent present,

37 Medusoid_stage_36 / absent present,

38 'Through-gut 37' / absent present,

39 'U-shaped gut 38' / absent present,

40 Dorsal_anus_or_anal_pore_39 / absent present,

41 Mouth_40 / ventral terminal,

42 body_divided_with_distinct_inflated_head_and_neck_region_42 / absent present,

43 bipartite_gut_with_cuticular_pharynx_43 / absent_ present,

44 Nephridia_44 / protonephridia metanephridia transition_from_proto_to_metanephridia absent,

45 Fate_of_blastopore_45 / protostomy deuterostomy amphistomy unique,

46 annelid_type_cuticle_46 / absent present,

47 Body_cuticle_with_chitin_47 / absent present,

48 'Body cuticle with ?-chitin 48' / absent present,

49 Body_cuticle_molted_49 / absent present,

50 lorica_50 / absent present,

51 lobopods_or_segmented_limbs_51 / absent present,

52 limb_type_52 / lobopods arthrodized_limbs,

53 anteriorly_facing_last_pair_of_limbs_53 / absent present,

54 Slime_papillae_54 / absent present,

55 Telescoping_mouth_cone_with_protrudable_stylets_55 / absent present,

56 Respiration_via_metameric_tracheae_and_spiracles_56 / absent present,

57 'Mixocoel (haemocoel) surrounded by segmented mesoderm 57' / absent present,

58 Teloblastic_segmentation_58 / absent present,

59 'Longitudinal ventral nerve cord(s) 59' / absent present,

60 paired_vnc_60 / absent present,

61 circumoral_nerve_ring_61 / absent present,

62 'Stomatogastric ganglion(s) directly connected to the brain' / absent present,

63 stomatogastric_ganglion_with_ciliated_receptors / absent present,

64 Major_ventral_nerve_plexus_in_trunk_63 / absent present,

65 'Circum-pharyngeal, collar-shaped brain with anterior rings of perikary 64' / absent present,

66 Introvert_with_scalid_rings_65 / absent present,

67 Flosculi_66 / absent present,

68 Trochophores_68 / absent present,

69 apical_organ_70 / absent present,

70 Apical_organ_with_muscles_extending_to_the_hyposphere_71 / absent present,

71 Segmental_metanephridia_sacculus_72 / absent present,

72 chaetae_73 / absent present,

73 chaetae_in_bundles_74 / absent present,

74 mineralised_chaetae_75 / absent present,

75 serially_repeated_chaetal_bundles_76 / absent present,

76 Parapodia_with_dorsal_and_ventral_branches_terminated_by_?_77 / chaetae ' / absent present,' 78 Radula '/' absent present,

77 radula_78 / absent present,

78 Radula_tooth_rows_79 / few_rows many_rows,

79 sceletal_elements_in_the_anterior_digestive_tract_80 / absent present,

80 basal_plate / absent present,

81 'pharyngeal sceleton composed of rod-shaped elements' / absent present,

82 grasping_spines_82 / absent present,

83 teeth_83 / absent present,

84 anterior_teeth_84 / absent present,

85 posterior_teeth_85 / absent present,

86 internal_jaws / absent present,

87 jaws_with_symphysis / absent present,

88 Ctenidia_89 / absent present,

89 Mantle_cavity_90 / absent present,

90 Eversible_proboscis_surrounded_by_rhynchocoel_91 / absent present,

91 tripartite_body_plan_with_septum_93 / absent present,

92 trunk_fins_94 / absent present,

93 anterior_lateral_fins_95 / absent present,

94 caudal_fin_96 / absent present,

95 fins_supported_by_rays_97 / absent present,

96 Phragms_98 / absent present,

97 phragms_in_trunk_99 / absent present,

98 phragms_in_tail_100 / absent present,

99 head_hood_106 / absent present,

100 AP_axis_107 / absent present,

101 dorsoventral_axis_108 / absent present,

102 coelom_111 / absent present,

103 Coelom_formation_112 / schizocoely enterocoely,

104 serially_repeated_paired_coelomic_cavities_113 / absent present,

105 Trimeric_coelom_114 / absent present,

106 Pharyngeal_slits_115 / absent present,

107 'Endostyle (or homologues) 116' / absent present,

108 Notochord_117 / absent present,

109 Stomochord_118 / absent present,

110 Haemal_system_with_axial_complex_119 / absent present,

111 Calcareous_endoskeleton_composed_of_separate_ossicles_120 / absent present,

112 Tornaria_type_larva_121 / absent present,

113 Longitudinal_dorsal_nerve_cord_122 / absent present,

114 Zig_zag_myomeres_123 / absent present,

115 Endothelium_that_lines_the_inner_wall_of_blood_vessels_124 / absent present,

116 Neural_crest_125 / absent present,

117 Neurogenic_placodes_126 / absent present,

118 Body_symmetry_127 / radial bilateral biradial,

119 Mesoglea_128 / absent present,

120 Cydippid_larvae_129 / absent present,

121 Ciliary_rosettes_130 / absent present,

122 Lophophore_131 / absent present,

123 'Hox/ParaHox genes 132' / absent present,

124 ftz_133 / absent present,

125 'Ubx/abd-A 134',

126 AbdB_135 / absent present,

127 lox5_136 / absent present,

128 duplication_of_UbdA_into_lox4_and_lox2_137 / absent present,

129 Post2_138 / absent present,

130 MedPost_140 / absent present,

131 rotifer_corona / absent present,

132 terminal_cells_of_protonephridia / monociliated biciliated 'multiciliated (irregular)' 'multiciliated (flame)',

133 cellularity_of_the_nephridium_collecting_canal / unicellular multicellular syncytial,

134 shape_of_nephridial_canal / straight branching,

135 Intracellular_lamellar_cuticule / absent present,

136 Epidermis_structure / cellular syncytial,

137 Female_organs_with_germovitellarium / absent present,

138 Retrocerebral_organ / absent present ;

MATRIX

Bryozoa -01101111111100111000010100?0000000001110003000-
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0111001000000000000001000110?010100---0000

Brachiopoda -0110111111110010100001010-0000000000(0
1)100001200-000--000001100-000001-0110(0 1)0--0--------01000-0-0--
0111011000000000000001000110?010100-100000

Phoronida 20110111111110010000001010-00000000001110002000-
000--000001100-000001-00---0--0--------00000-0-0--
011100100000000000000100011???01?00110000

Annelida 2011011111111001100000101111000000000001000002210-
000--000011110-0000111011011--0--------00000-0-0--
01110100000000000000100001000111000100000

Sipuncula -011011111111001110000101111000000000001110001?10-
000--000?01010-000011100---0--0--------00000-0-0--
0111010000000000000100001???????0-100000

Mollusca 20110111111110011001101011110000000000100000220(0 1)0000--0000011110?0001100(0 1)1(0 1)001110000--0011000-0-0--01110000000000000000100001000111002100000

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Canadia ???????????????????0?????????0???????100000???0-?00--000?11110-000????11011--0-------?10000-0-???011????0????0?0????1???0????????0???????

Entoprocta 20110111111110011?0110101111000000000110?0000010000--000-00-00-000011100---0--0-------?00000-0-0--0110---000000000000100001????11?02??0000

Platyhelminthes 101101111111100110010010111100000000000--0000300-000--000-01100-000001-00---0--0-------?00000-0-0--0110---0000000000001000010001010031l-000

Gastrotricha 0011011111111001100100101--00000000001001?10?00-000--000-01100-000000-00---0--0-------?00000-0-0--0110---000000000000100001???????00000000

Gnathostumulida 0011011111111001000100101111?0000000000--0(0 1)00?00-000--000-011111000000-00---00-11100--1100000-0-0--0110---000000000000100001???????0000-000

Micrognathozoa 001?????1?11?001100100?01???0000000000--0100?00-000--000-0111?100000??00---00-10100--1100000-0-0--0110---00000000000010000????????00111001

'Inquicus-fellatus'????????????????????0????????0??0????10??1????????0--?0??0???????0?????0--?00-10?00--10?0?00-0-???011????0????0??0???1???0????????0???????

Amiskwia ????????????????????0????????0??0????10?010???????0--?0??0???????0?????0---00-10?00--1000?01010???111????0????0??0???1???0????????0???????

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Spadella -011011111111001011000101--00000000001000101100-000--0000011110100000-00---00-10011110-0001101111011110010000000000000100001000000010-100000

Eukrohnia -011011111111001011000101--00000000001000101100-000--0000011110100000-00---00-10011010-00011111110111100100000000000001000001??0????0-100000

Protosagitta ?????????????????????????????0???????100?1????????0?-?0??0?????????????0---?0-10?11??0-???????????111?????????0??0???1???0????????0???????

Ankalodous

???1?????????????????????????

?????????0-10?10??0-

???????????111?????????0??????1???0????????0???????

Capinatator

?????????????????????????????0???????10??1????????0--

?0??0???????0?????0---00-10?10??0-

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Adineta 1011011111111001110000101--00000000001?10000??0-

000--0000011011000000-00---00-10100--1100000-0-0--0110---

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Branchionus 1011011111111001110000101--00000000001?10000??0-

000--0000011011000000-00---00-10100--1100000-0-0--0110---

000000000000100001000000113211111

Nemertea 201101111111100110010010110100000000001000000000-

000--000001100-0000?1?00---0--0--------00100-0-0--011100-

00000000000010000100011100211-000

Onychophora -011011111111000----00101--

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011100-00000000000010000111100000-100000

Arthropoda -011011111111000----00101--

0000000000100100120111011000(0 1)111110-000000-10---00-10000--0-00000-0-

0--011100-00000000000010000111100000-100000

```
        Tardigrada      -011011111111000----00101--
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011100-0000000000000100001???????0---0000
        Nematoda        -011011111111000----00101--
00000000001001003201 0100--000-01000-011000-00---00-10000--0-00000-0-0--
0110---0000000000000100001???????0---0000
        Nematomorpha     -011011111111000----00101--
000000000010010031010100--000-01000-001000-00---00-10000--0-00000-0-0--
0110---0000000000000100001???????0---0000
        Priapulida      2011011111111000----00101--0000000000100100010111 10-
-000-01000-011100-00---00-10000--0-00000-0-0--0110---
00000000000010000110100000 0110000
        Kinorhyncha     1011011111111000----00101--
00000000001001000??10100--000-?1000-011100-00---00-10000--0-00000-0-0--
0110---0000000000000100001???????01100000
        Loricifera      0011011111111000----00101--00000000001001000??10110--
000-01100-011100-00---00-10000--0-00000-0-0--0110---
0000000000000100001???????00100000

;

END;
```

Supplementary Information III

Careful amendment of morphological datasets improves phylogenetic frameworks: re-evaluating placement of the fossil *Amiskiwia sagittiformis*

Nicolas Bekkouche[a] and Ludwik Gąsiorowski[b]

[a] Institut de Systématique, Evolution, Biodiversité, ISYEB–UMR 7205 MNHN CNRS UPMC EPHE, Sorbonne Universités, 45 rue Buffon, 75005 Paris, France

[B] Department of Tissue Dynamics and Regeneration, Max Planck Institute for Multidisciplinary Sciences, Am Fassberg 11, 37077 Göttingen, Germany

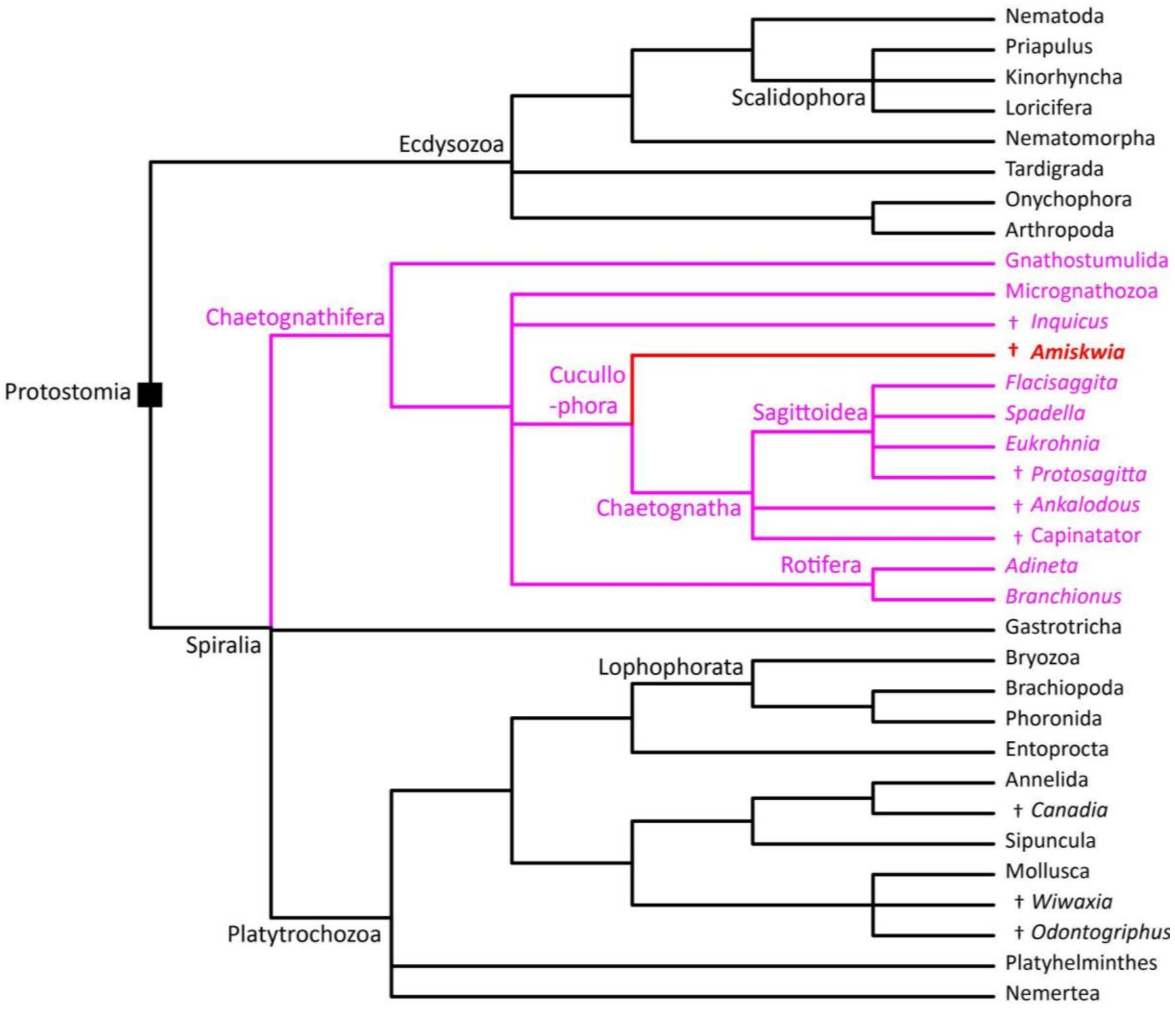

Figure S1. Strict consensus of the parsimony analysis of the original matrix from Vinther and Parry 2019 (O-VP).

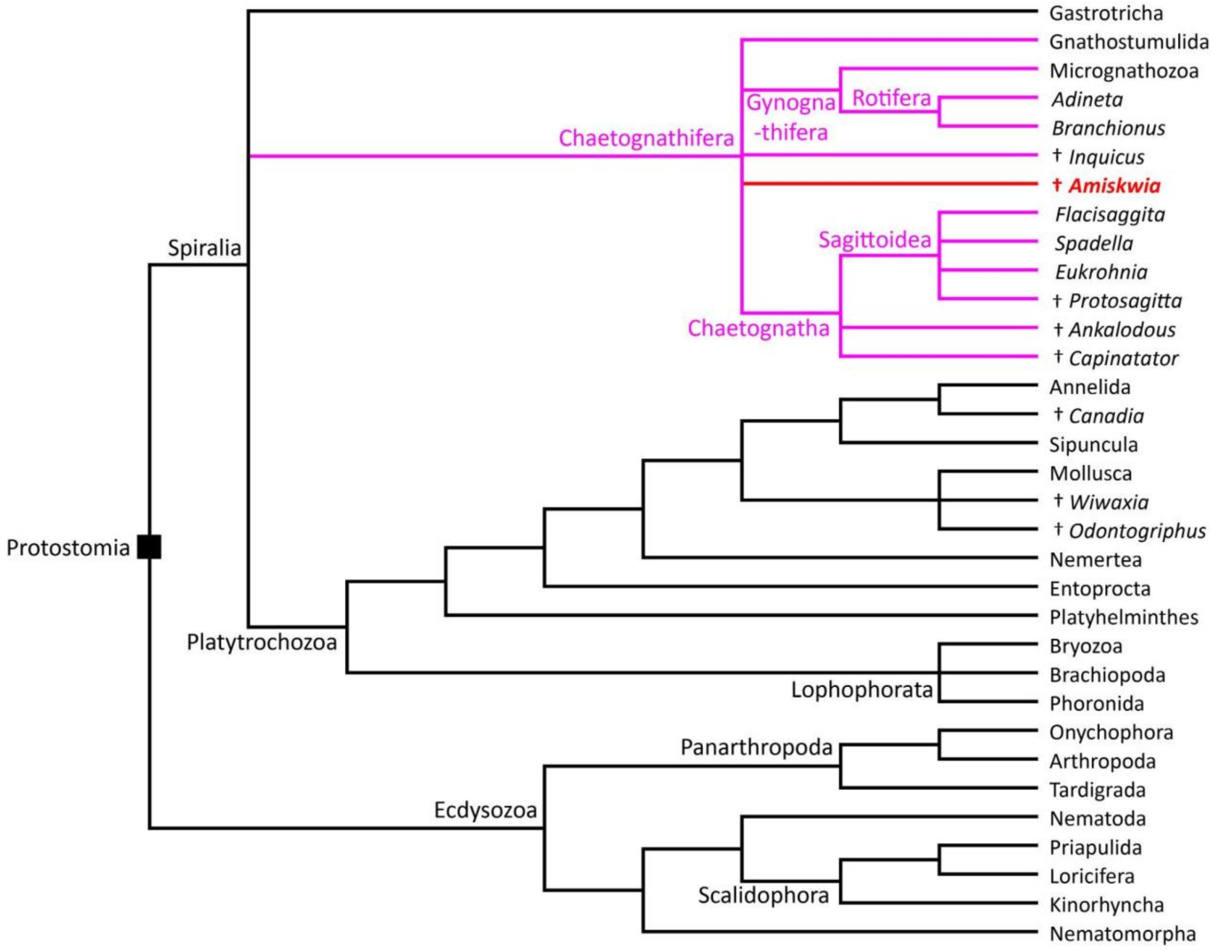

Figure S2. Strict consensus of the parsimony analysis of the modified matrix, following the Caron and Cheung interpretation of the jaws of *Amiswkia* including parsimony informative characters only (M-CC-PI) with the three characters on fins fused as one character.

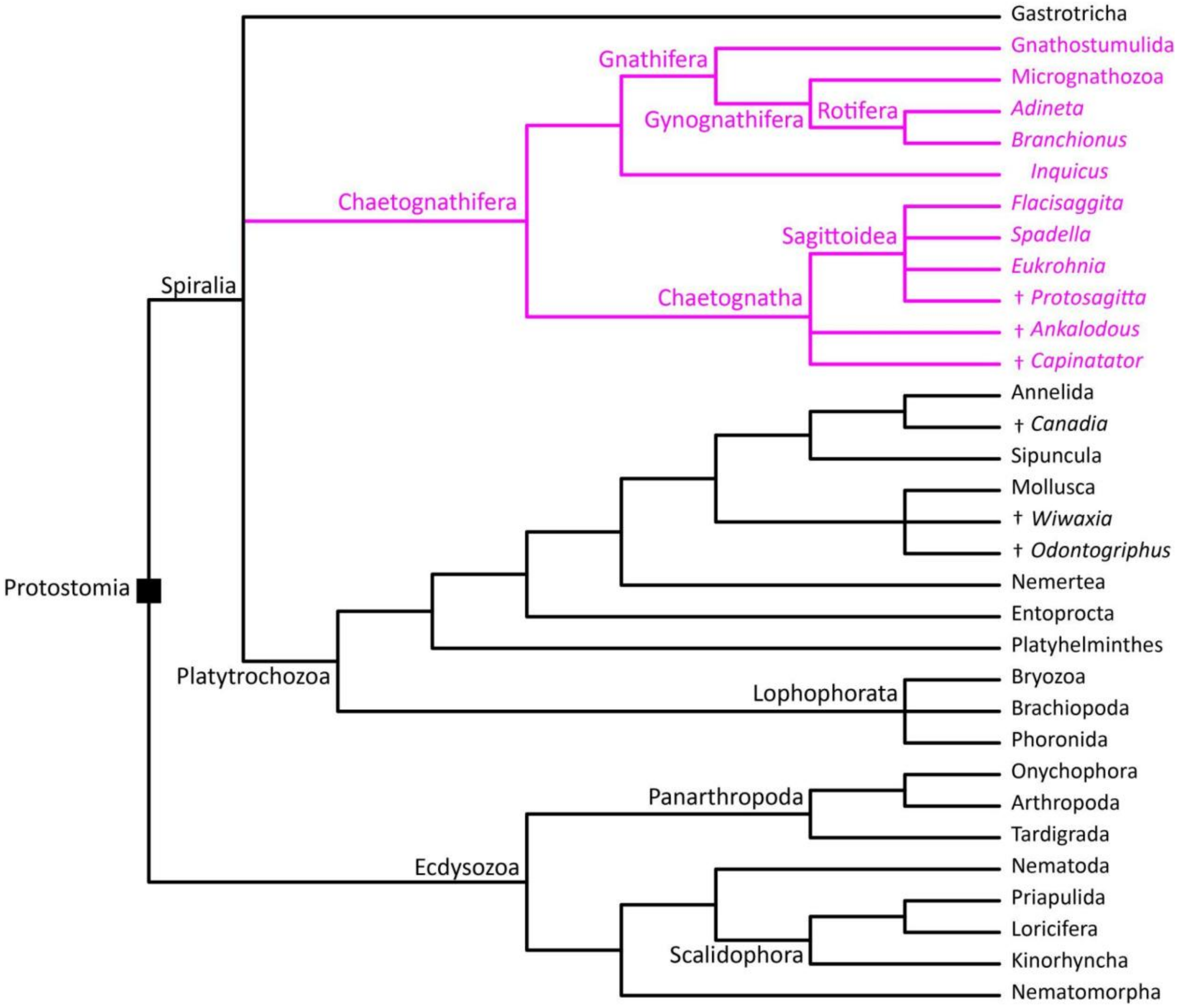

Figure S3. Strict consensus of the parsimony analysis of the modified matrix, following the Caron and Cheung interpretation of the jaws of *Amiswkia* including parsimony informative characters only (M-CC-PI) but with *Amiswkia* removed from the matrix.

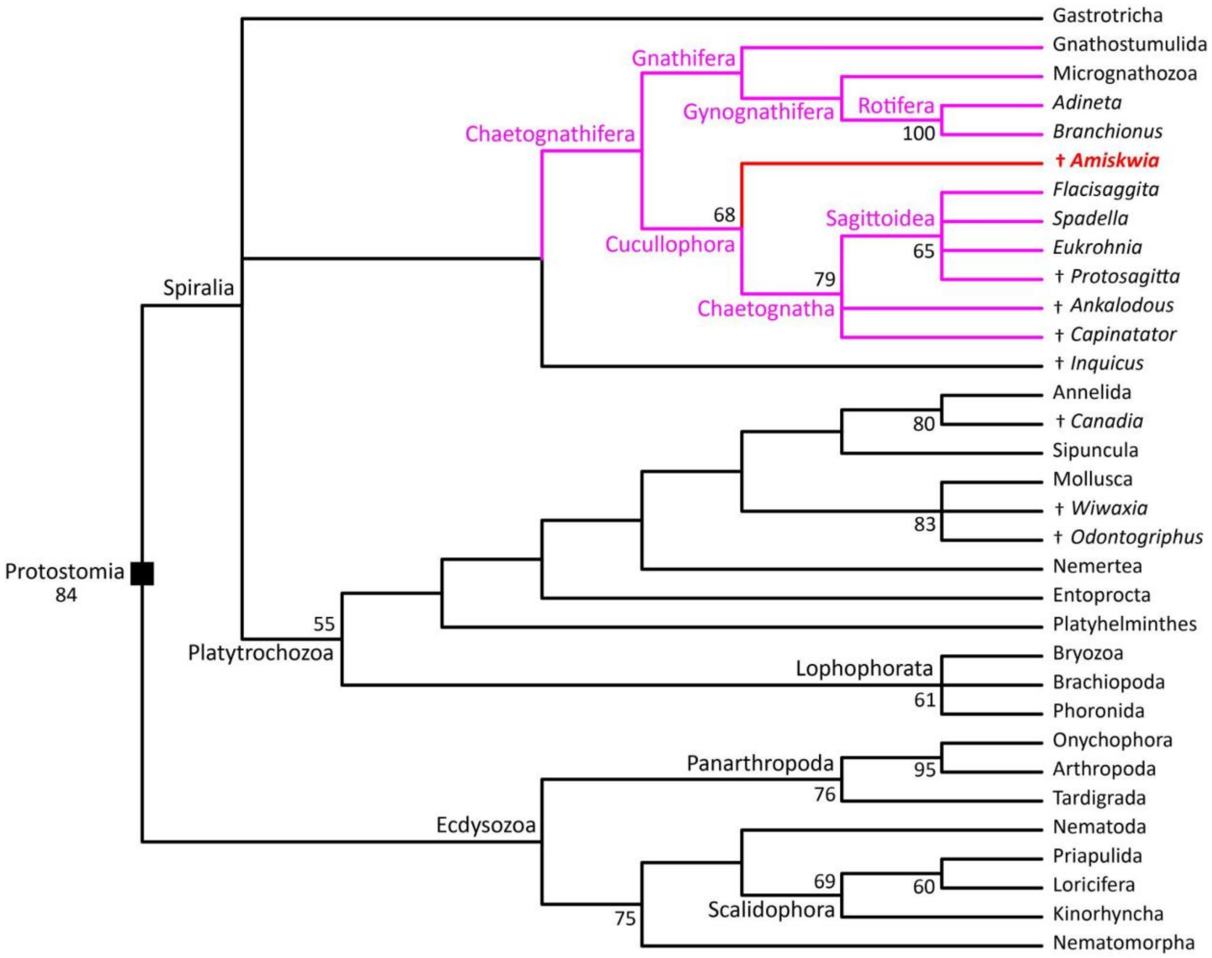

Figure S4. Strict consensus of the parsimony analysis of the modified matrix, following the Caron and Cheung interpretation of the jaws of *Amiswkia* including parsimony non-informative characters (M-CC-PN). Node values are bootstraps. Nodes without bootstraps have a value under 50%.

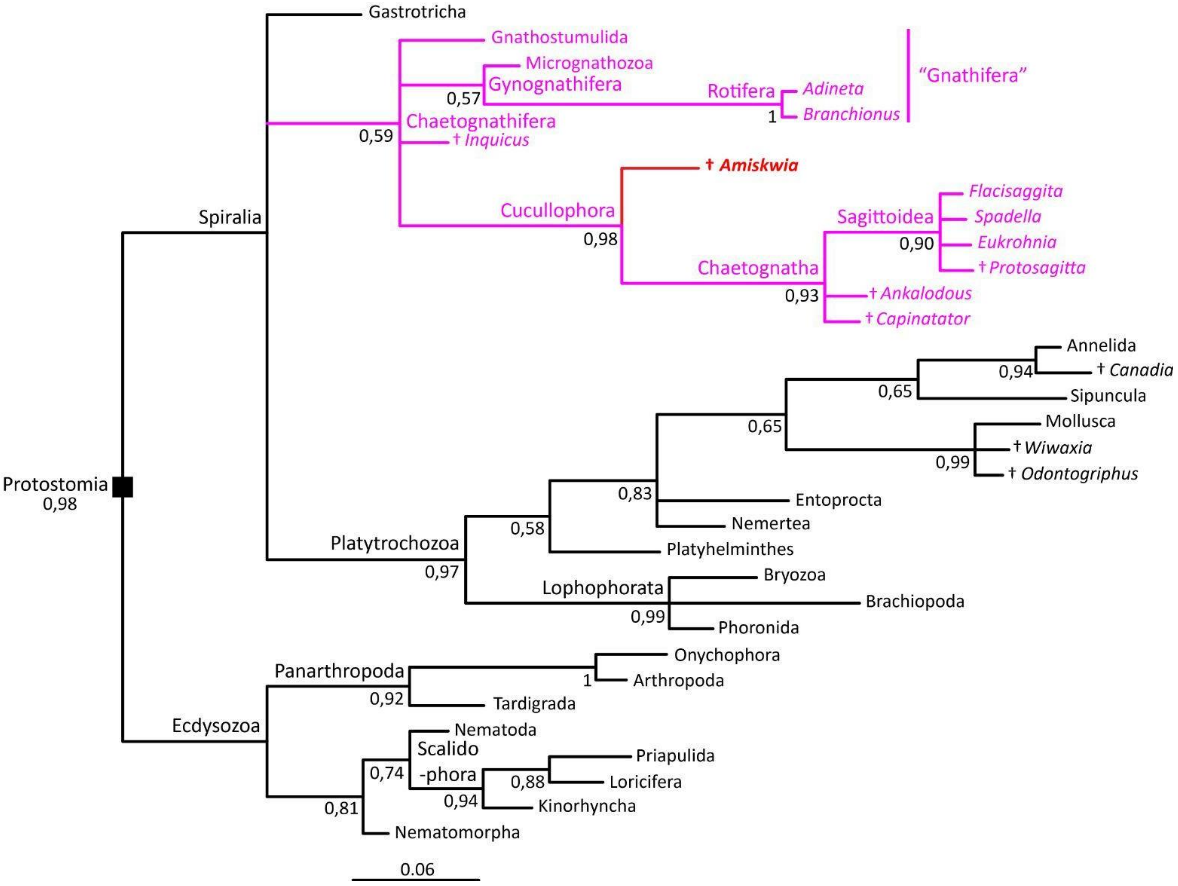

Figure S5. Results of the bayesian analysis of the modified matrix, following the Caron and Cheung interpretation of the jaws of *Amiswkia* including parsimony non-informative characters (M-CC-PN). The branch numbers are the posterior probabilities.

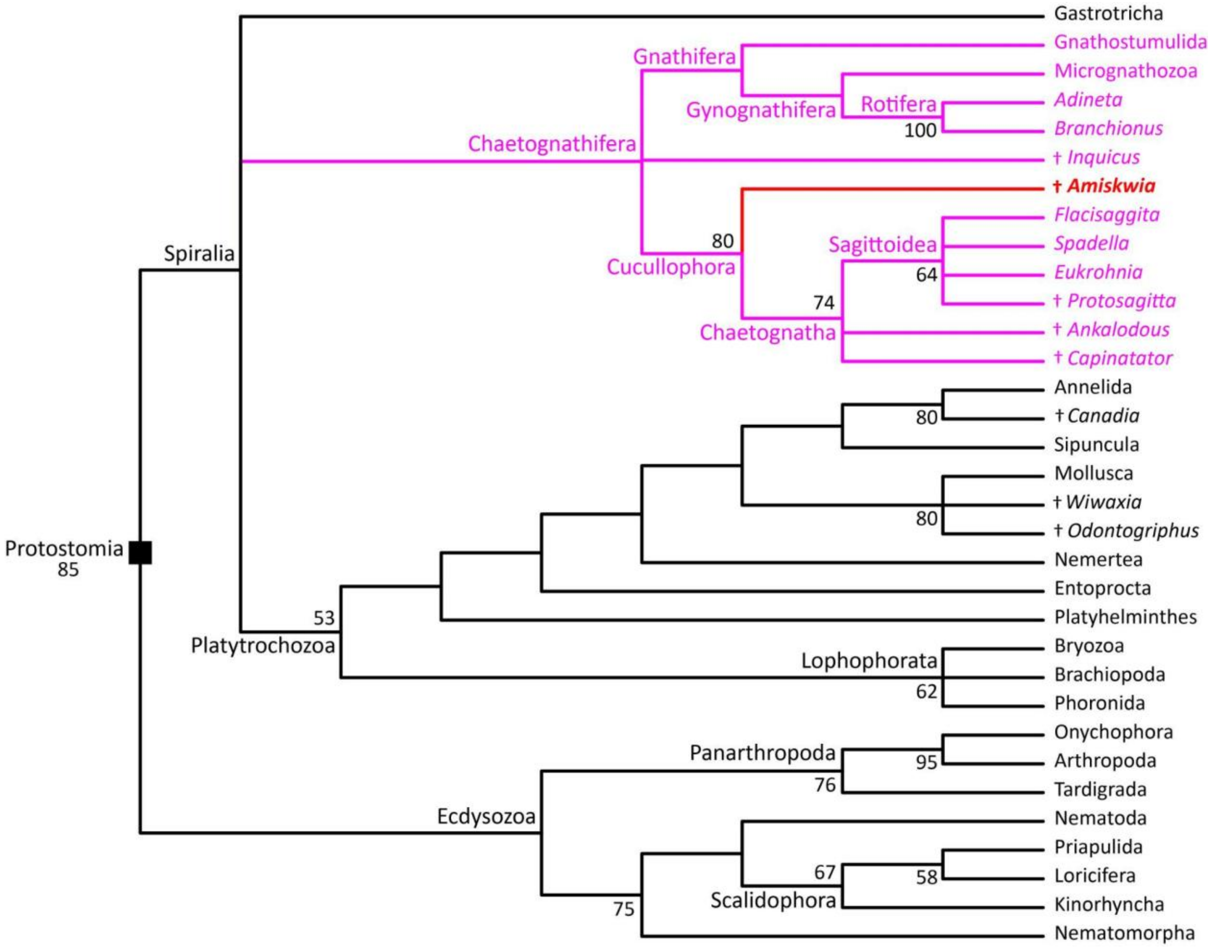

Figure S6. Strict consensus of the parsimony analysis of the modified matrix, following the Vinther and Parry interpretation of the jaws of *Amiswkia* including parsimony non-informative characters (M-VP-PN). Node values are bootstraps. Nodes without bootstraps have a value under 50%.

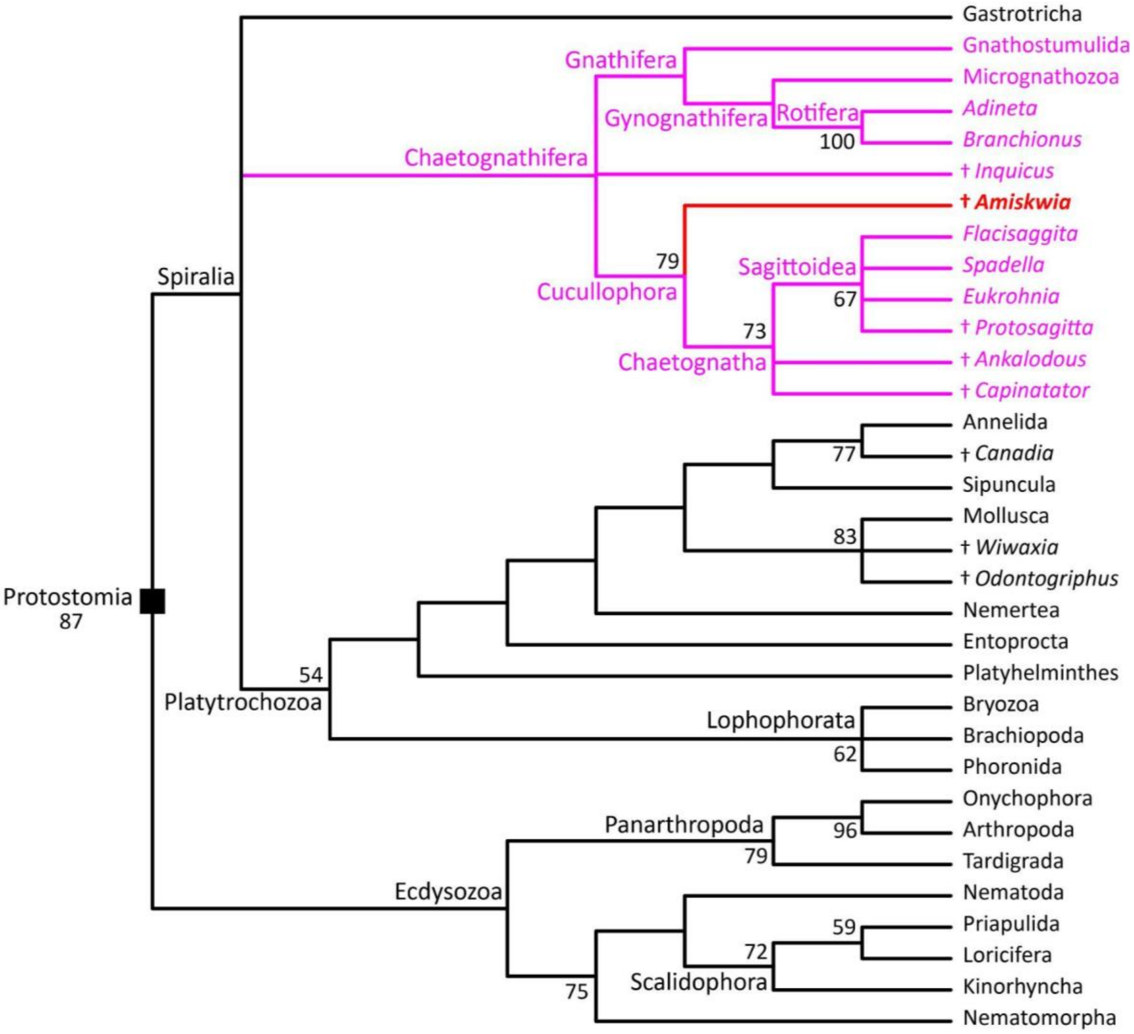

Figure S7. Strict consensus of the parsimony analysis of the modified matrix, following the Vinther and Parry interpretation of the jaws of *Amiswkia* including parsimony informative characters only (M-VP-PI). Node values are bootstraps. Nodes without bootstraps have a value under 50%.

Table S1. Retention index for individual characters of the strict consensus resulting from the result of the parsimony analysis of the modified matrix with Caron and Cheung interpretation of the jaws of *Amiswkia* including parsimony informative characters only (M-CC-PI) estimated with Mesquite. Numbers into brackets for each characters are the character numbers from the matrix of Vinther and Parry 2019.

Character	**RI**
1: cilia-microvilli arrangement in protonephridia	0,5
2: ciliated epidermis (15)	1
3: multiciliated epidermal cells (16)	0,4
4: ciliation restricted anteriorly (17)	0,42
5: corona ciliata (18)	1
6: ventral locomotory cilia (19)	0,5
7: ventral surface modified into foot (20)	0,666
8: Spiral or radial cleavage (25)	1
9: apical cross (26)	0
10: 4d mesentoblast (27)	1
11: Through-gut (37)	0
12: U-shaped gut (38)	0,5
13 : Dorsal anus or anal pore (39)	0,25

14: Mouth position (40)	0,875
15: body divided with distinct inflated head and neck region (42)	0,875
16: Nephridia (44)	0,45
17: Fate of blastopore (45)	0,5
18: annelid type cuticle (46)	1
19: Body cuticle with chitin (47)	0,875
20: Body cuticle with α-chitin (48)	0,666
21: Body cuticle molted (49)	1
22: lorica (50)	1
23: lobopods or segmented limbs (51)	1
24: Mixocoel (haemocoel) surrounded by segmented mesoderm (57)	1
25: Teloblastic segmentation (58)	0,666
26: Longitudinal ventral nerve cord(s) (59)	0
27: paired ventral nerve cords (60)	0,5
28: circumoral nerve ring (61)	0,7
29: Stomatogastric ganglion(s) directly connected to the brain	0,833
30: stomatogastric ganglion with ciliated receptors	1
31: Major ventral nerve plexus in trunk (63)	1
32: Circum-pharyngeal, collar-shaped brain with anterior rings of perikary	1

(64)	
33: Introvert with scalid rings (65)	1
34: Flosculi (66)	1
35: Trochophores (68)	1
36: apical organ (70)	1
37: Segmental metanephridia sacculus (72)	1
38: chaetae (73)	0,33
39: Parapodia with dorsal and ventral branches terminated by chaetae (77)	1
40: radula (78)	1
41: sclerotized elements in the anterior digestive tract (80)	0,777
42: basal plate	0
43: pharyngeal sclerotized elements composed of rod-shaped structures	1
44: grasping spines (82)	1
45: teeth (83)	1
46: internal jaws	0,666
47: jaws with symphysis	1
48: Ctenidia (89)	0,5
49: Mantle cavity (90)	0,666
50: tripartite body plan with septum (93)	1

51: trunk fins (94)	1
52: anterior lateral fins (95)	0
53: caudal fin (96)	1
54: Phragms (98)	0
55: head hood (106)	1
56: coelom (111)	0,727
57: serially repeated paired coelomic cavities (113)	0,5
58: Trimeric coelom (114)	1
59: Lophophore (131)	1
60: ftz (133)	1
61: Ubx/abd-A (134)	1
62: AbdB (135)	1
63: lox5 (136)	1
64: duplication of UbdA into lox4 and lox2 (137)	1
65: Post2 (138)	1
66: MedPost (140)	1
67: rotifer corona	1
68: terminal cells of protonephridia	0,5
69: cellularity of the nephridium collecting canal	0,5

70: shape of nephridial canal	0,33
71: Intracellular lamellar cuticule	1
72: Epidermis structure	1
73: Female organs with germovitellarium	1
74: Retrocerebral organ	1