The first leech body fossil predates estimated hirudinean origins by 200 million years (#88062)

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The first leech body fossil predates estimated hirudinean origins by 200 million years

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Clitellata is a major Annelid clade comprising oligochaetes (e.g., earthworms) and hirudineans (e.g., leeches). Due to their scant fossil record, the origins of clitellates, particularly Hirudinea, are poorly known. Here, we describe the first unambiguous leech body fossil, *Macromyzon siluricus*, gen. et sp. nov., from the Brandon Bridge Formation (Waukesha Lagerstätte). This fossil, which is preserved in exceptional detail, possesses several hirudinean soft-tissue synapomorphies–including a large sucker at the posterior end and sub-divided segments–and phylogenetic analyses resolve *Macromyzon siluricus* as a stem leech. Its age, 437.5-436.5 Ma, predates the estimated origins of Hirudinida by at least 200 million years. These findings indicate that the earliest true leeches were marine and that, contrary to prevailing hypotheses, were unlikely to have fed on vertebrate blood.

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Abstract

Clitellata is a major Annelid clade comprising oligochaetes (e.g., earthworms) and hirudineans (e.g., leeches). Due to their scant fossil record, the origins of clitellates, particularly Hirudinea, are poorly known. Here, we describe the first unambiguous leech body fossil, *Macromyzon siluricus*, gen. et sp. nov., from the Brandon Bridge Formation (Waukesha Lagerstätte). This fossil, which is preserved in exceptional detail, possesses several hirudinean soft-tissue synapomorphies–including a large sucker at the posterior end and sub-divided segments–and phylogenetic analyses resolve *Macromyzon siluricus* as a stem leech. Its age, 437.5-436.5 Ma, predates the estimated origins of Hirudinida by at least 200 million years. These findings indicate that the earliest true leeches were marine and that, contrary to prevailing hypotheses, were unlikely to have fed on vertebrate blood.

Introduction

Annelida, a speciose and morphologically disparate phylum within the animal group Lophotrochozoa, possesses a fossil record stretching back to the Cambrian. This consists primarily of polychaetes, non-biomineralized examples of which are recorded from deposits of exceptional fossil preservation such as the Burgess Shale in British Columbia, Canada (Conway Morris, 1979; Nanglu & Caron, 2018); Sirius Passet (Buen Formation) in North Greenland (Conway Morris & Peel, 2008); and both the Chengjiang (Maotianshan Shale) and Guanshan Formation biotas of South China (Liu et al., 2015; Han et al., 2019). The fossil record of scolecodonts (polychaete jaw elements) indicates that the first major polychaete radiation occurred during the Ordovician (Hints & Eriksson, 2007).

These fossils, in conjunction with novel modes of analysis and more robust molecular datasets (Parry et al., 2016; Weigert & Bleidorn, 2016; Nanglu & Caron, 2018), are helping to clarify some of the broad phylogenetic interrelationships among annelids; however, the origins of the major annelid clade Clitellata remain uncertain. In part, this is due to the uncertain phylogenetic placement of Clitellata (Weigert & Bleidorn, 2016), but, a more significant impediment is the exceedingly poor published fossil record of clitellates. This paucity is unsurprising given that clitellate anatomy consists almost entirely of non-biomineralized tissue. Taphonomic studies have shown that polychaetes decay rapidly post-mortem: only their sclerotized jaws possess high decay resistance (Briggs & Kear, 1993). Although branchiobdellidans and some leeches possess hardened jaws, these structures are absent in the vast majority of clitellates, which reduces the likelihood that they will fossilize under most aqueous depositional circumstances.

Clitellata comprises roughly one-third of all extant annelid species, and they can be found in nearly every conceivable habitat on Earth (Sawyer, 1986; Martin et al., 2008; Sket & Trontelj, 2008). The group is characterized by the possession of a clitellum, a glandular region in the anterior part of the body which secretes a cocoon into which eggs are deposited (Sawyer, 1986). They are readily distinguished from other annelids by their lack of parapodia, the reduction or lack of chaetae, and the presence of certain autapomorphic internal reproductive characteristics

(Brinkhurst, 1982; Rouse & Fauchald, 1995; Westheide, 1997). Clitellates are generally split into two groups: the paraphyletic Oligochaeta (earthworms and their relatives), and Hirudinea (Branchiobdellida, the crayfish worms; Acanthobdellida, the hook-faced fish worms; and Hirudinida, the true leeches) (Tessler et al., 2018; Erséus et al., 2020).

Today, clitellates are of great ecological, economic, and evolutionary importance. Non-hirudinean clitellates are among the most important ecosystem engineers in aquatic and terrestrial sedimentary environments (Lavelle et al., 1997), and hirudinean taxa fill a huge diversity of ecological niches as parasites, commensal symbionts, predators, and disease vectors (Sawyer, 1986; Martin et al., 2008). With the acquisition of a caudal sucker, and reduction of the clitellum, chaetae, and internal segmentation, hirudineans represent a unique annelid body plan (Purschke et al., 1993). Furthermore, acanthobdellidans and leeches represent a novel radiation of parasitic annelids, and as a result, the latter have been used as medical tools for thousands of years, continuing to the present day. Based primarily on morphological evidence and ancestral state estimation, most authors have converged on the hypothesis that the earliest clitellates were freshwater organisms, but the precise timing of the transition to a terrestrial habitat is not known (Manum, Bose & Sawyer, 1991; Rousset et al., 2008; Erséus et al., 2020).

Shcherbakov et al. (2020) provide a comprehensive summary of the clitellate fossil record, which largely consists of poorly preserved oligochaetes; these authors do not recognize any leech body fossils. A specimen putatively labelled (but not formally described) as “?Leech” by Mikulic et al. (Mikulic, Briggs & Kluessendorf, 1985a) and cited by Briggs (Briggs, 1991) lacks the necessary characteristics that are definitively diagnostic of leeches, most notably having segments which are entirely uniform and too numerous for this group (de Carle, 2022). An argument may be made for a general clitellate affinity, however, the lack of any visible clitellum, in addition to the overall incompleteness of the fossil, precludes confident placement (de Carle, 2022). Shcherbakov et al. suggested a possible cycloneuralian affinity for this fossil; while a formal description is beyond the scope of this paper, we agree that any relation with Annelida is unsubstantiated at present. Exceptionally preserved leech cocoons have been documented in deposits dating to the Triassic (Manum, Bose & Sawyer, 1991; Bomfleur et al., 2012; Steinthorsdottir, Tosolini & McElwain, 2015), but no body fossils of leeches were found in association with them. Because cocoon morphology has low taxonomic specificity and cocoon-building behaviour predates the origins of Clitellata (Manum, Bose & Sawyer, 1991; Bomfleur et al., 2012), these trace fossils provide little insight into early clitellate evolution, ecology, and anatomy.

The scant fossil record of Clitellata leaves a number of questions unresolved; a better understanding of early clitellate history would elucidate the timing of important evolutionary events including terrestrialization, development of the hirudinean body plan, and the origins of parasitism.

Here, we report the first unambiguous fossil leech from the lower Brandon Bridge Formation (Silurian: Llandovery, Telychian) of Wisconsin, USA. The Brandon Bridge biota is diverse, including the remains of biomineralising, non-biomineralising and lightly biomineralising organisms (Wendruff et al., 2020a,b). This array of anatomies with varying taphonomic potentials includes even the most labile tissues found in non-sclerotised animals, such as leeches (Briggs, 1991; Briggs & Kear, 1993; Saleh et al., 2020). The exceptionally preserved form described herein shows clear evidence of synapomorphic hirudinean characters, including a large caudal sucker and segments sub-divided into annuli. This new species allows for a re-evaluation of the timing of clitellate origins, as well as new consideration of ancestral hirudinean ecology.

Materials & Methods

Specimen preparation and imaging

Our fossil specimen was exposed using a freeze-thaw technique to split the dolostone along the bedding-plane-parallel fossil. The tissue has been preserved as a thin organic film, and lacks any secondary phosphate or other diagenetic overprinting; compare with other fossils in Wendruff et al. (2020a,b). It is deposited in the collections of the University of Wisconsin-Madison Geology Museum, accession number UWGM 4001_14.

The specimen was photographed with a Canon EOS Rebel T3i Digital SLR using a Canon MP-E 65 mm macro lens and full spectrum lighting. Images were stitched using Adobe Photoshop CC 2015. Measurements were made using ImageJ ver. 1.53, and figures were assembled using Adobe Illustrator ver. 19.2.0.

Nomenclatural Acts

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: ***[XXX]***. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central SCIE and CLOCKSS.

Phylogenetic analyses

Our phylogenetic matrix (supplemental file 1) was adapted from Ou et al. (2017), which was designed to accommodate a wide sampling of metazoan taxa. A broad selection of body plans was deemed necessary to accommodate for the potential variety of affinities of this new taxon: see the last paragraph of the Results sub-section, “Description”. Non-bilaterian taxa were removed, as *Macromyzon siluricus* is clearly bilaterian in nature. Four further groups – Xenocoelomorpha, Gastrotricha, Gnathiferea, and Chaetognatha – were removed for two reasons. First, like the coelenterates, there is no apparent connection between these taxa and *M. siluricus*, making their inclusion unnecessary. Second, as their systematic positions remain heavily debated based on both morphological and molecular data (Simakov et al., 2013; Cannon et al., 2016; Caron & Cheung, 2019; Philippe et al., 2019; Kapli & Telford, 2020), their inclusion could skew our analyses without providing meaningful data for resolving the phylogenetic affinities of *Macromyzon*.

A number of taxa were added to the character matrix to test our hypotheses of *Macromyzon*'s affinities. The polychaete *Capitella* was added, as the family Capitellidae has been hypothesised to be among the closest relatives of Clitellata (Weigert & Bleidorn, 2016). *Eunice* and *Harmothoe* were added to accommodate for the possibility that *Macromyzon siluricus* may have a phylogenetic affinity with a polychaete body plan. The fossil forms *Esconites* and *Dryptoscolex* were added as their relationships to the polychaete families Eunicidae and Polynoidea are relatively well established; an analysis which recovers the *a priori* known relationships among these five polychaete groups to the exclusion of the clitellates increases our confidence that *Macromyzon* has not been erroneously placed. Based on external similarities, five clitellate taxa were added: the sub-class Oligochaeta, and the orders Acanthobdellida, Branchiobdellida and Hirudinida (the true leeches, represented by and *Hirudo*, and *Glossiphonia*). Together, these four taxa comprise the subclass Hirudinea. We have added the fossil palaeoscolecid *Cricocosmia*, to corroborate our hypothesis that *Macromyzon siluricus* lacks any palaeoscolecid affinity.

Addition of new taxa required that we add new characters to the phylogenetic matrix. In total, we added 19 new characters (char. 56-74; supplemental file 1): the majority of which are designed to infer annelid relationships (*e.g*., segmentation patterns, and morphology of jaws, clitellum, and reproductive anatomy). Notes on these characters and their coding are included in the supplementary information. Finally, invariant characters were deleted from the character matrix to avoid overestimation of branch lengths that results from acquisition bias (Lewis, 2001).

Phylogenetic analyses were performed under Bayesian inference in MrBayes ver. 3.2.7a (Ronquist & Huelsenbeck, 2003) and maximum parsimony in TNT ver. 1. 5 (Goloboff, Farris & Nixon, 2008). Bayesian analysis used the mkv+gamma model, and a symmetrical Dirichlet hyperprior for all characters. Tracer ver. 1.7.1 (Rambaut et al., 2018) was used to determine whether the simulations had converged (*i.e.,* effective sample sizes for all parameters > 200; sampling reached stationarity; average standard deviation of split frequencies < 0.01). Parsimony heuristic searches ran for 10,000 iterations each with 5 rounds of ratcheting. Support values were calculated from 1,000 rounds of bootstrapping.

The resulting Bayesian (supplemental file 2) and parsimony (supplemental file 3) tree files, as well as the complete phylogenetic matrix and commands used to run MrBayes (supplemental file 1), are included as supplementary information.

Results

Systematic palaeontology

Phylum: Annelida, Lamarck 1809

Class: Clitellata, Michaelson 1919

Subclass: Hirudinea, Lamarck 1818
Genus: *Macromyzon* gen. nov.
Type Species: *Macromyzon siluricus*, sp. nov.
urn:lsid:zoobank.org:act:***[XXX]***

Etymology

Macromyzon (gender: masculine), from Greek, *makros,* "large" + *myzon*, "sucker" in reference to the large caudal appendage. The specific epithet refers to the Silurian age of the fossil.

Material examined

Holotype: University of Wisconsin Geology Museum (UWGM) 4001_14.
Locality: Waukesha Lime and Stone Company, west quarry, north of State Highway 164, Waukesha, Wisconsin, USA.
Stratigraphic occurrence: Lower part of the Brandon Bridge Formation (Silurian: Llandovery, Telychian). Details of the palaeoenvironmental context of the Brandon Bridge Formation and the Waukesha fauna are included in the supplement.
Stratigraphic context and taphonomy: The fossil material examined here was collected from the Waukesha Lime and Stone Company west quarry in Waukesha, Wisconsin, USA (Wendruff et al., 2020a). It is from the lower part of the Brandon Bridge Formation (Silurian: Llandovery, Telychian), better known as the Waukesha Lagerstätte. Taxa from this locality were first reported by Mikulic et al. (1985a,b). Subsequently, various taxa have been discussed in a number of papers, and summarised by Wendruff et al. (2020a). The taxonomic composition of the biota and some taphonomic pathways leading to exceptional fossil preservation were reviewed by Wendruff et al. (2020a), who also reviewed available biostratigraphic information bearing on the age of the Waukesha Biota. Conodonts provide the most precise biostratigraphic guides known from the lower Brandon Bridge Formation near Waukesha, Wisconsin. According to Kleffner et al. (2018), the euconodont *Pterospathodus eopennatus*, the eponymous indicator of the *P. eopennatus* Superzone, is present. This guide fossil constrains the deposit to the Telychian Stage (Silurian System, Llandovery Series, approximately 437.5-436.5 Ma).

Palaeoenvironmentally, the Brandon Bridge deposit represents a tropical, carbonate marine platform setting (Wendruff et al., 2020a). Remains of organisms were transported into intertidal to supratidal sedimentary traps associated with karstic topography adjacent to the shoreline. Some remains were quickly covered by cyanobacterial mats. Early diagenetic activity within the mats helped preserve some bodily remains. Replication by thin calcium phosphate (apatite) encrustation is common, but some non-biomineralised parts are preserved as carbon films.

Diagnosis

Hirudinean having a vermiform-sublanceolate shape, truncated anteriorly, width expanding toward posterior, reaching maximum width slightly forward of margin. Posterior margin approximately twice the width of anterior margin. Body segmented, with regularly spaced, sexannulate divisions. Posterior terminates in a large caudal sucker. The genus is monospecific; the diagnosis applies to both genus and species.

Description

The holotype, and only known specimen, of *Macromyzon siluricus* (Fig. 1A-C) is 51 mm in length. As preserved, the specimen is twisted near midlength; the narrower anterior portion exposes the dorsal side, and the wider posterior portion exposes the ventral side.

Outline is vermiform-sublanceolate, truncated at anterior end; width expands toward posterior, reaching a maximum width of 18.9 mm at the posterior end somewhat forward of the posterior terminus, then narrowing slightly toward anterior margin. Integument divided into numerous, narrow, sexannulate divisions, subequal in length (sag.); divisions covered with semi-circular protrusions (Fig. 1C-E). A large ellipsoid structure, which we interpret as a caudal sucker, encompasses approximately the entire width of the posterior end (Fig. 1A-B, 2A). It is preserved more darkly in comparison with the surrounding material, and thin, concentric striations around the caudal sucker give it a wrinkled appearance.

Remarks

Clear external evidence of segmentation, coupled with the lack of a chitinous cuticle as is seen the diverse arthropod fauna of Waukesha (Wendruff et al., 2020a), identifies *Macromyzon siluricus* gen. et sp. nov. as an annelid. While segmentation is also a feature of arthropods, *M. siluricus* is divided into thinner, non-sclerotized segments compared with any co-occurring arthropod taxa. Further, these segments do not bear limbs, nor do they appear to have any features indicative of limbs that may have been preferentially lost due to taphonomic factors. The combination of a prominent caudal sucker, lack of external appendages, and the subdivision of segments into annuli strongly supports an affinity with the Clitellata, specifically the hirudineans (Sawyer, 1986; Purschke et al., 1993; Westheide, 1997). Some polychaete lineages have lost parapodia and chaetae independently of the clitellates, and sub-divided segments are present in many members of Scalibregmatidae and Opheliidae, but no non-hirudinean annelids possess suckers (Rouse, Pleijel & Tilic, 2022).

The large caudal sucker of *M. siluricus*, which occupies approximately 3/4 of the posterior body width (Fig. 1A-B, 2A), is similar to the well-developed caudal suckers that characterize members of Hirudinea (Fig. 1F-G, 2) (Sawyer, 1986). Depending on their location on the body, caudal suckers can be considered either terminally or ventrally positioned. Terminally positioned caudal suckers are directed anteriorly or posteriorly on the end of the posterior-most somite, and ventrally positioned suckers are attached to the ventral surface of the body. The caudal sucker of *M. siluricus* is in a terminal position, and the width of the posterior region at the point of attachment of the caudal sucker to the body is uniform. Many modern leech lineages possess such characteristics – *e.g.,* Praobdellidae, Haemadipsidae, and some members of Piscicolidae (Sawyer, 1986; Utevsky, Solod & Utevsky, 2021) – as do branchiobdellidans and acanthobdellidans (Fig. 2)(James et al., 2015; Parpet & Gelder, 2020; de Carle et al., 2022). Terminal caudal suckers and a sublanceolate body shape are common features of leeches belonging to the family Glossiphoniidae (Fig. 2G) (Sawyer, 1986).

In Hirudinea, segments are sub-divided into rings, or annuli, which do not correspond to internal segmentation. The holotype of *M. siluricus* shows external signs of segmentation as well as annuli. Each annulus that is externally visible (Fig. 1C-E) seems to be secondarily sub-divided on both the dorsal side (expressed anteriorly) and ventral side (expressed posteriorly); this is particularly evident between annuli 13 and 26 (Fig. 1C,D). The subdivision of the annuli in *M. siluricus* suggests that the segments are primarily triannulate, as in most extant leeches (Fig. 1D,E,G) (Sawyer, 1986), with each primary annulus being sub-divided, resulting in a total of six annuli per segment. This pattern is further supported by the presence of metameric circular organs on the anterior end, on every sixth annulus. Eleven of these metameric circular organs can be distinguished by average size, position, and metameric distribution. They are distributed on both margins of the anterior (dorsal) region at annuli 3, 13 and 14, 19 and 20, 27 and 28, 33 and 34, 37 and 38, 46 and 47, and 51 (Fig. 1C-E). Six of them are paired. External markers of the segmentation pattern are common in leeches (Fig. 1G). The most common ornaments found on leech integument are tubercles and sensillae. Both occur in metameric patterns that repeat on each midbody segment.

In leeches, the number of annuli is highly variable among species, and sexannulate segments, like those observed in *Macromyzon*, are common (Sawyer, 1986). In total, the new species has 109 visible annuli amounting to 15 visible segments. As is in most Hirudinea, a clitellum is not visible. Presuming that *Macromyzon* has a fixed number of segments, and that the number is similar to other hirudineans (n = 15–33) (Purschke et al., 1993), the number of visible segments (n = 15) in our fossil is sufficient to claim that an externally visible clitellum is absent in *M. siluricus*, as it would be preserved if it were present.

In addition to the diagnostic hirudinean characteristics noted above (*i.e.*, caudal sucker, sub-divided annuli, and absence of and lateral appendages), *M. siluricus* notably lacks clear, defining features of any other plausible vermiform clade. The apparent absence of chaetae indicates that *Macromyzon* is not a polychaete, as polychaetes with chaetae preserved are present in the Waukesha Biota (Wendruff et al., 2020a). Because they are reduced in both size and number, chaetae of oligochaetes might be less likely to fossilize; however, the large and well-preserved caudal sucker of *M. siluricus* argues against an oligochaete affinity.

Although the surface texture of *M. siluricus* bears some superficial similarity to palaeoscolecids, morphological and taphonomic evidence conclusively dismiss any affinity between these taxa. Palaeoscolecids have elongate bodies which characteristically curl ventrally inwards post-mortem (Topper et al., 2010; García-Bellido, Paterson & Edgecombe, 2013) (Fig. 3A-C), unlike the sub-lanceolate and straight pose of *Macromyzon* (Fig. 1A,B). Palaeoscolecids also have thickened cuticles with an elaborated series of hardened sclerites comprising a comprehensive scleritome; imagery of the palaeoscolecid *Hadimopanella* shows these sclerites in greater detail (Fig. 3D). These features are clearly visible in other, currently undescribed, palaeoscolecids from Waukesha (Wendruff et al., 2020a) (Fig. 3A,B), but completely absent in *Macromyzon*.

The possibility that it may represent a palaeoscolecid moult is similarly unconvincing: *M. siluricus* lacks features that would presumably be evident in such remains (e.g., sclerites, scalids, posterior hooks), and no palaeoscolecid moults have ever been confidently identified (García-Bellido, Paterson & Edgecombe, 2013). When they are tentatively identified, they typically take the form of disarticulated cuticular fragments, which is logical for discarded exuviae (Zhang & Pratt, 1996; García-Bellido, Paterson & Edgecombe, 2013; Daley & Drage, 2016), but incongruous with the entirely articulated and well preserved specimen illustrated here (Fig. 1). Here, it may also behoove us to consider the manner in which extant priapulids–the best extant models for palaeoscolecids–undergo ecdysis. *Priapulus* moults through the formation of a longitudinal split that begins at the proboscis and gradually lengthens (Wang et al., 2019). Shed cuticle near the caudal appendages also wrinkles and folds as the animal emerges from its exuvia; the morphology of such exuviae would not be consistent with *Macromyzon*. Finally, our phylogenetic analyses, the results of which are presented below, included several vermiform ecdysozoans (including both extant priapulids and the palaeoscolecid *Cricocosmia*), and argue strongly against any ecdysozoan relationship.

We have also considered the possibility that *M. siluricus* represents a fragment of another fossil but have found that argument unconvincing after careful consideration of morphology. The overall outline of the fossil is sharp and well defined without any appearance of breakages or ragged edges that might be interpreted as tearing points. It is difficult to conceive of a taphonomic setting that would result in the sub-division of a soft-bodied, vermiform animal into perfectly smooth constituent parts without any evidence of breakage, and we do not know of any such examples in similar soft-tissue lagerstatten. There is also the fundamental issue that no currently described taxon from Waukesha–or the Silurian for that matter–has an anatomy of which *M. siluricus* could logically represent a fragment. In addition, the soft-tissues of polychaetes from Waukesha have been identified, although not formally described (Wendruff et al., 2020a; Fig. 7F), indicating that their cuticular body plan falls within the taphonomic window of the Waukesha locality. Indeed, even more taphonomically labile body plans such as non-cuticularized early chordates have been recognized from this biota (Wendruff et al., 2020a; Fig. 8F, G).

Phylogenetic analyses recover *Macromyzon* as a stem leech

Phylogenetic analysis resolves *Macromyzon siluricus* as sister to extant leeches (posterior probability = 0.60) within a strongly-supported clade consisting of *Macromyzon* and the three extant orders of Hirudinea (posterior probability = 1; Fig. 4A, supplemental file 1). Although the parsimony tree is less resolved, the Hirudinea + *M. siluricus* clade is still present and well-supported (bootstrap = 80%; supplemental file 3). Taken together, our analyses indicate that *Macromyzon* is, at minimum, a stem hirudinean, but most likely a stem leech.

Discussion

Morphology and phylogenetic analyses suggest that *Macromyzon siluricus* is a stem leech (Fig. 1,2,4C). The caudal sucker is well-developed (Fig.1A-B,2), contrasting the modern acanthobdellidan condition in which the sucker is not clearly demarcated from the body, and has been considered rudimentary (Fig. 2E) (Utevsky, Sokolov & Shedko, 2013; Bielecki et al., 2014; de Carle et al., 2022). Although the lanceolate body shape of *M. siluricus* is similar to modern leeches in the family Glossiphoniidae (Fig. 2G), we are unable to address the finer-scale phylogenetic affinities of this new taxon due to the lack of internal characters and the absence of the anteriormost region of the body. Nevertheless, the discovery of *Macromyzon* has implications for aspects of clitellate evolution, including timing, habitat transitions, and the evolution of parasitism.

Evolutionary Origins of Leeches

Macromyzon siluricus represents a critical data point for constraining the timing of clitellate and hirudinean diversification. Three fossil-calibrated molecular clock estimates have placed the origins of Clitellata between the Silurian (Llandovery) and the Late Devonian, 444 - 359 Ma (Edgecombe et al., 2011; Erwin et al., 2011; Erséus et al., 2020). These analyses also estimate that the split between Hirudinea and oligochaetes occurred 300 - 200 Ma, and that true leeches appeared 230 - 140 Ma, but most likely in the Jurassic or Cretaceous (Fig. 4B). The identification of *Macromyzon* as a stem leech pushes the minimum date for the appearance of true leeches back by more than 200 million years. The presence of *M. siluricus* in the Waukesha Biota (Silurian: Llandovery, Telychian), constrained to 437.5-436.5 Ma, is consistent with earlier molecular clock estimates for divergence of clitellates. Given the relatively derived position of this taxon within that class, the origins of Hirudinea and Clitellata as a whole may precede the Llandovery.

It must be noted that the discrepancies between our findings and these previous studies are to be expected. First, and most significantly, because the accuracy and precision of node age estimates improve as the number of fossil calibrations increases (Warnock, Yang & Donoghue, 2017), we should not expect accurate or precise age estimates for lineages – such as clitellates – with sparse geologic records. Second, none of the three studies was principally interested in the origins of Hirudinea. One focusses on Clitellata as a whole (Erséus et al., 2020), while the others focus on Metazoa broadly, and the nearest calibration points were phylogenetically distant from Clitellata: either at the base of Annelida, or within Mollusca (Edgecombe et al., 2011; Erwin et al., 2011).

At least three scenarios may explain the paucity of described leech fossils. These hypotheses are not necessarily mutually exclusive. 1) Leeches may have originated early in the Palaeozoic, but remained at low diversity and abundance for most of their evolutionary history, reducing their contribution to the fossil record. 2) Localities in which leeches thrived or diversified were not conducive to exceptional fossil preservation: this hypothesis is consistent with the proposed diversification of some leech lineages – and clitellates in general – in freshwater ecosystems. 3) For a variety of reasons, crucial diagnostic characters are not visible or present in collected specimens, precluding their identification as hirudineans.

Habitat

The most recent clitellate ancestor was an aquatic organism. The common consensus is that the earliest clitellates inhabited freshwater environments, with some lineages secondarily becoming marine (Rousset et al., 2008; Erséus et al., 2020). Clitellate cocoons have been interpreted as a necessary pre-requisite for freshwater and terrestrial life, as they protect eggs in hostile, non-isotonic environments (Manum, Bose & Sawyer, 1991; Westheide, 1997). Additionally, most modern aquatic leeches live in freshwater, with the exception of the principally marine suborder Oceanobdelliformes. The marine palaeoenvironmental setting in which *Macromyzon* was discovered gives cause for re-examining these long held assumptions.

Given the phylogenetic placement of *Macromyzon*, there are three possible ways to explain the distribution of marine taxa within Hirudinea. First, *Macromyzon* and the hirudinidan clade Oceanobdelliformes–which is sister to all other leeches (Trontelj, Sket & Steinbrück, 1999; Tessler et al., 2018)–may each represent independent transitions from freshwater to marine environments. Second, it is possible that a freshwater-to-marine transition occurred on the branch leading to Hirudinida, with a subsequent recolonization of freshwater in the clade encompassing the non-oceanobdelliform true leeches. The third, and most plausible, possibility is that the ancestor of Hirudinea was marine, and that the non-leech, freshwater orders Branchiobdellida and Acanthobdellida represent independent transitions to freshwater. Both of these orders are separated from other taxa by long branches (Tessler et al., 2018; de Carle et al., 2022). For the branchiobdellidans, this implies a recent and rapid radiation; the Acanthobdellida is incredibly species-poor, with only two known extant species. Acanthobdellidans are primarily parasites of salmonid fishes, and branchiobdellidans are obligately commensal on astacoid crayfish (Skelton et al., 2013; Utevsky, Sokolov & Shedko, 2013; Bielecki et al., 2014; de Carle et al., 2022). Both hosts stem from marine lineages; therefore, these taxa may have accompanied their hosts on their freshwater transitions while the earliest leeches remained marine.

Parasitism

Leeches are unique among annelids as a radiation of blood-feeding parasites. Although not directly used in feeding, it is tempting to speculate that the posterior sucker, which defines Hirudinea, could be an adaptation to parasitism due to its use in host-attachment; however, some aspects of hirudinean ecology cast doubt on this speculation. Many extant leeches are non-blood-feeding, using their suckers to manipulate prey or to attach to substrates (Sawyer, 1986). Furthermore, branchiobdellidans, which have tight associations with their crayfish hosts, are broadly ectocommensal rather than parasitic (Skelton et al., 2013); however, evidence suggests that their “suckers” are homoplastic with those of Hirudinida and Acanthobdellida (Weigl, 1994).

Other features–the sub-division of annuli, elaboration of the crop, and lack of septa in mid-body segments–have been postulated to facilitate sanguivory in leeches by allowing them to expand to store copious quantities of blood (Sawyer, 1986). Because these, in addition to several anticoagulants implicated in blood-feeding (Iwama et al., 2019, 2021; Iwama, Tessler & Kvist, 2022), are present in all leeches regardless of feeding mode, most authors have postulated that the ancestral hirudinidan fed on vertebrate blood (Trontelj, Sket & Steinbrück, 1999; Tessler et al., 2018), rather than parasitizing invertebrates or engulfing them wholesale as many extant leeches do (Sawyer, 1986). Supposing that the most recent common ancestor of leeches was indeed parasitic, the composition of the Waukesha Biota and the age of *Macromyzon* call the validity of this hypothesis, and the distinction between vertebrate and invertebrate parasitism, into question.

No crown vertebrates have been recovered from Waukesha. Chordate remains, such as conodonts and other undetermined chordates, are present in the Waukesha Biota (Wendruff et al., 2020a), but are extremely rare, and were likely too small to serve as viable hosts.

The most abundant animals at the Waukesha locality are arthropods, with trilobites being the most numerous of these (Wendruff et al., 2020a). Given that some modern leeches obligately or facultatively feed on arthropod haemolymph and other bodily tissues (Sawyer, 1986; Nakano et al., 2017), trilobites and other arthropods represent the most plausible hosts for *M. siluricus*. Interestingly, certain extant hirudineans also have non-parasitic associations with arthropods: some marine leeches deposit their cocoons on the hard shells of arthropods and bivalves, and branchiobdellidans live exclusively on freshwater crustaceans (Sawyer, 1986; Skelton et al., 2013). It is therefore possible that these associations, both parasitic and commensal, share a common origin.

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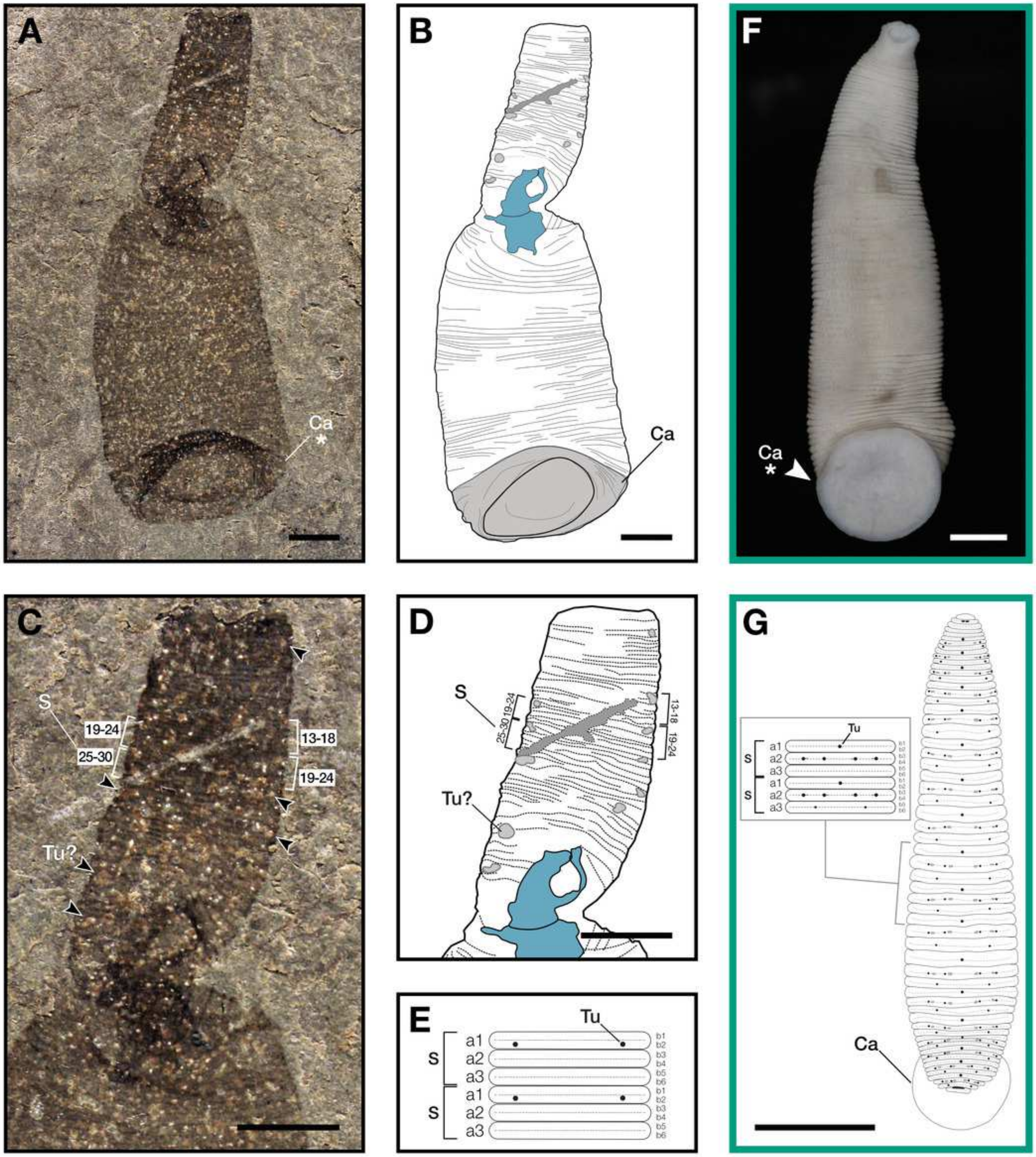
Figure 1

Macromyzon siluricus gen. et sp. nov. from the Lower Brandon Bridge Formation (Waukesha Lagerstätte), Silurian (Llandovery: Telychian), Waukesha, Wisconsin, USA

Holotype specimen UWGM 4001_14 (B) Schematic of the external morphology of *Macromyzon siluricus* based on the holotype. (C) Detail of the anterior region, dorsal view, showing sexannulate segments with annuli numbered; black arrows indicate putative tubercles. (D) Schematic of the anterior region showing tubercles in light grey and sexannulate segments with annuli numbered. (E) Segmentation pattern of *M. siluricus*. Green borders indicate extant Hirudinida introduced for comparison: (F) Ventral view of *Myxobdella sinanensis*, white arrow indicates caudal sucker; photo by Takafumi Nakano. (G) Schematic of *Haementeria lutzi* (dorsal view) with inset showing the species' segmentation pattern. This specimen is deposited in the collections of the Museum of Zoology of the University of São Paulo (MZUSP 0026). Abbreviations: Ca, caudal sucker; S, segment; Tu?, putative tubercles. The point of torsion is shown in blue; breakage in the specimen is indicated in dark grey. Detail of starred features is shown in Fig. 2A,B. Scale bars: 5mm.


A
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a1
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s
a1
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s
a1
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Ca

Figure 2

Comparison of caudal suckers (green arrows) in *Macromyzon* and extant Hirudinea.

(A) Detail of the caudal sucker in *Macromyzon siluricus*, whole specimen shown in Fig.1A. (B) Detail of the caudal sucker in the hirudiniform leech *Myxobdella sinanensis*, whole specimen shown in Fig.1F. (C) Cladogram showing relationships between taxa represented in this figure. (D) Branchiobdellidan species *Cambarincola* aff. *okadai* and *Triannulata magna*; photos by Andrew S. Y. Mackie (James et al., 2015) and Jean- François Parpet (Parpet & Gelder, 2020). (E) Full body (top) and scanning electron micrograph (bottom) of the acanthobdellidan *Acanthobdella peledina*; images by P. Swiątek. (F) The oceanobdelliform leech *Pterobdellina vernadskyi*; photo by A. Utevsky (31). (G) The glossiphoniiform leech *Torix* sp.; photo by Takafumi Nakano. (H) The hirudiniform leech *Haemadipsa japonica*; photo by Takafumi Nakano.

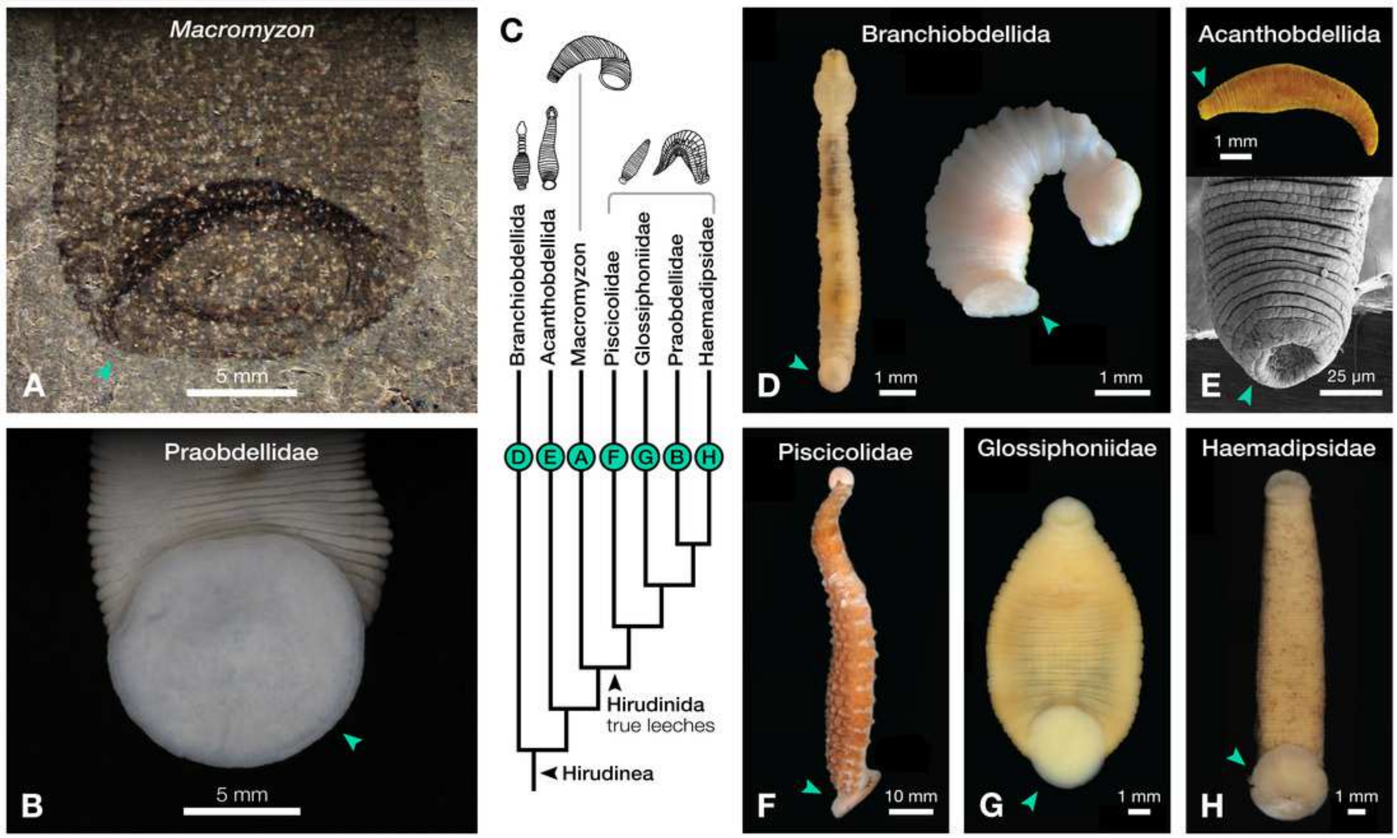
Macromyzon
A
5 mm
Praobdellidae
B
5 mm
C
Branchiobdellida
Acanthobdellida
Macromyzon
Piscicolidae
Glossiphoniidae
Praobdellidae
Haemadipsidae
D
E
A
F
G
B
H
Hirudinida
true leeches
Hirudinea
Branchiobdellida
D
1 mm
1 mm
Acanthobdellida
1 mm
E
25 μm
Piscicolidae
F
10 mm
Glossiphoniidae
G
1 mm
Haemadipsidae
H
1 mm

Figure 3

A selection of palaeoscolecids for comparative purposes, refuting any affinity between *Macromyzon* and vermiform ecdysozoans.

(A) An undescribed palaeoscolecid from Waukesha showing a toughened, plate-like arrangement of the cuticle and scleritome. (B) Another undescribed palaeoscolecid from Waukesha, with tightly packed, regularly arranged, dome-shaped sclerites. A and B demonstrate that diagnostic palaeoscolecid characters readily preserve at Waukesha, and thus their absence in *Macromyzon* is not a function of taphonomy. (C) A specimen of *Wronascolex antiquus* from García-Bellido et al. (2013), the most convincing possible palaeoscolecid moult known due to the breakage (white arrow) at the midline; however, the authors acknowledge that this is not a surety. (D) A close up on a piece of the scleritome of *Hadimopanella* from Topper et al. (2010) showing the interlocking articulation of the sclerites. The sclerites of palaeoscolecids in general are unlike the more labile tubercles found in leeches and *Macromyzon* in particular.

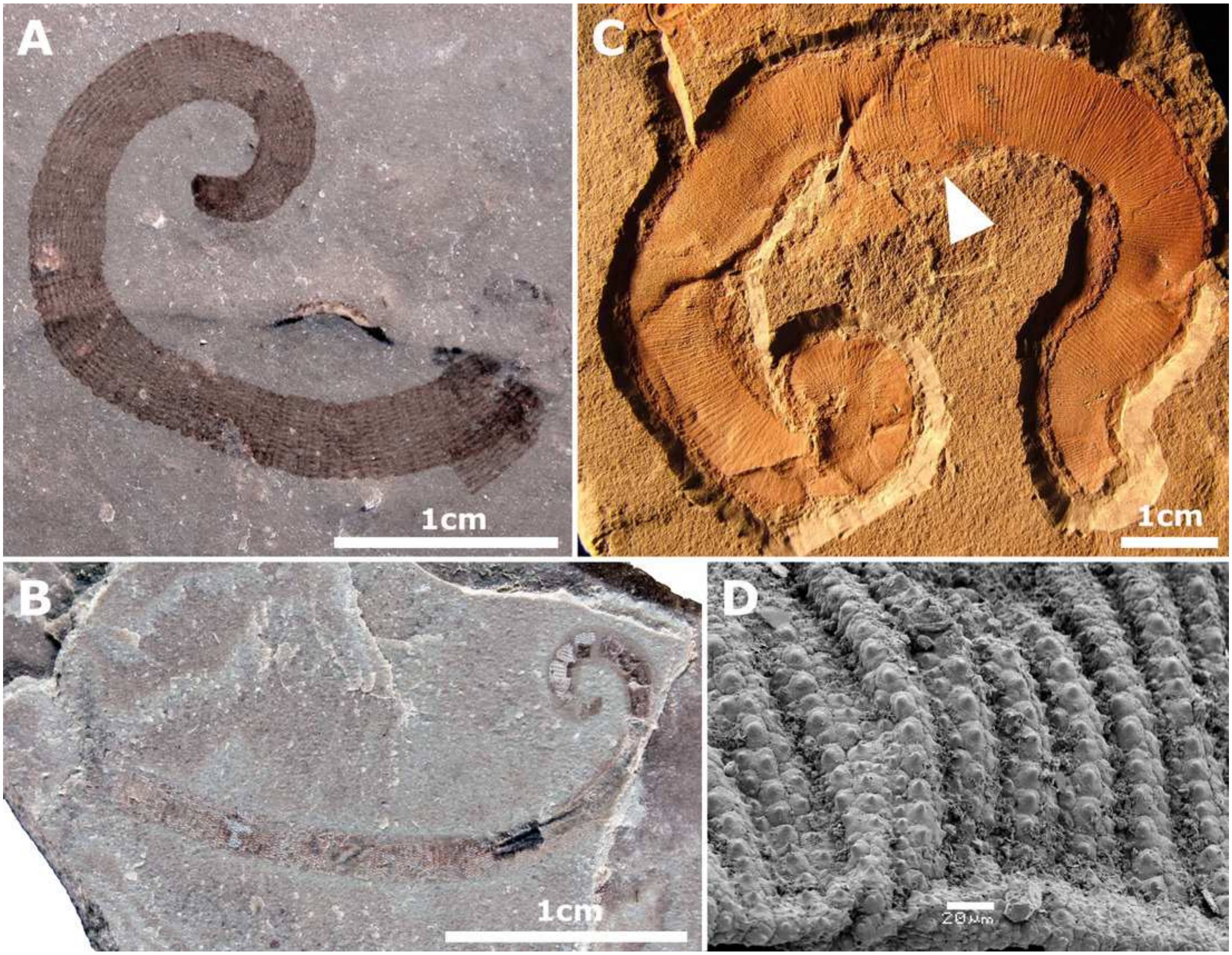
A
1cm
C
1cm
B
1cm
D
20 µm

Figure 4

Phylogenetic analyses recover *Macromyzon* as a stem leech.

(A) Bayesian inference tree: black circles on branches indicate nodes at which posterior probability (PP) = 1, other PP values are displayed below branches. Triangles represent collapsed clades with the length of the triangle corresponding to the length of the longest branch. (Uncollapsed topology is available as supplemental file 2. Maximum parsimony topology is shown in supplemental file 3.) Illustrations show representative taxa for each clade. (B) Geologic timescale comparing clade age estimates from molecular clock analyses (grey bars) above, age of cocoon fossils (black bar), and age of *Macromyzon siluricus*. (C) Life reconstruction of *M. siluricus*. Illustration by E. K. Chan.