

DISPATCH

Evolution: Arrow Worms Find Their Place on the Tree of Life

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A new phylogenomic study places the erstwhile enigmatic chaetognaths, also known as arrow worms, within a subgroup of lophotrochozoans, the gnathiferans.

Chaetognaths, also known as 'arrow worms', have been important predators in the world's oceans since the Cambrian. While there are only around 130 chaetognath species known, they are nevertheless hugely abundant, showing the success of their life style. Most chaetognaths are pelagic predators, ranging in size from a few millimeters to a few centimeters and feed on fish fry, copepods and other plankton making them a key link in the ocean's food web. The chaetognath body plan has been tinkered with remarkably little over the past half a billion years. The living taxa are physically all very similar: with a streamlined, tripartite, arrow-like body with fins and a horizontal tail at one end and a head with very obvious grasping spines at the other (hence the name chaetognaths, meaning 'spiny jaws'; Figure 1). The very same morphological characters are immediately recognisable in fossil chaetognaths from the early [1] and mid-Cambrian [2], and the widespread existence of protoconodonts (fossilised chaetognath teeth) betrays their success in Cambrian oceans [3]. Despite their abundance and ecological importance, where exactly arrow worms sit in the animal tree of life has remained enigmatic. A recent phylogenomic study in *Current Biology* by Ferdinand Marlétaz, Daniel Rokhsar and colleagues [4] resolves this puzzle.

There aren't many animal phyla left without a home, but the chaetognaths were one [5]. Chaetognaths have generally been placed towards the final chapters in zoological textbooks, fitting with the view that they may be close relatives of the deuterostomes, a long-established major animal group that includes chordates, such as humans and echinoderms, such as sea urchins. The idea of a close

relationship between chaetognaths and deuterostomes is based principally on a set of embryological characters: radial cleavage of the early embryonic blastomeres, deriving the mesoderm from out-pocketing of the embryonic gut (enterocoely) and the derivation of the anus, rather than the mouth, from the blastopore of the gastrula (deuterostomy). These traits had been thought to be defining characteristics of the deuterostomes and to differentiate them from the other big animal subdivision, the protostomes. The enigma of the evolutionary affinities of the chaetognaths has been the focus of multiple molecular phylogenetic studies over the years. The first of these concluded that the link with deuterostomes was incorrect [6], and over the years it has become clear that the arrow worms are protostomes [7-9]. This, however, is where the consensus ended.

Within the protostomes there is a major split into two large groups — the ecdysozoans (including arthropods, nematodes and other moulting animals) and the lophotrochozoans (including annelids, molluscs, flatworms, rotifers and other less familiar phyla) [5]. Chaetognaths have been proposed in all possible relationships to these two groups — as ecdysozoans, lophotrochozoans or as the sister group of both these clades. This ambiguity seemed also to be reflected in the sequences of their Hox genes, in which both ecdysozoan and lophotrochozoan signatures have been described [10].

In their new study, Marlétaz and colleagues [4] have produced transcriptome sequences from 10 chaetognath species and included these in phylogenomic data sets containing over 1000 genes from species covering the majority of animal phyla, with a focus on deep sampling across the protostomes. Their conclusions with regards to the position of the chaetognaths were consistent across different data sets (with or without fast sites pruned) and using different methods of analyses including those designed to minimise artefacts from compositional bias (identifying and removing the more biased taxa and recoding the data to bin frequently exchanging amino acids) and systematic differences in rates of evolution (removing fast taxa and using site heterogeneous models of evolution).

The principal finding of Marlétaz and colleagues [4] clearly supports the notion that chaetognaths are lophotrochozoans (Figure 1). More precisely, chaetognaths are part of a previously identified monophyletic group containing three phyla: the rotifers, gnathostomulids and micrognathozoans. Rotifers are tiny animals easily found in pond water and readily recognized by the wheel of cilia found around their mouth. The gnathostomulids and micrognathozoans are similarly tiny but much less familiar: gnathostomulids live in the small interstitial spaces between grains of marine sand and the micrognathozoans are a recently described phylum discovered in mosses in a cold spring in Greenland [11]. The clade containing these phyla has been named 'Gnathifera' (jaw bearers) after their most obvious shared characteristic — a pair of chitinous jaws found in all members [12]. The obvious conclusion is that the jaws of chaetognaths are homologs of the jaws of the other gnathiferans. A relationship with rotifers

had been suggested before. It has been claimed, for example, that the intracellular cuticle found in rotifers and acanthocephalans (a parasitic group within the rotifer phylum) is also present in chaetognaths (though this seems not to have been studied further). A chaetognath–rotifer relationship also fits with a recent comparison of rotifer Hox genes, which found unique amino acid signatures shared with the previously puzzling chaetognath Hox genes [13].

A second paper in this issue brings further evidence for this Chaetognatha-Gnathifera connection but from a very different point of view. Vinther and Parry [14] have reexamined the mid Cambrian (Burgess Shale) fossil *Amiskwia sagittiformis*, which has previously been interpreted either as a chaetognath or a pelagic nemertean worm (ribbon worm). While having a clearly chaetognath shaped body, *Amiskwia* lacks certain characteristics of living chaetognaths, most notably the obvious grasping jaws and a transverse septum. In living chaetognaths, the septum divides the posterior part of the body into two compartments (with female gonads anterior and male gonads posterior) and the anus terminates at this septum. In *Amiskwia* the gut appears to terminate closer to the tail. Vinther and Parry show that internal kidney shaped structures, found at the anterior end of the gut in *Amiskwia*, project slightly from the rest of the fossil suggesting that, in life, these were hardened structures. They interpret these as internal jaws homologous to those of other Gnathifera [14]. In this interpretation, *Amiskwia* is a stem chaetognath which branched from the lineage leading to modern (crown group) chaetognaths prior to the evolution of the grasping spines, transverse septum and non-terminal anus. Their phylogenetic analysis of a matrix of morphological characters supports this idea and, congruent with the work of Marlétaz et al, places the chaetognaths within the Gnathifera [14].

Gnathiferans are positioned as the sister group to all the other lophotrochozoans [4], which share a clear unique character, namely that almost all the phyla within it have members with a spiral pattern of cleavage in the early embryo [15]. Hence, they are known as spiralian. Early development leads, in many spiralian phyla, to a ciliated larva similar to the classic trochophore larva of annelids and molluscs. By contrast, all gnathiferans, develop directly into the adult. Interestingly, Marlétaz and colleagues [4] find evidence for a close relationship, within the spiralian, between nemerteans (ribbon worms) and platyhelminths (flatworms). The ciliated larvae of these two phyla have specific similarities, differing from the canonical trochophore larvae in the elaboration of the ciliary band around extensions of the larval body. Flatworms and ribbon worms have been linked in the past in a group called the Parenchymia due to the extensive parenchymatous tissue they possess. Marlétaz and colleagues [4] also group the molluscs and a phylum of tiny, sessile, filter feeding animals called entoprocts in a previously proposed clade named 'Tetraneuralia' after the four longitudinal nerve chords observed in both groups (Figure 1) [16].

Recently, there has been a trend towards renaming the monophyletic sister group of ecdysozoans as spiralian and to reserve the previously popular name lophotrochozoans for just a subset of this large clade. This change made some sense, when the earliest split within this large clade had spirally cleaving platyhelminths in one branch and spirally cleaving annelids and molluscs in the other, implying spiral cleavage was a primitive state for this clade. Recent results, including those of Marlétaz and colleagues [4], showing that spirally cleaving animals are a subset of the larger group, suggests that the name 'Spiralia' would be sensibly used for this smaller, spirally cleaving clade of animals, and that we should return to the widely understood use of Lophotrochozoa for the sister group of Ecdysozoa (Figure 1B.).

One important use of an accurate phylogenetic tree is as a framework for a better understanding of how certain traits evolved [17]. Clarification of the position of the chaetognaths suggests different interpretations of the characteristics of the phylum in the wider context of animal evolution. The position of gnathiferans as the sister group of spiralian has been used to suggest that the ancestor of lophotrochozoans was a miniature animal, as all members gnathiferans (apart from the parasitic acanthocephalans within the rotifers) are tiny and meiofaunal [18]. The addition of the chaetognaths to this clade make this idea somewhat less parsimonious as both Cambrian [2] and modern chaetognaths can grow to more than 10 cm.

Additionally, the characteristics that were once used to link chaetognaths to the deuterostomes are clearly not specific to that clade, they are characteristics also found in some lophotrochozoans. Indeed, radial cleavage, enterocoely and deuterostomy have all also been described in various ecdysozoans [19]. These erstwhile deuterostome synapomorphies are now more reasonably interpreted as primitive characteristics of all bilaterian animals that have been lost in some protostomian clades — in this sense, the ancestor of all bilaterians (Urbilateria) was a deuterostome. This might even be literally true if the result of the analysis of wider metazoan relationships by Marlétaz and colleagues [4] is correct: they show that the old concept of a group of deuterostomes comprising chordates and ambulacrarians (echinoderms and hemichordates) might not exist. Instead, they find limited evidence that the chordates are actually more closely related to the protostomes than to the ambulacrarians. Their analysis suggests that the ambulacrarians plus xenacoelomorph worms (collectively the Xenambulacraria) are the sister group of all other bilaterians. This intriguing result, which has been seen before in studies using site-heterogenous models of tree reconstruction which are expected to minimise certain systematic errors [20], would mean the direct ancestor of chordates and ambulacrarians is in fact Urbilateria, the common ancestor of all bilaterian animals.

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Figure 1. Chaetognaths and their place in protostome phylogeny.

Left: the chaetognath *Pterosagitta draco* showing the arrow like body, flattened tail and the jaws visible at the anterior end (photo: Katja Peijnenburg & Erica Goetze). Right: phylogeny of the protostomes as supported by Marlétaz and colleagues [9]. The major groups are shown in shaded boxes. Within the

Lophotrochozoa there are two clades: the Gnathifera (for which schematics of their jaws are shown) and the Spiralia (phyla with members possessing spiral cleavage are indicated). Marlétaz and colleagues [9] found support for some clades within Spiralia as indicated (thanks Anne Zakrzewski for the outlines of gnathiferan jaws.)