Correspondences

Broad taxon and gene sampling indicate that chaetognaths are protostomes

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Despite advances in phylogenetic methods, there are still a number of enigmatic phyla whose affinities remain poorly resolved. One of the most recalcitrant of these is a group of small predatory marine invertebrates, the chaetognaths (arrow worms). Resolution of the phylogenetic position of the chaetognaths is key for reconstructing the evolutionary history of some of the most fundamental features of animals, including those that have been used to delineate two major clades of animals — the protostomes and deuterostomes. An affiliation of chaetognaths to deuterostomes was inferred from a number of shared embryological and morphological features including radial, indeterminate cleavage, a posterior position of the blastopore (deuterostomy), enterocoelous coelom formation, and a tripartite adult body plan with a post-anal tail [1,2]. Various other features (e.g., a ventrally positioned nervous system, ecdysozoan-like spines, and a lack of circular body wall muscle) have been interpreted as evidence of a protostome affinity [3].

Here, we analyse the phylogenetic position of the chaetognaths using existing datasets, i.e. small and large subunit nuclear ribosomal RNAs (SSU and LSU), and complete mitochondrial genomes. We also report a new dataset from the tropomyosin gene of many Metazoa. Each of these three datasets is broadly sampled across the Metazoa. We have also sequenced ~ 5000 ESTs from the pelagic chaetognath *Flaccisagitta enflata*, recovering 72 of the genes used by Philippe *et al*. [4]. Preliminary analyses of SSU and LSU ribosomal RNAs showed chaetognath sequences clustering with taxa that also show long phylogenetic branches and a higher than average GC content (see Supplemental data published with this article online). In order to address the possibility that this grouping was an artifact of GC content, we recoded all nucleotides as purines (A,G = R) and pyrimidines (C,T/U = Y). Maximum likelihood and Bayesian analyses of this recoded dataset grouped the chaetognaths with the priapulids within the Ecdysozoa (Supplemental data). However, with priapulids excluded, chaetognaths grouped with the Lophotrochozoa (100% posterior probability), without resolving their position within the group (Supplemental data). The association with the Ecdysozoa, therefore, depends on a single taxon and is not likely to reflect a general affinity for the group.

Furthermore, we analyzed two complete chaetognath mitochondrial genomes in combination that previously were analyzed independently with differing results [5,6]. Bayesian analysis of the nucleotide matrix places chaetognaths within the Lophotrochozoa allied to molluscs, and Bayesian analysis of the amino acid matrix places them as sister to the Lophotrochozoa (Supplemental data). Maximum likelihood analyses of nucleotide and amino acid datasets support the hypothesis that chaetognaths are sister to lophotrochozoans albeit incongruously allied with the long branched arthropod *Ornithoctonus huwena* using nucleotide data (Supplemental data). Bayesian and likelihood analyses of tropomyosin from 56 taxa (Supplemental data) show unequivocal support for the new metazoan phylogeny within the Bilateria, recovering a monophyletic Protostomia clade (100% posterior probability), as well as Ecdysozoa (88%) and Lophotrochozoa (100%). Chaetognaths are placed as sister to lophotrochozoans (100%) (Figure 1A).

Bayesian analyses of the 72 genes in the EST dataset (11,650 amino acids) positioned the chaetognaths as sister to the lophotrochozoan taxa (annelids and molluscs) (Figure 1B; Supplemental data), while maximum likelihood analyses placed them within the protostomes but did not resolve their relationship to other protostome taxa (Supplemental data). Most features of this tree were consistent with the ‘new’ metazoan phylogeny [4,7–9], with the notable exception that the platyhelminths grouped with nematodes. To eliminate long branch attraction artefacts, we analyzed subsets of taxa to determine the effects of taxon sampling (Supplemental data). Regardless of the taxa removed (nematodes, platyhelminths or tardigrades) the chaetognaths remained as sister to annelids and molluscs with varying degrees of support in Bayesian analyses and as sister to the Ecdysozoa (arthropods, nematodes and tardigrades) with weak bootstrap support in likelihood analyses (Figure 1B; also see Supplemental data).

Topological tests on all four datasets did not reject the hypotheses that chaetognaths form a clade with ecdysozoans. Only the Tropomyosin dataset rejected the positioning of chaetognaths as sister to the protostomes as significantly worse. (Shimodaira-Hasegawa test using RELL bootstrapping; Supplemental data).

All of our analyses indicate that chaetognaths are protostomes, consistent with other recent studies [5,6,10] and the accompanying paper [11]. This has implications for our understanding of the evolution of morphological characters and for the reconstruction of the last common ancestor of protostomes and deuterostomes. The early development of chaetognaths resembles a radial cleavage

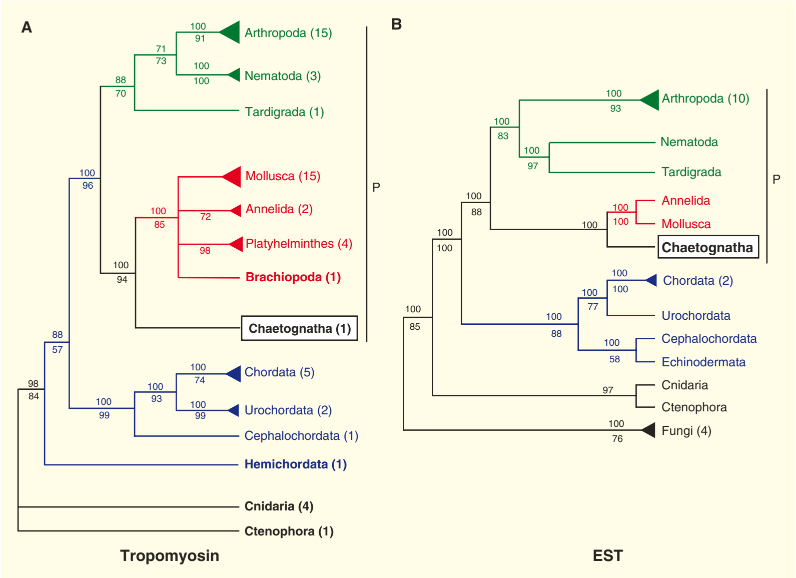

Figure 1. Phylogenetic analyses suggest a relationship between chaetognaths and lophotrochozoans.

(A) Bayesian and maximum likelihood analysis of a 56 taxa metazoan tropomyosin data set. Phylogenetic analyses recover the main protostome (P) clades of Ecdysozoa (green) and Lophotrochozoa (red) as well as the deuterostomes (blue), all with significant support. Chaetognath tropomyosin shows a sister-group relationship to all other lophotrochozoan tropomyosin genes (100% posterior probability). Some clades have been collapsed, with the number of species represented indicated in parentheses. (B) Bayesian consensus tree based on 72 genes from the EST dataset (26 taxa) groups the chaetognath Flaccisagitta with the Lophotrochozoa. Posterior probabilities are shown as percentages. All platyhelminths and nematodes, except for the slowly evolving *Trichinella*, have been excluded. Bootstrap support values from maximum likelihood analyses are included below relevant nodes where significant. Amino acid data matrices were used to build both trees (under a WAG substitution model). Four runs of four chains each were run for one million generations and sampled every 100 generations (a 50,000 generation burn-in was found to be sufficient for both datasets).

program. As an irregular radial cleavage pattern seems to be present in the ecdysozoan ground pattern [12], it seems that spiral cleavage is an innovation of the spiralian lophotrochozoans and that the deuterostome – protostome ancestor may have possessed regulative radial cleavage. The phylogenetic position of the direct developing chaetognaths might also suggest that larvae with ciliary bands evolved more than once, reinforcing doubts over the homology between dipleurula and trochophora larvae [13].

The deuterostomy and enterocoely of chaetognaths could be convergent on that of the deuterostomes. For example, coelom formation in chaetognaths has been described as fundamentally different from that seen in the Deuterostomia [14] and the site and fate of the blastopore has changed often in the protostomes [3].

The affinity of chaetognaths with the protostomes has strong support, but their placement within this group remains uncertain. The mitochondrial and tropomyosin analyses, and — less credibly — rRNA dataset did support a clade of Lophotrochozoa and chaetognaths. Bayesian EST analyses also support a Lophotrochozoan relationship; however, the sensitivity of these analyses to the removal of taxa suggests that broader taxon sampling is required – particularly of the poorly represented Lophotrochozoa.

Supplemental data

Supplemental data including experimental procedures are available at http://www.current-biology.com/cgi/content/full/16/15/R575/DC1/

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