

Reclassification and Ctenophores as the Sister Group to All Metazoans.

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INTRODUCTION

When talking of early animal evolution, 4 phyla are most commonly referred to: Porifera, Choanoflagellates, Ctenophora, and Cnidarians. Commonly, ctenophores are known as comb jellies or sea gooseberries, Porifera are known as sponges, and cnidarians are known as jelly fish. These three are debated in which is most closely related to the ancestor of all Metazoans, while Choanoflagellates are understood as being a common outgroup to Metazoans.

Early animal evolution is a controversial subject amongst many evolutionary biologists. There are two main schools of thought, that of the Porifera-sister hypothesis (1) where the phylum Porifera is the sister group to all Metazoans and the second more controversial hypothesis of Ctenophora-sister (2-5), where the phylum Ctenophora is the sister group to all Metazoans (Figure 1). This controversy stems partially from the traditional teaching that animals became more ‘complicated’ through evolution, so therefore the simplest phylum should be placed as the sister group to the Metazoans. More recently, the concept of Ctenophora being the sister group arose due to phylogenetic support (2-5). Here, the Ctenophora-sister hypothesis will be tested to determine the sister group to all animals.

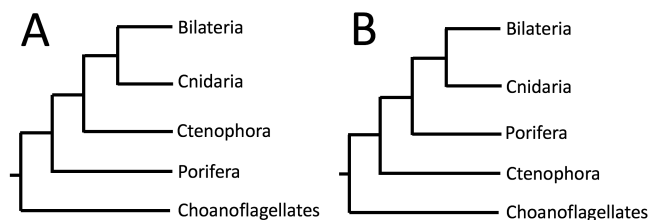


Figure 1 Alternative hypotheses from previous studies. (A) Porifera-sister hypothesis. (B) Ctenophora-sister hypothesis.

METHODS

Dataset Assembly.

I used complete non-coding DNA sequences of internal transcribed spacer 1 (ITS1), 5.8S, and internal transcribed spacer 2 (ITS2) rRNA, and partial coding DNA sequences of the small sub-unit (18S) and large sub-unit (28S) rRNA of 76 selected species (6). ITS1 and ITS2 were chosen due to high variability from nucleotide substitutions, deletions, and insertions and are more recently being used to phylogenetically place species in eukaryotes (7). The 5.8S rRNA region is a highly conserved site (in addition to the ITS regions) amongst all eukaryotes, but especially Metazoans, which are composed of 3 of the 4 phyla analyzed here. Selected sequences include 29 Ctenophores, 39 Porifera, 7 Cnidarians, and 1 Choanoflagellate (Table S1). All data was downloaded from GenBank, the NIH genetic sequence database, and accession numbers are located in Table S1.

Alignment.

Sequences were aligned using MAFFT (8) version 7.245 using localpair iterative refinement method (L-INS-i) with a maxiterate of 16 and input order phylip output (script available in Figure S1).

Maximum Likelihood.

Alignments were analyzed using RAxML (9) version 8.2.9 using the GTR+ Γ model with 30 stepwise additions and using *Salpingoeca kjevrii* as an outgroup to root the tree. The GTR+ Γ model is suited to DNA data. In addition, the alignments were analyzed using the same software and model to generate 30 maximum likelihood trees on distinct starting trees with the best likelihood saved with the same outgroup chosen to root the tree. Script available in Table S2.

Bootstrapping.

Alignments were analyzed for bootstrap values using the GTR+ Γ and GTR+CAT models and a random seed number of 12345 and 100 replicates was used. The GTR+CAT model is considered the most suitable model to use when looking at deep phylogeny and ancestral groups as it accounts for site heterogeneity (5, 10, 11). Bipartitions were then drawn on the best maximum likelihood tree and viewed using FigTree v1.4.3 (Rambaut 2016). Tree was transformed using the function 'cladogram' and node labels instead of branch lengths of the bootstrap values were included due to rooting of the tree with *Salpingoeca kjevrii*.

RESULTS

Ctenophora is Sister to All Metazoans.

Using complete ITS1, 5.8S and ITS2 sequences for 76 species, the tree topology for the phyla Porifera, Ctenophora, and Cnidaria was determined and is presented in Figure 2. Figure 2 indicates that Ctenophora is the sister group to all other metazoans as indicated by being the first branch point as compared to the Choanoflagellate outgroup. In addition, this is the first time the Ctenophora phylum has been analyzed using ITS1 and ITS2 and the first time the Porifera phylum has been analyzed with this many species using ITS1 and ITS2.

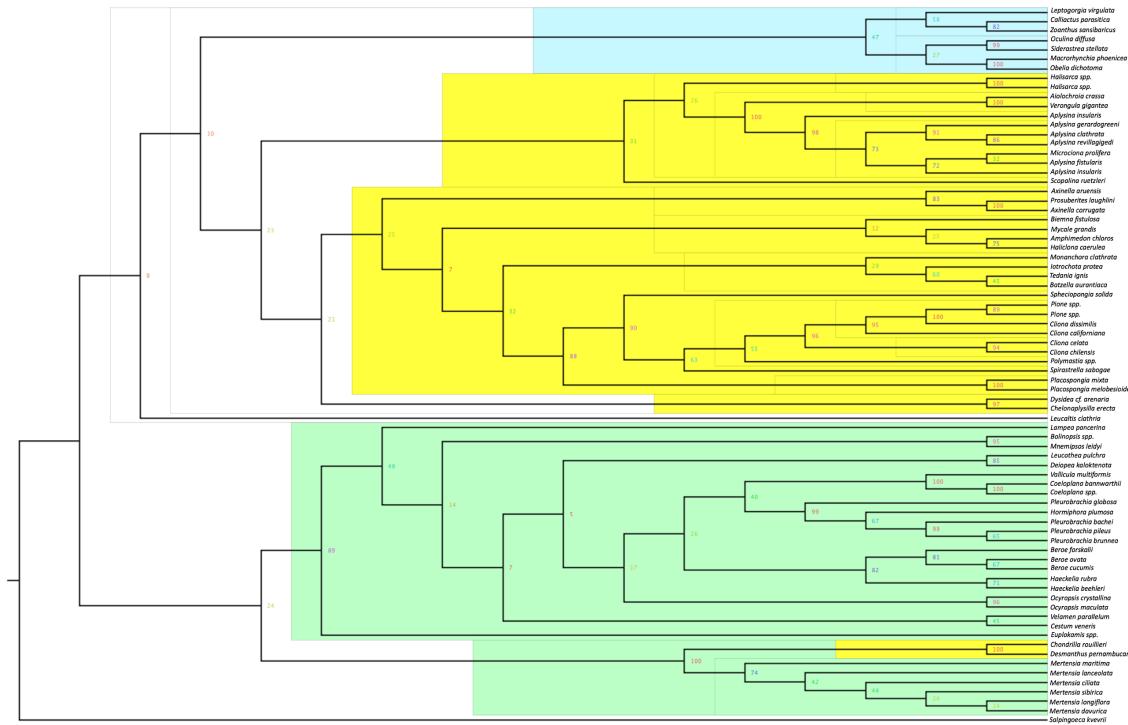


Figure 2: Bootstrap support value tree of all 76 species with outgroup consisting of *Salpingoeca kvevrii*, a choanoflagellate. Green highlighting indicates species classified in the phylum Ctenophora, yellow highlighting indicates species classified in the phylum Porifera, and blue highlighting indicates species classified in the phylum Cnidaria.

The tree in Figure 2 reveals numerous interesting relationships, including possible misidentifications and a need for reclassifications. The need for reclassifications of some genera of sponges to others is present especially in the species *Prosuberites laughlini*, which has high bootstrap support for being in the genus *Axinella* instead of *Prosuberites* due to its similarity. There are additional reclassifications necessary such as the high bootstrap support for the genus *Cliona* and the genus *Pione* being the same genera.

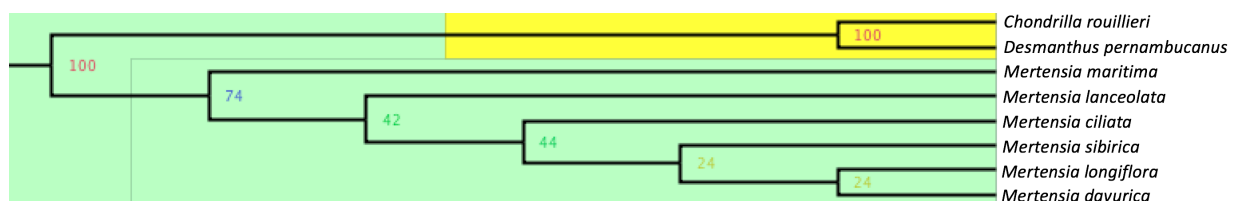


Figure 3: Close up of one branching section of the complete tree seen in Figure 2. Yellow highlighting indicates species classified in the phylum Porifera, and green highlighting indicates species classified in the phylum Ctenophora. Bootstrap support values are presented at the nodes.

In addition to these reclassifications, Figure 3 shows the lower branches of Figure 2, which indicates that there are two species of sponges that are very closely related to a genus of comb jellies. *Chondrilla rouillieri* and *Desmanthus pernambucanus* both have complete bootstrap support for being related to the genus of comb jellies *Mertensia* instead of sponges.

DISCUSSIONS AND CONCLUSIONS

Reclassifications.

Phylogenetic analysis of the ITS1, 5.8S, and ITS2 rRNA regions revealed the need for reclassification of two genera. The species *Prosuberites laughlini* has a high bootstrap support (>80%) as being related to the genus *Axinella*, and complete support (100%) as being related to *Axinella corrugata*. These high support values indicate a need for reclassification of *Prosuberites* to *Axinella*. Research also suggests that these sponges reside in similar locations (12) which could lead to the original misidentification. Additional sequences and phylogenetic topology will be necessary before official reclassification, but with high support, it is expected that this is necessary.

Furthermore, the genera *Cliona* and *Pione* share numerous similarities. In common language, these two genera are often confused with each other indicating a high similarity between these two Porifera. After analysis, these two genera had very high bootstrap support (>85%) as well as complete support between *Pione spp.* and *Cliona dissimilis* indicating these two genera are very closely related. Again, additional sequences and phylogenetic topology will be necessary to reclassify, but the data presented here indicates an expected need to reclassify the genus *Pione* to *Cliona*.

Through the use of site-heterogenous gene sequences and model selection, the overall findings indicate high support for the reclassification of the genus *Pione* to *Cliona* and the genus *Prosuberites* to *Axinella*.

Ctenophora is the Sister to All Metazoans.

One major current debate amongst evolutionary biologists is whether early animal evolution branches from Porifera or Ctenophora. Based on the rRNA genes ITS1, 5.8S, and ITS2 and the analyses, the Ctenophora-sister hypothesis is most strongly supported. The use of the GTR+ Γ and GTR + CAT models of maximum likelihood are previously strongly supported and resistant to systematic errors (13) and further supported with the addition of more taxa and different outgroups. The high bootstrap values and placement in relation to the outgroup Choanoflagellate, a known and commonly used outgroup for metazoan phylogenies, indicates support for the phylum Ctenophora as the sister to all metazoans. This Ctenophora-sister support makes major implications in early animal evolution, indicating that both neural and muscular systems were lost in Porifera and another phyla Placozoa instead of originating from a common ancestor to Ctenophora (14). While this may seem unlikely at first, in reality using complexity as a measure of character in evolution is flawed due to an inability to define what complexity is. Additionally, most models of the Porifera-sister hypothesis use parsimonious methods which while useful in some scenarios, again defines that complexity is a character and therefore the least complex should be at the base of early animal evolution (15). It is known that convergent evolution is a relatively common phenomenon in evolution, as this has been described for over a decade on eye evolution alone (16), so the concept the neural and muscular systems also evolved through convergent evolution is not a reach. Other papers support that ctenophores are the sister to all Metazoans (17, 18). Through the use of proper model selection and high bootstrap support, the

overall findings presented here also support that ctenophores are the sister to all Metazoans.

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