

**Replication Study of “Ancient gene linkages support ctenophores as sister to other animals” Write-up**

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## Introduction/Background

The study “Ancient gene linkages support ctenophores as sister to other animals” examines the phylogenetic relationship between sponges, ctenophores, and all other animals. Sponges are organisms of the sea that play an important role in marine ecosystems and phylogenetic contexts due to their early emergence (Wörheide et al., 2012). Sponges maintain a structured composition of silica and calcium carbonate. They have specialized cells that perform functions within feeding, reproduction, and defense. Despite these specialized cells, sponges lack neurons and muscle cells (Schultz et al., 2023). This is an important point, as it distinguishes the sponge from the other organism of focus in this study: ctenophores. Ctenophores are gelatinous sea creatures, also known as comb jellies. Much like sponges, ctenophores posit important phylogenetic implications due to their early emergence. Ctenophores have an amorphous structure, and in contrast to sponges, they possess multiple interconnected neurons, or nerve nets (Schultz et al., 2023; Moroz et al., 2014).

While both organisms are shown to have important phylogenetic positions, the specific order and relationship between the two organisms and all other animals is heavily debated. In a phylogenetic tree, all other animals (parahoxozoa) form a clade distinctly separated from sponges and ctenophores. However, the specific positioning of the two organisms in relation to parahoxozoa is unknown. Among this debate, there are two competing hypotheses: sponge sister hypothesis and ctenophore sister hypothesis. As the names suggest, the sponge sister hypothesis positions the sponge as the sister to all animals, while the ctenophore sister hypothesis positions the ctenophore as the sister to all animals (Schultz et al., 2023). The sponge sister hypothesis would imply that the origin of neurons is a single point on the ctenophore-parahoxozoan stem in a phylogenetic tree. The ctenophore sister hypothesis would imply that ancestral metazoan

neurons were lost in the sponge lineage or there was a convergence event of neurons from ctenophores and parahoxozoan lineages (Schultz et al., 2023). The implications of these two opposing hypotheses are quite different and can imply varying things for evolutionary neuronal development.

### **Statement of Need**

While the debate over the two hypotheses is very active, it is difficult to prove one or the other due to the nature of the research question as well as the previous methods that were being employed. Physical fossil records have provided minimal insights because both sponge and ctenophore fossils are difficult to obtain and read accurately. Furthermore, comparing physical characteristics of currently living organisms is not directly attributable to homology (Schultz et al., 2023). Previous studies exploring this phylogenetic question have yielded inconclusive results, possibly due to weak phylogenetic signals and inconclusive molecular data. Confounding variables such as “how sequence evolution is modelled, which taxa or sites are included, and the effects of long-branch artifacts and nucleotide compositional variation” have added to this difficulty. By employing a more robust genomic approach, the researchers address the need for definitive evidence that could resolve these questions and enhance our understanding of animal evolution.

### **Original Researchers’ Hypothesis**

The researchers of this paper hypothesize that ctenophores are the sisters to all other animals, supporting the ctenophore sister hypothesis. A pair of gene duplications present among sponges and Parahoxozoa but absent in ctenophores supports the ctenophore sister hypothesis

(Schultz et al., 2023). However, as mentioned before, the previous sequencing methods introduced confounding variables that weaken the evidence for this genomic evolutionary connection. Researchers in this study intend to substantiate this evidence by providing a more rigorous and insightful approach.

### **Substantiating/Corroborating Information**

To support their findings, the researchers of this study employ synteny analyses, phylogenetic analyses, and comparative genomics.

The foundation for the genomic and phylogenetic evidence in this study is grounded in synteny, or chromosome-scale gene linkage. Synteny is the conservation of genes on the same chromosomes across different species, regardless of order (Ghiurcuta et al., 2014; Schultz et al., 2023). By analyzing syntenic relationships, the researchers can infer evolutionary connections that may not be evident from sequence data alone. This method is particularly powerful due to the speed at which chromosomal patterns of gene linkage evolve. The slower evolutionary speed makes them less susceptible to rapid changes that can affect individual gene sequences. The authors argue that this slow evolution of synteny provides a more stable framework for understanding phylogenetic relationships (Schultz et al., 2023).

Using conserved synteny as a phylogenetic character to test the ctenophore-sister versus sponge-sister hypotheses, the researchers constructed evolutionary trees. By identifying shared derived syntenic characters, they could infer evolutionary relationships based on the presence or absence of these syntenic blocks across different species. The analysis focused on identifying synteny groups that are shared among metazoans but not present in unicellular relatives, which helps to establish the evolutionary history of these groups. The study also used Bayesian

phylogenetic analysis, a statistical method that allows for the incorporation of previous knowledge and the estimation of probabilities for different phylogenetic trees. Bayesian analyses was run on both constrained and unconstrained tree topologies. The constrained analysis tested specific hypotheses about the relationships among taxa, while the unconstrained analysis allowed for an exploratory approach to determine the best-fitting tree based on the data. The results of the Bayesian analyses provided posterior probabilities for the different phylogenetic relationships. Higher probabilities supporting the grouping of all other animals (including sponges) and exclusion of ctenophores were found (Schultz et al., 2023). This finding is significant as it provides strong evidence against the sponge sister hypothesis and for the ctenophore sister hypothesis.

The study involved a comparative genomic analysis of multiple species across different animal phyla, including ctenophores, sponges, and other metazoans. By examining the genomes of these organisms, the authors identified conserved gene families and syntenic blocks that support the proposed phylogenetic relationships. The comparative approach allows researchers to highlight shared genetic traits that may indicate common ancestry, as well as unique adaptations that have arisen in specific lineages. The comparative genomics approach involved analyzing genomic data from multiple species, including ctenophores, sponges, and other metazoans. The authors utilized publicly available genomic data and sequencing reads from various repositories, allowing for a comprehensive comparison. This integration of genomic data from diverse species enabled the researchers to identify patterns of synteny and gene conservation that are critical for understanding evolutionary relationships.

## **Validity of Methods and Results**

The methods employed by the researchers to explore the sponge-ctenophore phylogeny to all other animals are robust and valid. Particularly, the use of synteny as the foundational phylogenetic character proves to be a stable and insightful benchmark for evolutionary relatedness. Furthermore, the integration of computational tools and modeling to corroborate the evidence supporting the ctenophore sister hypothesis adds value to the validity of the results. Ultimately, the researchers provided a rigorous and insightful approach to support the ctenophore sister hypothesis that previous research fell short of.

## **Drawbacks and Oversights**

While this study provides strong evidence for the ctenophore sister hypothesis, there are potential drawbacks and oversights that the researchers may have missed. While genetics is the foundation of evolutionary relationships, environmental and ecological factors can also drastically impact the trajectories of evolutionary lineages. Understanding how ecological niches and environmental pressures have influenced evolution could provide additional context to the findings.

## References

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