

***Licnophora* (Spirotrichea, Licnophoria): a hypotrich-like spirotrich**

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The ciliate *Licnophora* was classified for some years as a heterotrich ciliate, related to *Stentor* because of its body shape. However, research confirmed the presence of a replication band in the macronucleus, placing it in the spirotrich clade. Small subunit rRNA gene sequences confirmed its affiliation with the spirotrichs but at a basal position in the clade. Is this basal position a true reflection of its phylogenetic affinities or is it placed there due to a long branch attraction artefact that is a result of its unusual symbiotic relationship with a variety of invertebrates?

To test these alternatives, we have undertaken a genomic and transcriptomic study of *Licnophora macfarlandi*, a symbiont from the respiratory trees of the sea cucumber *Polystichopus*. Ciliates were collected from the respiratory tree fluid, briefly washed in filtered sea water, and collected in ethanol for DNA extraction or transferred to Triton X-RNaseOut lysis buffer and then immediately frozen at -20C. The transcriptomes of 4 single cells and the genome amplified from the DNA of 20-30 cells were assembled and analyzed.

Using BLASTX on polyA mRNA or genome sequences, the codons (UAA/UAG > Q, UGA > STOP) tended to give more significant hits than the codon assignments for *Euplotes* or for canonical codon assignments. Again using BLASTX, a selection of polyA transcripts was compared to an annotated database of proteins from *Oxytricha trifallax*. Of 40 transcripts that map to the ends of 23 *Oxytricha* proteins, 38/41 unambiguously had UGA as the stop codon. We conclude that *Licnophora* uses the hypotrich codon assignments.

Next, we did a BLAST search of *Licnophora* genes against a ciliate database of 8 hypotrichs, 2 *Euplotes* spp., all *Euplotes* proteins available from NCBI, 3 oligohymenophorean species, *Stentor*, *Favella*, and *Chilodonella*. Of the top 3 hits for 390 sequences, 79.7% are to hypotrichs, 3.9% are to *Euplotes* 9.5% are to Oligohymenophoreans, 4.9% are to *Stentor*, 2.0% are to *Favella*, and 0% are to *Chilodonella*.

Based on these data, we predict that our phylogenomic analyses of the position of *Licnophora* will place it close to the hypotrichs and separate from oligotrichs and euplotians.

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