



Phylogenetic analyses of Ophiothrix (Echinodermata: Ophiuroidea) [↗](#)

Version 3

PLOS One

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ABSTRACT

Using 16S and COI fragments in Bayesian Inference, Maximum Parsimony, and Maximum Likelihood.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

Working

Align Sequences

- 1 All 16S and COI sequences were aligned with the MUSCLE algorithm, providing a unified matrix. All the sequences from *Ophiothrix angulata* (16S) and additional sequences from others Ophiotrichidae (16S, COI) available on GenBank were added to the matrix for comparison. *Amphipholis squamata* (Amphiuridae) was used as outgroup.

Datasets

- 2 Three datasets were considered: i) 16S, ii) COI and iii) concatenated (COI+16S). Phylogenetic reconstructions were made on the basis of two different optimality criteria, Maximum Parsimony (MP) and Bayesian Inference (BI), to assess whether there were any differences in the trees recovered with regard to the method used.

Maximum Parsimony (MP)

- 3 The MP was performed with the TNT 1.5 program. Most parsimonious trees were obtained by a heuristic search (best length was hit 100 times), using the new technology search option, which included sectorial searches, ratchet, tree drifting and tree fusing. The gaps were considered as fifth state. The cladograms had their nodes evaluated by the *Bootstrap* resampling test, based on 1000 pseudoreplicates using the *Traditional Search*.

Bayesian Inference

- 4 For BI, the best-fitting model of molecular evolution was GTR+G, chosen based on the AIC and Hierarchical Likelihood Ratio Tests according to the estimation by MR.MODELTEST v.2.3. The BI analyses used one cold and three incrementally heated Monte Carlo Markov chains (MCMC) on two simultaneous runs. The standard deviation of the split frequencies between the two runs reached a value lower than c. 0.005 at two million generations, with one tree sampled every 100th generation, each using a random tree as a starting point and a temperature parameter value of 0.2 (the default in MrBayes). The first 25% of the total sampled trees were discarded as 'burnin' to achieve the MCMC log-likelihoods that had become stationary and converged. The analyses resulted in similar likelihood scores, with ESS > 200, as verified using TRACER.

Maximum Likelihood (ML)

- 5 The ML was performed with the MEGA v.7.0 program using the Kimura 2-parameter model. The statistical support was obtained with a bootstrap function using 1000 replicates.

Edition of phylogenetic trees

- 6 The phylogenetic trees were visualized and edited in FIGTREE v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).



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