Towards a molecular phylogeny of the fish parasitic family Cymothoidae Leach, 1818 (Cymothooidea: Isopoda): Cymothoids from Moreton Bay, Australia and the South Coast of South Africa as a case study.

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Introduction

Representatives of the isopod family Cymothoidae are diverse, remarkably large fish parasites that are known to infect the skin, mouth, and gills of their marine, brackish, and freshwater hosts. To date, evolutionary biologists have been intrigued by this family, and have sought to understand how this family of parasites has evolved. Because one of the more obvious ways to categorise these taxa is by their attachment location, most molecular phylogeny papers seek to understand their phylogeny with respect to attachment site. Interestingly, these papers have not reached a consensus on the evolution of attachment. It is most likely that the disparate phylogenetic results for the family is related to the use of genetic data that have been extracted from specimens which have not been correctly morphologically identified.

To work towards a more complete, accurate, and reproducible molecular phylogeny for this family, the aim of this paper was to use cymothoids from Moreton Bay (Fig. 1), Australia, and the southern coast of South Africa as a case study to provide best practice guidelines for generating a morphological and molecular cymothoid dataset for future phylogenetic work. As morphological and molecular phylogenies of the Cymothoidae have been documented to contradict each other, we seek to specifically discuss which aspects of morphological and molecular taxonomic data should be paired for the most accurate results.

Materials & Methods

To achieve this aim, sequences of the partial mitochondrial genes cytochrome c oxidase subunit 1 (COI) and 16S, and the partial ribosomal gene 18S were generated for 25 cymothoid taxa. Based on the criteria formulated in the present study (Table 1), only 40 cymothoid sequences from Genbank (out of 407) were included in the phylogenetic analyses. The newly generated sequences were aligned, using Aegidae and Corallanidae representatives as outgroups. Three alignments were created: COI (43 taxa, 678 bp long), 16S (30 taxa, 500 bp long), and 18S (14 taxa, 1521 bp long). Phylogenetic analyses were run under Bayesian and Maximum Likelihood inference, generating trees for each alignment.

**Table 1.** Sequence criteria applied in the phylogenetic analyses of the present study.

| Sequence Criteria	Criteria applied
Associated or not with peer-reviewed publication	Associated
Taxonomic level	Species
COI	
Sequence length	Minimum of 550 base pairs
Genetic code	Invertebrate Mitochondrial
Presence or absence of ambiguities	Ambiguities must be absent
Presence or absence of stop codons the sequence	Stop codons must be absent
Gaps in the alignment	Gaps must be absent in the alignment
Presence or absence of stop codons in alignment	Stop codons must be absent in alignment
16S	
Sequence length	Minimum of 300 base pairs
18S	
Sequence length	Minimum of 1000 base pairs

Fig. 1. Cymothoid species from Moreton Bay, Australia, sequenced as part of the present study. **A**. *Ceratothoa banksii*, **B**. *Ceratothoa imbricata*, **C**. *Cymothoa indica*, **D**. *Cymothoa vicina*, **E**. *Elthusa sigani*, **F**. *Mothocya karobran*, **G**. *Mothocya halei*, **H**. *Nerocila monodi*. Photos not to scale.

Results & Discussion

Phylogenetic relationships for COI (Fig. 2) and 16S (Fig. 3) showed a pattern congruent with the morphological classification of the genera *Anilocra*, *Ceratothoa*, *Cinusa*, *Cymothoa*, *Mothocya*, and *Nerocila*, in which congeneric species clustered together in supported clades. However, species of *Elthusa* did not cluster together in the COI analyses, which corroborates morphological evidence that this genus should be split into at least two. At present, there are only a few ribosomal gene sequences for cymothoids. Based on the available data, the 18S tree (Fig. 4) depicted two main clades: one clustering together two freshwater species from Brazil, and the other uniting 12 marine species. This is the first study to compare the phylogenetic relationships of cymothoid species using the most current taxonomic classifications available, as well as the first to include 18S sequences for seven species of cymothoids. This research provides guidance for moving forward towards good practice in morphological and molecular studies on cymothoids.
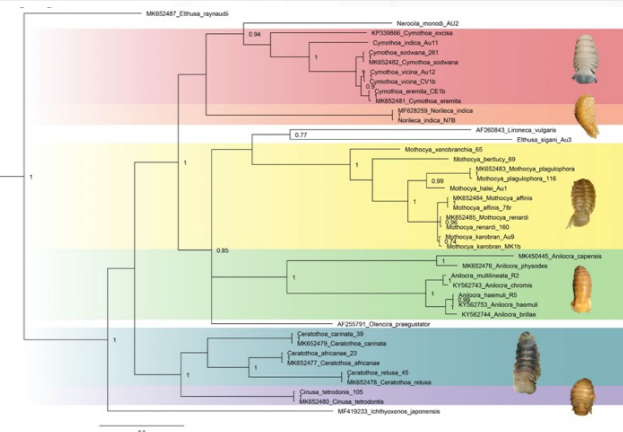
Fig. 2. Bayesian Inference phylogram based on partial COI sequences of cymothoid isopods.
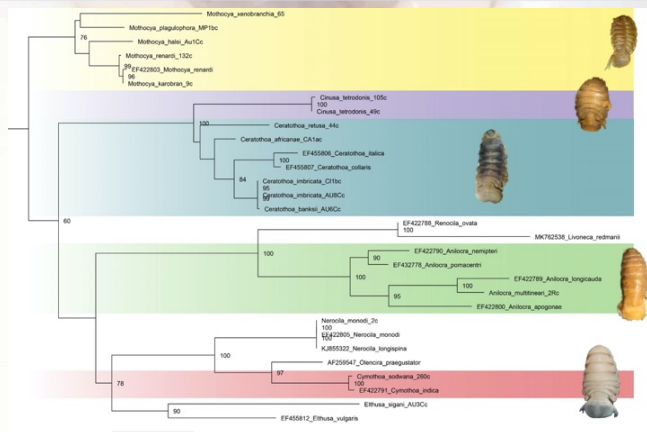
Fig. 3. Maximum likelihood phylogram based on partial sequences of 16S of cymothoid isopods.
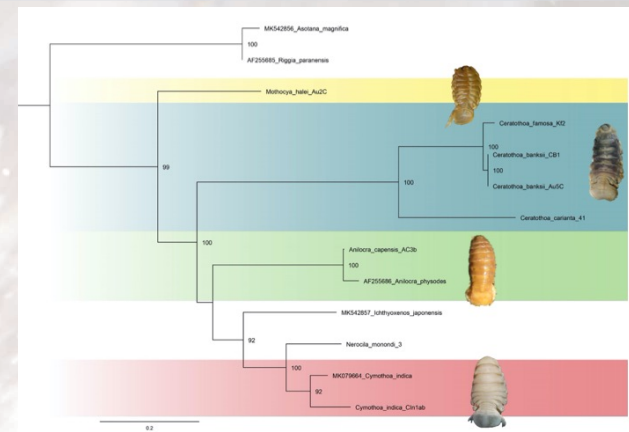
Fig. 4. Maximum likelihood phylogram based on partial sequences of 18S of cymothoid isopods.

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