

# DNA barcoding of oomycetes with the Cytochrome Oxidase I (COI) gene

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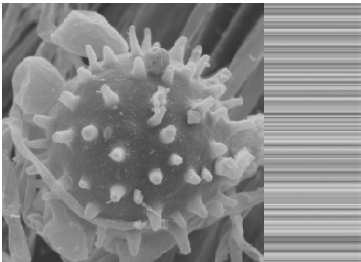
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## Introduction

Oomycetes, which include water moulds and downy mildews, are terrestrial and aquatic fungi-like organisms belonging to the kingdom Stramenopila. They include important plant pathogens such as the causal organisms of the potato famine in Ireland (*Phytophthora infestans*), and Sudden Oak Death Syndrome (*Phytophthora ramorum*) devastating California. This group also includes fish pathogens (*Saprolegnia parasitica*) and mammalian pathogens (*Pythium insidiosum*), as well as biological control agents such as *Lagenidium giganteum*, which infects the larvae of mosquitoes, or the mycoparasite *Pythium oligandrum*. Oomycetes are commonly found in fresh water, oceans, soil, and can also be airborne. Our research to date has focussed on the genera *Pythium* and *Phytophthora*. The current taxonomic groupings of *Pythium* and *Phytophthora* are supplemented significantly by molecular data, specifically from nuclear DNA sequences of the ITS (Internal Transcribed Spacer) and LSU (Large Subunit) regions of rDNA (Lévesque and de Cock 2004). While analyses of these genes and spacers have produced phylogenetic relationships that are generally consistent with morphological species concepts, there are still potential weaknesses in these genes in terms of their usefulness for identification. For example, some species of *Pythium* share the same ITS sequence. The use of a different gene that may provide resolution to species-level identification is therefore of great interest, especially considering that in *Pythium*, identification to the species level is crucial in determining the ecological role of the organism. Our research to date has focussed on the validity of COI barcode sequencing for oomycete species delimitation.



## Materials and Methods

PCR amplification of COI was performed using primers designed to amplify the barcode region of all oomycetes. The forward primer designed was 5'-TCAWCWGMGATGGCTTTTTTCAAC-3'. The primary reverse primer used was 5'-RRHWACKTGACTDATRATACCAAA-3' which was modified from Fm85 (Martin and Tooley 2003). In a few cases an internal reverse, 5'-CYTCHGGRTGWCCRAAAAACCAAA-3', was used when the primary reverse did not yield a good PCR product. Sequencing of PCR products was performed with an ABI Prism® 3130XL.

## Results and Discussion

COI barcode sequences were easily obtained using the primers designed. The barcode region of the oomycetes sequenced did not contain any introns, and in the vast majority of samples, a 727bp fragment was sequenced as opposed to some samples for which a 680bp fragment was obtained with the internal reverse primer. The sequences of the barcode region were highly variable, with many species showing intraspecific variation in COI. The barcode region still exhibits ability in species identification however, because conspecific barcode sequences clustered together, as seen in Figure 1. The phylogenetic relationships created with the barcode region did not match the previously established ones that matched morphological characters in the genus *Pythium* (Lévesque and de Cock 2004), but this was not an unexpected result, as short DNA regions do not generally provide enough variable characters to produce accurate phylogenies. Resolution of *Pythium* species boundary ambiguities that ITS cannot discriminate was not achieved with COI. In cases where two *Pythium* species shared identical or similar (≥95%) ITS sequences, they also had similar or identical COI sequences. In many cases, the classification of an ambiguous pair as separate species was based on subtle and questionable morphological differences. It is possible that with further study and multi-gene analysis, these species with identical barcodes may be conspecific.

## Conclusions

Although COI relationships are not congruent with existing phylogenies, our results have provided unique COI sequences for most species of *Pythium* and *Phytophthora* sequenced so far. We have also discovered intraspecific variation of COI sequences in some species studied, including the recently defined species of *Pythium attrantheridium*. Although intraspecific variation exists in COI, the clustering of conspecific COI sequences is reliable for identification. Future investigation will be made into species complexes such as *Pythium irregulare*, and the ability of COI to discriminate among members of such complexes.

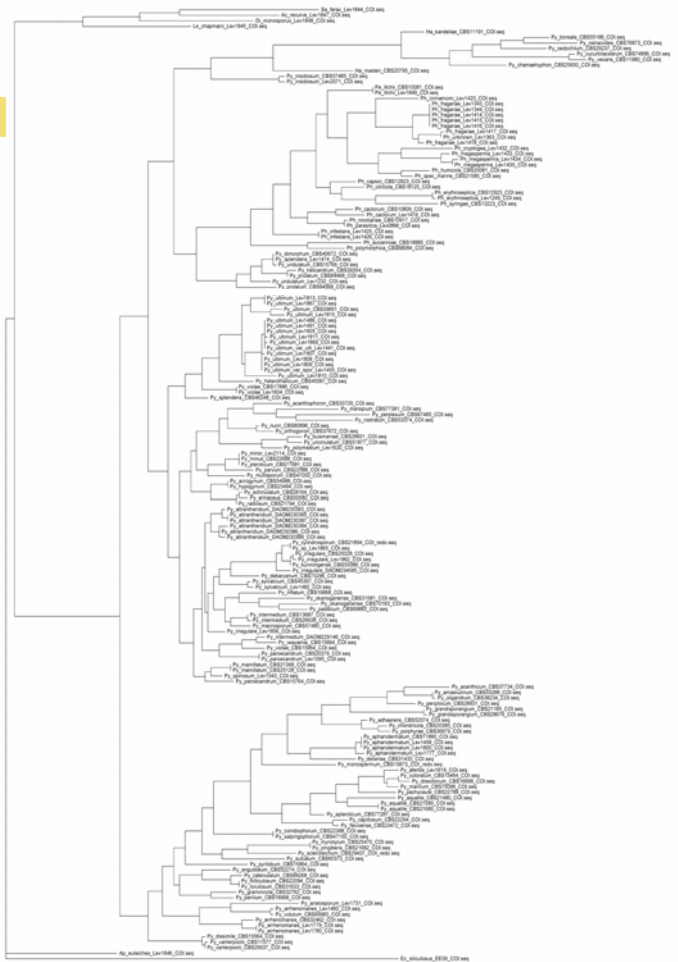


Figure 1. Phylogenetic tree of oomycetes based on a 679bp fragment of COI. A heuristic search was performed on 181 representative sequences out of 700 available using parsimony analysis. A brown algae was used as outgroup. Genus abbreviations: Ac = *Achlya*, Di = *Dictyuchus*, Ha = *Halophytophthora*, Pe = *Peronophythora*, Py = *Pythium*, Ap = *Aphanomyces*, Ec = *Ectocarpus*, Le = *Leptolegnia*, Ph = *Phytophthora*, Sa = *Saprolegnia*

## Acknowledgements

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## References

Lévesque, C. A., and A. W. de Cock. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycological Research* 108:1363-1383.  
 Martin, F.N., and P.W. Tooley. 2003. Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia* 95 (2):269-284.