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A Preliminary Phylogeny of Rhyacophilidae with Reference to *Fansipangana* and the Monophyly of *Rhyacophila*

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Abstract

The phylogeny of Rhyacophilidae was explored with 28S ribosomal RNA (rRNA) and *Cytochrome Oxidase Subunit I* (COI) mitochondrial DNA (mtDNA). Eighty one rhyacophilids were included in the analysis. We found that although Rhyacophilidae was recovered as monophyletic, intrafamilial relationships are not well-resolved using this dataset. Bootstrap support was poor for intrageneric relationships and additional data will be required to present a more robust hypothesis. The recovered phylogeny places *Fansipangana* as the sister taxon of the rest of Rhyacophilidae. We found that *Himalopsyche* was nested inside the genus *Rhyacophila* with the *verrula* group sister to *Himalopsyche* and remaining *Rhyacophila*. These results and possible relationships should be tested with a more extensive data set.

Key words: *Rhyacophila, Fansipangana, Himalopsyche,* phylogeny, Trichoptera, rRNA, mtDNA, barcodes

Introduction

Rhyacophilidae is primarily Holarctic, consisting of only 4 extant genera. With 479 known species, *Rhyacophila* is the largest genus in the family, and was only recently surpassed as the largest genus within Trichoptera by *Chimarra* (Philopotamidae). The other three genera are much smaller: *Himalopsyche*, which includes 53 species, and two monotypic genera, *Philocrena*, and *Fansipangana.* We did not sample *Philocrena*, but were able to sequence a fragment of DNA from *Fansipangana*, from which only four specimens exist in collections. *Fansipangana vernalis* Mey, 1996 was recently described (Mey, 1996) from the Fan Si Pan mountain range in North Vietnam.

As we move toward very large datasets from genomes and transcriptomes (e.g. Trautwein *et al.* 2012; Misof *et al.* 2014), we are interested in whether smaller datasets from Sanger sequencing can be useful in providing phylogenies from a larger number of taxa. Because Kjer *et al.* (2014) generated a reasonable phylogeny of *Chimarra*, using nuclear rRNA and a fragment of cytochrome oxidase c, subunit I (COI: the barcode fragment) we decided to explore the phylogeny of *Rhyacophila*, Trichoptera's second largest genus, with the same approach. This approach combines "deep genes" found in ribosomal RNA, and the faster-changing genes, found in COI.

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Methods and Materials

Approximately 1000 nucleotides of 28S RNA, spanning the D1-D3 hypervariable regions, were chosen for the analysis of rRNA. This region should be the most phylogenetically informative because it is one of the fastest-changing regions of relatively slow-evolving rRNA. Because this is a relatively long fragment of about 1000 nucleotides, some of the lower-quality template DNA had to be sequenced in pieces. If the D1-D3 primers failed (D1-3F CGAGTAGCGGCGAGCGAAACGGGA and D1-3shortR CGTGYRCGCTCTCAGTGCGT), then a D2 primer (GAGTTCAAGAGTACGTGAAACCG) was combined with the D1-3 shortR primer. At times, only the D2 region could be recovered. Sequences will be made available upon publication of a publication in preparation that includes morphological data by J. Giersch.

Standard PCR methods were used: the annealing temperature was 55º C and the extension time was 2 minutes. Successfully amplified reactions were sent to the GeneWiz (Piscataway, NJ) for sequencing. Sequences were viewed and manually edited in the plasmid editing program, ApE. Editing DNA involved removing illegible or poor DNA reads with either high background or irregular spacing and checking DNA sequences with their complement for verification. Sequences were then checked for contamination with a preliminary phylogenetic analysis. If two different species of *Rhyacophila* were identical, contamination was strongly suspected, and any such sequences were marked for re-sequencing. Following this, sequences were converted to FASTa format and a preliminary alignment was created in the program SeaView (Gouy *et al.* 2010). Alignment of COI was unambiguous, with no length variation. Ribosomal data were aligned manually in MS Word, as described in Kjer *et al.* (2009). Aligned data were used to create a phylogenetic tree, using the GTR+ CAT model, with RaxML (Stamatakis 2014).

Results and Discussion

The combined data tree exhibits high bootstrap support on a few deep nodes, but very poor support throughout most of the backbone (Fig. 1). Both Hydrobiosidae and Rhyacophilidae are monophyletic. The Hydrobiosidae are strongly divided into their two subfamilies, Apsilochoreminae and the larger Hydrobiosinae. The *Fanispangana* specimens we received for sequencing were single legs, and an abdomen in very poor condition for DNA work after storage in 75% ethanol for over 10 years. Over 100 PCR reactions were performed, and two short fragments of rRNA were amplified and sequenced. These two short fragments were sufficient to place *Fansipangana* as the sister taxon of both *Himalopsyche* and *Rhyacophila* with moderate support. *Himalopsyche* are weakly grouped as monophyletic, as are all *Rhyacophila*, except for *R. verrula* Milne, 1936 and *R. rickeri* Ross, 1956, which are placed as the sister to *Himalopsyche*, and the rest of *Rhyacophila*. Despite low bootstrap values for intragenic relationships throughout most of the tree, the placement of *Fansipangana*, and the non-monophyly of *Rhyacophila*, due to the *verrula*-group, provides an interesting hypothesis, worthy of additional data. Our study shows that the two gene approach proposed by Kjer *et al.* (2014) is not a one-size-fits-all approach to resolve phylogenetic relationship in caddisflies at the genus or species level. This was already clearly stated by the authors in their paper. Kjer *et al.* (2014) concluded that using mtCOI and data from the rRNA can produce far from perfect but reasonable and useful phylogenies. The approach worked well in *Chimarra* when compared with a three gene molecular phylogeny of *Chimarra* (Wahlberg & Johanson 2014). Both studies show that despite the decreasing costs and increasing ease in generating and accessing phylogenomic data, integrative taxonomy and phylogenetics will continue to benefit from less expensive approaches that are applicable to many individuals. However, while the phylogeny presented here on Rhyacophillidae provides us with useful hypotheses to be pursued with more data, the two gene approach is clearly insufficient to actually resolve the phylogeny on its own. Caution should thus be used when planning, producing, and especially interpreting a two gene phylogeny in caddisflies.

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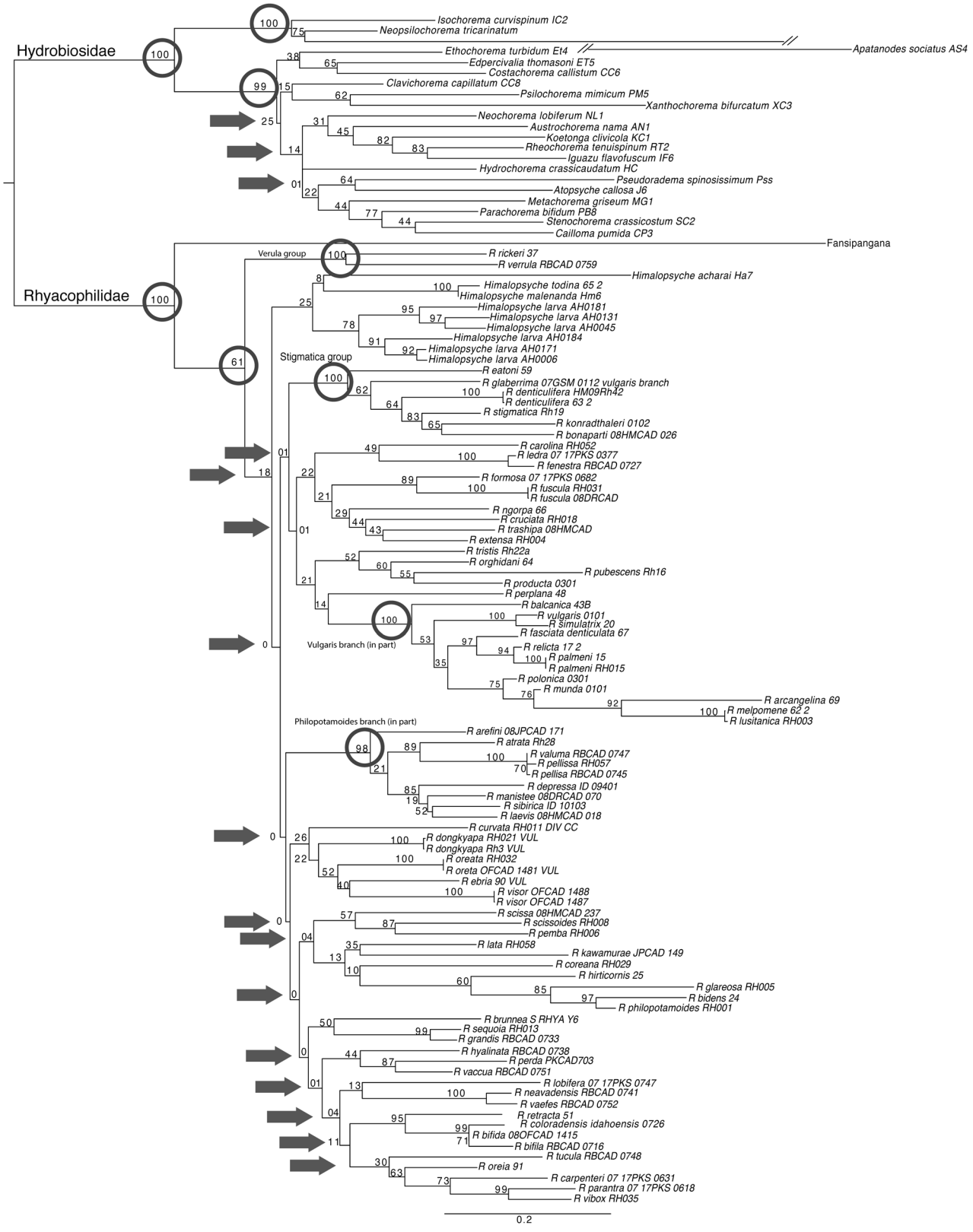

FIGURE 1. RaxML generated phylogeny from the combined data. Arrows draw attention to weakly supported nodes, which should be collapsed. Circled nodes are discussed in the text. Numerals at the internodes are bootstrap values.

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