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Phylogenetics of a small caddisfly genus (Thremmatidae: *Oligophlebodes*): comparison among multiple hypotheses from DNA barcode data

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Abstract

Barcoding datasets can serve as a resource for species associations and delineations, but single-gene trees estimated by distance methods do not provide strong estimates of phylogeny. Using DNA data from the Barcode of Life Database (BOLD), we calculate a phylogenetic tree for *Oligophlebodes* (Trichoptera: Thremmatidae), a small genus of caddisfly endemic to the Western United States. Here we estimate a preliminary phylogeny for Oligophlebodes using Bayesian likelihood, and compare it to trees produced by distance and standard likelihood methods. Using the barcode region of the cytochrome oxidase 1 (COI) gene, we analyzed 44 individuals representing five species (and 2 unknowns) and a sister-genus outgroup (*Neophylax*) from locations ranging from Southern New Mexico northwest into British Columbia. Partitioned Bayesian likelihood analysis under the F81 (1st codon positions) and HKY80 + I + Γ (for both the 2nd and 3rd codon positions) model gave the consensus topology (*Neophylax toshi*, (*O. sierra*, (*O. ruthae* inc. spc. 1 & 2, *O. sigma,* (spc. 3 & 4, (*O. ardis, O. minutus*)))). Species identifications were supported by monophyly of most species-level taxa. However, confirmation of species identifications of unknowns was complicated by incomplete taxon sampling for spc. 1 & 2. Placement of spc. 3 & 4 may serve as support for taxonomic review of *O. minutus*. Compared with an existing published phylogeny of *Oligophlebodes* BOLD sequences constructed under RAxML, the Bayesian hypothesis had higher resolution at the basal node of *Oligophlebodes*. Because of their support values, both likelihood trees are recommended over the BOLD TaxonID tree (an unrooted neighbor joining tree using the Kimura 2-parameter model). The novel topology produced in the Bayesian tree supports further explorations by likelihood-based methods, including partitioned analyses, of our preliminary *Oligophlebodes* dataset that can be used as additional lines of evidence to support morphological work.

Keywords: phylogeny, BOLD, Bayesian analysis, cytochrome oxidase 1, RAxML

Introduction

DNA barcoding (Hebert *et al.* 2003a) is an important and increasingly popular set of methods for species-level identification and has grown to incorporate genomic and environmental DNA sequencing techniques (Leray & Knowlton 2015). Barcoding uses particular reference genes such as Cytochrome Oxidase I (COI) and the Internal Transcribed Spacer gene (ITS; Irinyi *et al.* 2015) to assign unknown specimens to the correct genus and species, by comparing them to a database of homologous genes from close relatives, and produces a tree grouping species based on genetic distance. Barcoding also identifies candidate new species, which occurs when sequences of specimens are unusually divergent from their nearest sequenced relatives. 'Long

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branches' are often defined as having a greater than 2% pairwise distance (Johns & Avise 1998; Hebert *et al.* 2003b). One such resource, BOLD (Barcode of Life Database; Ratnasingham & Hebert 2007) allows users to contribute and use barcode sequences, some of which are either available to the public, or may be provided by the owner upon request.

DNA barcodes and available barcode libraries are exceptionally powerful tools for enhancing the associations among males, females, and larvae of a species. For example, the larval stage of Trichoptera is the longest-lived and most ecologically diverse life stage, however few species-level keys to North American larvae exist in the literature (Ruiter *et al.* 2013). Larval-adult associations are sometimes neglected because difficulty using the metamorphotype technique of Wiggins (1996). DNA barcodes have been successful in associating as many as 62 larval-adult caddisfly pairs from Manitoba, Canada that were used to develop larval keys (Ruiter *et al.* 2013) and much success has occurred worldwide using the barcode region along with other genes to associate larvae to adults (e.g, Zhou *et al.* 2007; Waringer *et al.* 2018). Barcode analyses also reveal cryptic species diversity and facilitate female species associations (Pauls *et al.* 2010; Giersch *et al.* 2015; Flint & Kjer 2011). For the insect order Trichoptera, BOLD includes over 45,000 barcode sequenced specimens from 5,847 species as of this writing (http://www.boldsystems.org accessed, December 10, 2015).

As the number of sequences in BOLD has grown, there is an increased ability to ask questions of a nature broader than species associations. However, the default barcode analysis procedures in BOLD are largely distance methods, which (although fast and computationally simple) have long been known to be potentially misleading for phylogenetic work (Hasegawa *et al.* 1991). Although the BOLD project managers are explicit about these methods being not designed for phylogenetic studies (BOLD database FAQ, accessed 12/21/2015, http://v3.boldsystems.org/index.php/resources/boldfaq?chapter=2_BolduserQuestions.html&section=q5), simple, more phylogenetically-robust analysis options should be considered for gathering phylogenetic information from barcoding datasets, especially if they are to be used for speculating cryptic species. Zhou *et al.* (2016) explicitly used Trichoptera as a model taxon for use of COI barcodes to fill out relationships between the "leaves" (terminal taxa) on the tree of life, and we continue in that vein.

Here we expand upon the effort of Zhou *et al.* (2016) under a different phylogenetic model of the "twigs" and the "leaves" representing the genus *Oligophlebodes,* a small genus of caddisflies. Using multi-model Bayesian analysis of COI barcode data from this group, we generate a DNA barcode phylogeny that will assist in partitioning species-level relationships within the genus. This model allows for making preliminary determinations of unidentified specimens within BOLD and to highlight potential new species in light of phylogeny, to be examined in further detail through future formal morphological species description.

Methods

Study group

Oligophlebodes Ulmer, 1905 (Trichoptera: Thremmatidae) is a genus of caddisflies endemic to the montane areas of Western North America (Southern New Mexico Northwest into British Columbia and Alberta) constrained to high altitude sites in cool fast-flowing streams (Wiggins 1996). In particular *O. sierra* Ross, 1944 and *O. minutus* (Banks, 1897) have large north-south distributions along the Sierra Nevada and Rocky Mountains, respectively. *Oligophlebodes ruthae* Ross, 1944, *O. mostbento* Schmid, 1968, and *O. zelti* Nimmo, 1971 occur in high-altitude sites on either side of the US-Canada border. Other species, such as *O. ardis* Ross, 1941 and *O. sigma* Milne, 1935 have much more constrained distributions.

Oligophlebodes is most closely related to the genus *Neophylax* McLachlan, 1871 (Schmid 1955, Wiggins *et al.* 1985, Holzenthal *et al.* 2007). The taxonomy of *Oligophlebodes* is limited to species descriptions and a key to adults by Ross (1944, 5 species, United States) and Nimmo (1971, 3 species, Alberta and Eastern British Columbia, Canada). The only published phylogeny of *Oligophlebodes* species (6 species + unknowns, 29 exemplars) occurs within the Limnephiloidea supplemental materials (https://github.com/pbfrandsen/trichoptera_barcodes/tree/master/subclades/Limnephiloidea) of the large-scale analysis of Trichopteran phylogeny of Zhou *et al.* (2016).

Taxon and Locus Selection

To examine the phylogenetic relationships among species of *Oligophlebodes*, we used existing BOLD sequences (private and public submission). We sampled 44 specimens of 5 (of the 7) described species in the genus (*O. ruthae*, *O. ardis*, *O. sierra*, *O. sigma*, and *O. minutus*) and 1 outgroup (*Neophylax toshioi* Vineyard & Wiggins, 1987), from southern New Mexico, United States to northern British Columbia, Canada. Unfortunately, sequences were not available for *O. mostbento* or *O. zelti*. We used multiple individuals from each species and included site replicates when available. We used a segment from the DNA barcode region of the cytochrome oxidase I (COI) gene (Hebert *et al.* 2003a), measuring 323–658 base pairs in length (see Appendix for lengths). Sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (3 sequences) and the BOLD database (http://www.boldsystems.org/) (41 sequences) (Ratnasingham & Hebert 2007), to build a matrix with 44 terminal taxa. All specimens, their unique labels (also used in tree diagrams), collection data (where available) and GenBank/BOLD. Accession numbers are included in the Appendix.

Molecular Methods

Alignment and model selection

We aligned sequences by-eye using codons and amino acid translation as references in Geneious v8.1 (Kearse *et al.* 2012). The aligned matrix was subjected to model testing using JMODELTEST 2 (Darriba *et al.* 2012) using the AIC criterion. Based on JMODELTEST outcomes, we chose the F81 model for 1st position codon and the HKY + I + Γ model for both the 2nd and 3rd codon positions in COI. This type of codon-based partitioning and model choice prior to analysis has been shown to significantly improve likelihood scores and bootstrap values for Bayesian analyses of mitochondrial data, as well as accounting for real differences in evolutionary patterns between the three codon positions (Brandley *et al.* 2005).

Phylogenetic analysis

We ran a Bayesian Markov Chain Monte Carlo (MCMC) analysis in MrBayes v3.2.4 (Ronquist & Huelsenbeck 2003) using three partitions (1st, 2nd, and 3rd codon positions, modeled as outlined above) for 1.1 million generations using 4 heated chains, with tree subsampling occurring every 200 generations. After 1.1 million generations the average standard deviation of split frequencies was below the threshold of 0.05, indicating convergence; we discarded the first 100,000 generations as burn-in. A majority-rule consensus tree, with posterior probabilities for each resolved node, was calculated based on the remaining sampled trees. We visualized the consensus tree using the tree-viewing software, FigTree v1.4.2 (Rambaut 2014). This analysis and tree will be referred to as the BAYESTREE for the remainder of this paper.

We examined two trees for comparison to BAYESTREE. We also produced a tree via the TaxonID method set as the default in the BOLD database (hereafter, BOLDTREE). Using the same data matrix as we used for BAYESTREE, we used the BOLD software (Ratnasingham & Hebert 2007) to produce an unrooted neighbor-joining tree under the Kimura 2-parameter model. We also compared trees to a section of the Limnephiloidea tree of Zhou *et al.* (2016), in which we extracted the *Neophylax* + *Oligophlebodes* branches (hereafter, ZHOUTREE), retaining the corresponding topology and branch support (available from https://github.com/pbfrandsen/trichoptera_barcodes/tree/master/subclades/Limnephiloidea). ZHOUTREE (complete with 29 ingroup and 3 outgroup terminals) was constructed by Zhou *et al.* (2016) using maximum likelihood as implemented in RAxML, with 1000 bootstrap replicates used to calculate branch support. The authors used the GTR + Γ maximum likelihood models, with 4 site-specific variation models. The ZHOUTREE contains additional specimens not available to our project: *O. mostbento* from MT and *O. minutus* from AZ.

Results

In BAYESTREE (Fig. 1), rooted with outgroup *Neophylax toshioi*, results were consistent with species taxonomy of all determined adult specimens. Support values for ancestral nodes for each species group, expressed as Bayesian posterior probabilities, are between 0.95 and 1.00, with the node separating *O. ruthae*

and *O. sigma* from the remainder of the taxa lacking statistical support (0.89), effectively making *O. ruthae* + *O. sigma* + (spc. 3 & 4, (*O. ardis*, *O. minutus*)) a tritomy. Within species groups, the lowest support values (0.63, 0.87) also occur within the *O. ruthae* group.

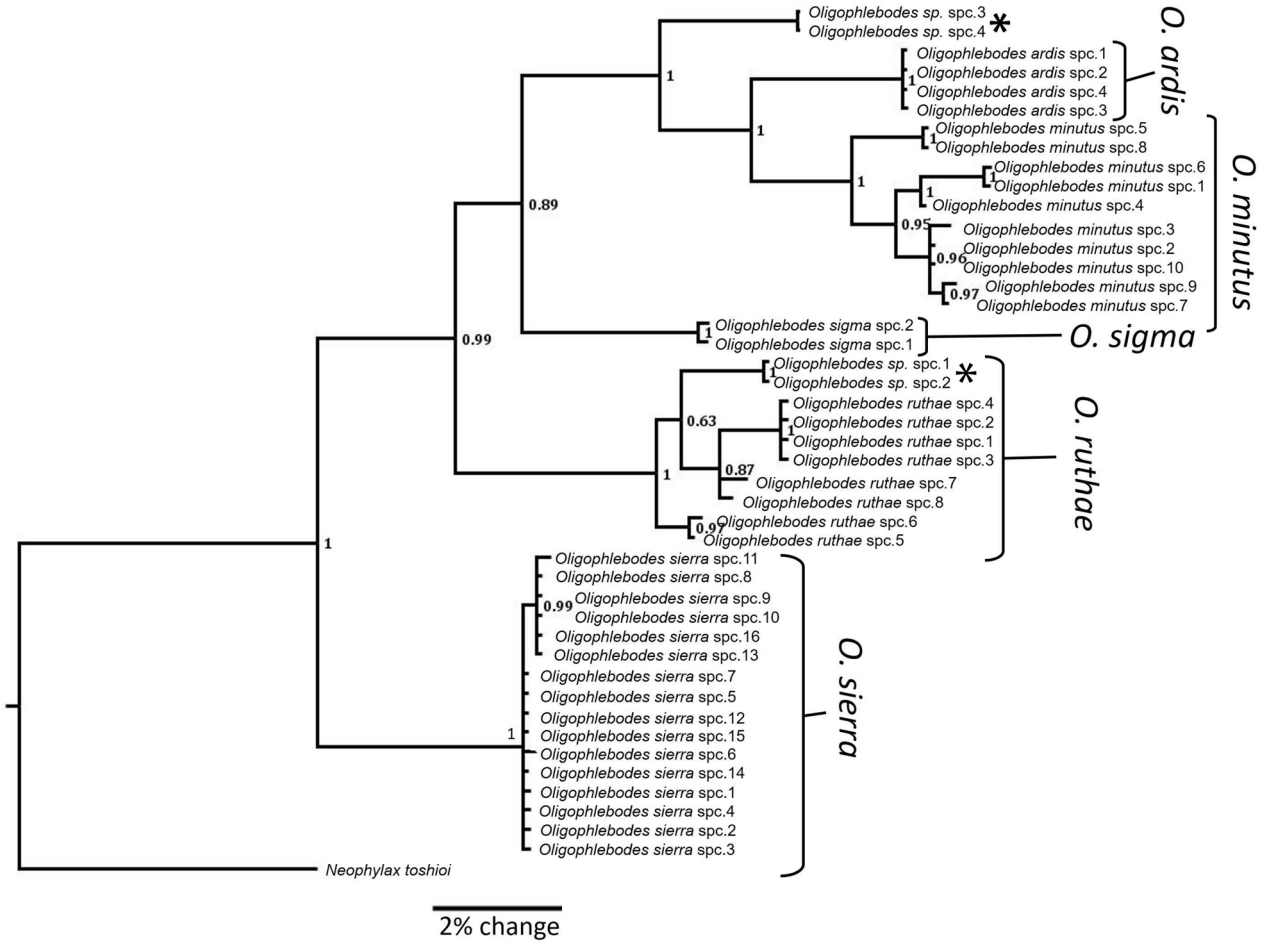

FIGURE 1. Bayesian Likelihood tree using F81 and HKY80 + I + Γ substitution models (BAYESTREE). Majority-rule consensus tree of dataset produced via Bayesian likelihood analysis. Rooted to outgroup *Neophylax toshioi*. Values on nodes represent Bayesian posterior probabilities (1.0 = 100%). Scale bar represents 2% genetic change along a branch. Terminal labels represent species ID's and individual specimen labels; collection information for all specimens is included in Appendix. Bracketed taxa are monophyletic groups including all specimens of single species. Asterisk (*) represents larval exemplars.

The topology of BAYESTREE places *O. sierra* sister to all other ingroup taxa. The next node is a tritomy consisting of a group of *O. ruthae* specimens (including spc. 1 & 2), *O. sigma* specimens, and a resolved clade of the rest of the taxa. This final clade consists of a branch with spc. 3 & 4 (species identified only to genus level in BOLD with a NM distribution) sister to an *O. ardis* + *O. minutus* clade supported by posterior probabilities ≥ 0.95 and ~2% genetic change along branches. Undetermined spc. 1 & 2, both larvae, nest within *O. ruthae*, however some of the lowest support values (0.63, 0.87) occur within the *O. ruthae* group making their within-group relationships uncertain. In contrast, spc. 3 & 4, also larval, do not nest within, or form an unambiguous monophyletic group with any single species in the containing clade, and would make *O. ardis* or *O. minutus* paraphyletic if identified as belonging to either of those groups. The final tree is (*O. sierra*, (*O. ruthae* inc. spc. 1 & 2, *O. sigma,* (spc. 3 & 4 , (*O. ardis, O. minutus*)))).

The unrooted BOLDTREE (Fig. 2) returned the same groups of identified species as monophyletic and undetermined specimens are placed in the same positions as in BAYESTREE (spp. 1 & 2 within *O. ruthae*; spc. 3 & 4 sister to *O. ardis* + *O. minutus*). However, this unrooted tree of the same ingroup taxa does indicate

different relationships among the species-level clades, albeit with no measures of support. The most notable difference in topology is the position of the *O. sigma* clade resolved as sister to *O. ruthae*. The final tree rotated to be similar to other trees is (*O. sierra*, ((*O. ruthae* inc spp. 1 & 2, *O. sigma*), (spc. 3 & 4, (*O. ardis, O. minutus*)))).

In the ZHOUTREE, *Oligophlebodes* is published as resolved sister to *Neophylax*. The final tree, where bootstrap values ≥ 50, is (*O. sigma*, *O. sierra,* (*O. ruthae* & *O. mostbento*), ((*O. minutus,* sp._1 & sp._2), (*O. ardis, O. minutus*))). The single exemplar of *O. mostbento* is nested within two clades of *O. ruthae*. BOLD identifiers for sp._1 & sp._2 of ZHOUTREE = spc. 3 & 4 of BAYESTREE/BOLDTREE. BOLD sequences for *O. minutus* that grouped with spp. 3 + 4 were not included in the BAYESTREE or BOLDTREE.

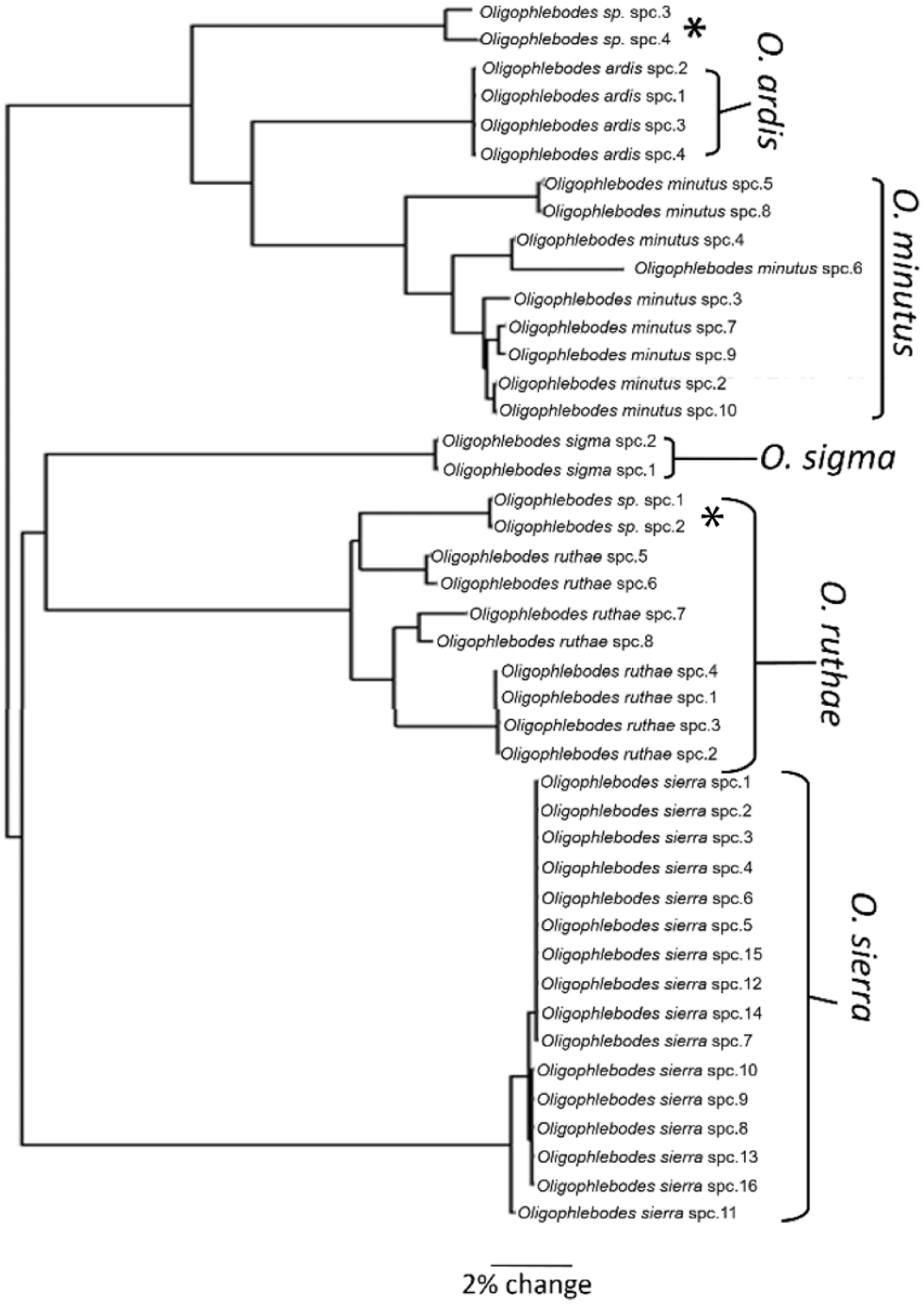

FIGURE 2. Taxon ID tree for *Oligophlebodes* (BOLDTREE), an unrooted neighbor-joining tree using the Kimura 2-parameter model generated by BOLD. All conventions as in Fig. 1.

Discussion

We recognize that single-gene trees reflect the history of the gene studied (Maddison 1997). They may not reflect the organismal phylogeny due to the long-recognized possibility of incomplete lineage sorting (Avise *et al.* 1983) and may have limited resolution. Moreover, with just one gene, there is no independent way to evaluate the gene tree's congruence with the species phylogeny. Despite these limitations, we find value in comparing the outcome of this analysis to the BOLD distance tree, or rapid ML algorithms (e.g. RAxML; Stamatakis 2006) to determine whether the Bayesian method provides any added value, in terms of changing the tree topology or improving support values, relative to the trees produced by those methods, and in comparison to the broader Zhou *et al.* (2016) analysis.

Oligophlebodes species definitions and phylogenetic relationships

BAYESTREE recovered the 5 sampled species (of seven described) of *Oligophlebodes* each on a monophyletic branch of the phylogeny. *Oligophlebodes sierra* from its northern Rocky Mountain distribution exhibited the lowest level of genetic differentiation between localities apparent by the extremely short branch lengths, however, exemplars from the Sierra Nevada were not available. *Oligophlebodes sigma* and *O. ardis* were each on well-supported branches. *Oligophlebodes ruthae* formed a well-supported clade, including spc. 1 & 2; however, no exemplars of *O. mostbento* or *O. zelti* were included in this analysis. *Oligophlebodes mostbento* and *O. zelti* are expected to place sister to *O. ruthae* based on similarity in morphological characters and do indeed place together in ZHOUTREE (Zhou *et al.* 2016), which similarly does not provide positive confirmation for spc. 1 & 2 to as *O. ruthae* or *O. mostbento*. *Oligophlebodes ruthae* is morphologically distinct and adult males can be identified using two resources with keys and illustrations: Ross (1944) and Nimmo (1971). However, despite these keys, males of *O. mostbento* and *O. zelti* are often difficult to confidently separate.

Problematic among these species is *O. minutus,* which has an extensive range from AZ to AK and morphological variation along the entirety of its north-south distribution. Undetermined spc. 3 & 4 likely are an undescribed species, occurring outside of *O. ardis* + *O. minutus* in BAYESTREE. These same BOLD sequences form a clade with part of the *O. minutus* in ZHOUTREE again suggesting that *O. minutus* may be paraphyletic. Moreover, in BAYESTREE a > 2% BP pairwise divergence occurs between the branch with spc. 3 & 4 and the other *O. minutus* + *O. ardis*. A 2% BP pairwise divergence is a reasonable cutoff between species, and works for many Trichoptera species, but a minority do show considerably greater intraspecific levels of variation (Zhou *et al.* 2011). Closer morphological examination of individuals from those localities paired with formal morphological comparisons is a logical next-step, following the model of Flint and Kjer (2011).

As a result of the placement of undetermined species outside of recognized species groups (in the case of spc. 3 & 4) and within a species group missing exemplars of closely related species, using this approach for positive species associations was problematic. Unless larval-male pairs or female-male pairs nest completely within recognized species without ambiguity, it is difficult to confidently make these associations without a more complete analysis of the haplotypes and increased taxon sampling. That said, there are many examples in the Trichoptera literature where larval-adult DNA associations have led to larval descriptions to pair with described adults (e.g. Zhou *et al.* 2007, Waringer *et al.* 2018).

More uncertain are the relationships among the long-recognized species. In BAYESTREE, *O. sierra* resolved basal to all other *Oligophlebodes*, respectively. Weak posterior probability support at one node (Fig. 1, p = 0.89) in the BAYESTREE resulted in collapsing *O. sigma*, *O. ruthae* and (spc. 3 & 4, (*O. ardis, O. minutus*)) into a tritomy. Collapsing support values below 50% bootstrap in the ZHOUTREE shows total lack of resolution between the four basal clades (*O. sigma, O. sierra*, (*O. ruthae, O. mostbento*), ((*O. minutus,* (sp._1, sp._2)), (*O. ardis, O. minutus*))). BOLDTREE, a result of neighbor-joining, and without support values, does not provide insight to resolve this issue. Similar to the pattern seen in caddisflies (Kjer *et al.* 2014) and gelechioid moths (Kaila & Ståhls 2006), we found the leaves of the tree and species groups resolved when using the barcoding region, while intermediate nodes showed much lower support values. Increased sampling from unsampled populations, expanding the gene sampling to include at least 3 loci, including markers such as *wingless* and a section 16S rRNA, which have been demonstrated to work well separating closely-related species in Trichoptera (Kjer *et al.* 2014; Pauls *et al.* 2008), and full consideration of

the morphological diversity across life stages are clear next steps in discerning the larger evolutionary and biogeographical patterns for species in this genus.

Bayesian likelihood vs. distance methods

The BAYESTREE and BOLDTREE are congruent at most nodes and we can draw two inferences from the differences. The most important contrast is the shift of the two *O. sigma* specimens, sister to *O. ruthae* in the BOLDTREE, to a position sister to a larger clade, including *O. minutus*, *O. ardis*, and two unidentified taxa, in the BAYESTREE. Without statistical support in the BAYESTREE and no support value in the BOLDTREE, the extent of these differences remains unclear. However, within *Oligophlebodes*, changing the methodology seems to have had little impact on affinities to species groups (with the exception of the topology within *O. ruthae*), but may change the interspecies relationships. Morphologically, *O. sigma*, *O. ardis* and *O. minutus* share the character of patterning on the wings, while *O. sigma* and *O. ruthae* share fewer characters. Despite these phylogenetic issues, species identification, the primary purpose of DNA barcoding (Hebert *et al.* 2003a, b), appears to be well-served by either method in this genus. Even with small differences between these trees, the established superiority of likelihood methods, including Bayesian likelihood over distance methods (Guindon *et al.* 2010; Huelsenbeck 1995; Mar *et al.* 2005) makes clear Bayesian methods provide better phylogenetic estimates at the internal nodes. The statistical support values estimated in these methods also improve interpretation of node support within the topology.

The topologies of the Limnephiloidea subclade in Zhou *et al.* (2016) and the BAYESTREE estimate are generally similar. Zhou *et al.* (2016) used RAxML with bootstrap support- a method optimized for rapid model-based analysis of hundreds of taxa- so the support values are not directly comparable to the BAYESTREE posterior probabilities, but BAYESTREE posteriors are nominally higher than their bootstrap supports, as predicted by Erixon *et al.* (2003). It is notable that two major internal nodes in the ZHOUTREE collapse under bootstrap standard of 50%, resulting in almost no intra-generic resolution, while the BAYESTREE has only one such weak node with a statistically marginal posterior of 0.89 (Fig. 1).

Conclusion

Barcoding sequences can be used to support morphological designations of recognized species and highlight potential new species. However, to include support from a phylogenetic hypothesis, it is best to analyze these with likelihood methods over distance methods, and Bayesian methods where possible.

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APPENDIX: Specimen codes, collection, and molecular accession data. * Denotes larval specimens.

Specimen designation	Length (b.p.)	Locality data	GenBank (GB) Accession Number
Neophylax toshioi	627	USA: Virginia, Smyth Co.	HQ654635
Oligophlebodes ardis spc.1	658	USA: Colorado, Clear Creek County, Hoop Creek	HM400215
Oligophlebodes ardis spc.2	658	USA: Colorado, Clear Creek County, Hoop Creek	HM400216
Oligophlebodes ardis spc.3	626	USA: Colorado, Clear Creek County, Hoop Creek	HM400213
Oligophlebodes ardis spc.4	658	USA: Colorado, Clear Creek County, Hoop Creek	HM400214
Oligophlebodes minutus spc.1	441	--	AF436509
Oligophlebodes minutus spc.2	658	USA: Colorado, Park County, Middle Fork of S. Platte River at Co. Rd. 14	HM400176
Oligophlebodes minutus spc.3	658	USA: Colorado, Park County, Middle Fork of S. Platte River at Co. Rd. 14	HM400178
Oligophlebodes minutus spc.4	658	USA: Colorado, Park County, Middle Fork of S. Platte River at Co. Rd. 14	HM400177
Oligophlebodes minutus spc.5	658	USA: New Mexico, Sandoval Co.	HM400192
Oligophlebodes minutus spc.6	441	--	AF436509
Oligophlebodes minutus spc.7	658	USA: Wyoming, Albany, Co. Sect. 14	HQ560547
Oligophlebodes minutus spc.8	658	USA: New Mexico, Sandoval Co.	HQ945582
Oligophlebodes minutus spc.9	658	USA: Wyoming, Albany, Co. Sect. 14, Telephone Creek South of Hwy 130	HQ560546
Oligophlebodes minutus spc.10	658	USA: Colorado, Park County, Middle Fork of S. Platte River at Co. Rd. 14	HM400175
Oligophlebodes ruthae spc.1	407	USA: Wyoming, Big Horn Co., Bighorn N.F.	JQ935399
Oligophlebodes ruthae spc.2	407	USA: Wyoming, Big Horn Co., Bighorn N.F.	JQ935400
Oligophlebodes ruthae spc.3	407	USA: Wyoming, Sheridon Co., Bighorn N.F.	JQ935401
Oligophlebodes ruthae spc.4	407	USA: Wyoming, Sheridon Co., Bighorn N.F.	JQ935404
Oligophlebodes ruthae spc.5	658	USA: Washington, Okanogan Co., NE Winthrop	JQ935402
Oligophlebodes ruthae spc.6	658	USA: Washington, Okanogan Co., NE Winthrop	JQ935403
Oligophlebodes ruthae spc.7	658	Canada: Alberta, Wateron, Coppermine Creek	KM533490
Oligophlebodes ruthae spc.8	658	Canada: Alberta, Wateron, Coppermine Creek	KM537818
Oligophlebodes sierra spc.1	407	USA: Washington, Skamania Co., E. Canyon Cr.	JQ935405
Oligophlebodes sierra spc.2	407	USA: Washington, Lewis Co., Yozoo Cr., Rt. 22	JQ935406
Oligophlebodes sierra spc.3	407	USA: Washington, Lewis Co., Yozoo Cr., Rt. 22	JQ935407
Oligophlebodes sierra spc.4	407	USA: Washington, Lewis Co., Yozoo Cr., Rt. 22	JQ935408
Oligophlebodes sierra spc.5	658	USA: Washington, Whatcom Co., off Rt. 542	JQ935409

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APPENDIX. (Continued)

Specimen designation	Length (b.p.)	Locality data	GenBank (GB) Accession Number
Oligophlebodes sierra spc.6	658	USA: Washington, Whatcom Co., off Rt. 542	JQ935410
Oligophlebodes sierra spc.7	658	USA: Wyoming, Whatcom, Bagley Creek at mile 49.1 on highway 542	KX292700
Oligophlebodes sierra spc.8	658	USA: Wyoming, Park Co., Gunbarrel Cr.	GU667912
Oligophlebodes sierra spc.9	658	USA: Wyoming, Park Co., Gunbarrel Cr.	GU667913
Oligophlebodes sierra spc.10	658	USA: Wyoming, Park Co., Gunbarrel Cr.	GU667914
Oligophlebodes sierra spc.11	624	Canada: Alberta, Wateron, Carthew Creek	GU711417
Oligophlebodes sierra spc.12	658	USA: Wyoming, Whatcom, Bagley Creek at mile 49.1 on highway 542	KX295735
Oligophlebodes sierra spc.13	624	Canada: Alberta, Wateron, Carthew Creek	GU711418
Oligophlebodes sierra spc.14	658	USA: Wyoming, Whatcom, Bagley Creek at mile 49.1 on highway 542	KX293615
Oligophlebodes sierra spc.15	658	Canada: British Columbia,Glacier NP,Hemlock Grove Trl.	KM535922
Oligophlebodes sierra spc.16	658	USA: Wyoming, Park Co., Gunbarrel Cr.	GU667916
Oligophlebodes sigma spc. 1	407	USA: Utah, Salt Lake Co., Thousand Springs	JQ935411
Oligophlebodes sigma spc. 2	323	USA: Utah, Salt Lake Co., Thousand Springs	JQ935412
Oligophlebodes sp. spc.1	658	Canada: British Columbia, Kootenay NP, Redstreak Creek Trl.	JF891080*
Oligophlebodes sp. spc.2	658	Canada: British Columbia, Kootenay NP, Redstreak Creek Trl.	JF891081*
Oligophlebodes sp. spc.3	658	USA: New Mexico, Lincoln, tributary to North Fork Rio Ruidoso	HQ945685*
Oligophlebodes sp. spc.4	658	USA: New Mexico, Lincoln, tributary to North Fork Rio Ruidoso	HQ945686*