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Moving toward species-level phylogeny using ribosomal DNA and COI barcodes: an example from the diverse caddisfly genus *Chimarra* (Trichoptera: Philopotamidae)

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Abstract

The genus *Chimarra* (Trichoptera, Philopotamidae) is a cosmopolitan genus with over 700 species. The taxonomic history of *Chimarra* is discussed, with reference to how large genera are best subdivided. We also examine the phylogenetic utility of the COI "barcode" fragment and find it to be phylogenetically useful, within limits. Adding a single fragment of nuclear rRNA (specifically the 28S D2 region) converts the barcode hypothesis into a strongly supported phylogeny that is corroborated by a morphologically derived subgeneric classification. This suggests that a simple two gene dataset could be combined with morphological data in order to rapidly and inexpensively include a molecular component to generic revisions. We confirm the monophyly of *Chimarra* (*Chimarra*), *C.* (*Curgia*), *C.* (*Otarrha*), and core *C.* (*Chimarrita*). The *C.* (*C.*) *tsudai* group is also recovered. We make use of web-based materials, including the BOLD website (http://www.boldsystems.org/), and keyhole markup language files (.kml format), which permit specimen data to be viewed on Google Earth. We suggest that static phylogenies presented in print could be dynamically updated with the use of these web materials.

Key words

Trichoptera, *Chimarra*, COI, barcode, keyhole markup language, phylogenetic utility, generic taxonomy.

1. Introduction

The genus *Chimarra* Stephens, 1829 (Philopotamidae: Chimarrinae), with over 780 described species (Wahlberg et al. 2014), is the largest in the order Trichoptera (recently surpassing *Rhyacophila*). Currently, about one species in 20 within Trichoptera belongs in *Chimarra*, making the genus larger than most families of Trichoptera. Unlike *Rhyacophila*, which is primarily Holarctic in distribution (extending into tropical Asia), *Chimarra* is nearly cosmopolitan, although only marginally represented in Europe (by *C. marginata* (Linnaeus), the type species) and northern Asia. It is absent from New Zealand and the Chilean region of South America and most abundantly represented in tropical regions, especially the Neotropics (229 species) and southern Asia (321 species; Morse 2014). A large number of additional species will likely be described, especially since some regions where

the genus occurs are incompletely inventoried (various tropical localities, and especially Africa, Madagascar and India). BLAHNIK (1998) estimated that an ultimate size of 1000 or more species was possible and probably represents a conservative estimate. One of the goals of this paper is to assess a relatively simple strategy of molecular sequencing in order to add to the phylogenetic infrastructure for this large genus. This strategy could then be applied to other Trichoptera genera.

1.1. On *Chimarra* and other large genera

We are also interested in how large genera come to be, and how they should be subdivided. It is acknowledged that the size of a genus has a subjective component. In Trichoptera, genera tend to be conservatively defined, often based on distinctive larval differences (WIGGINS 1981; SCHMID 1979). Many genera within Trichoptera probably have considerable antiquity (ROSS 1956; WAHLBERG & JOHANSON 2014) and it is understood that genera are not synchronized across orders. A large genus could potentially be the result of an old lineage, speciating at a normal rate, for a long time, without dramatic morphological change, or alternatively, a relatively young lineage that has experienced a rapid burst of speciation. The large number of species in *Chimarra* is mainly due to the fact that it has not been divided into additional genera, demonstrating how generic definitions are dependent on the uncoordinated decisions of systematists. The current size of the genus has resulted from recent descriptions of taxa (more than half the species described in just the last 50 years), and thus its size does not reflect some *a priori* decision to establish a large genus. There is some value in maintaining a broadly defined genus *Chimarra*. The genus has a number of distinctive apomorphies (BLAHNIK 1998) and its monophyly is not in question. Moreover, members of *Chimarra* are easily recognized wherever they occur, both in the adult and larval stages, and can be readily identified as *Chimarra* in ecological studies and biomonitoring programs. New species of *Chimarra* can be unambiguously assigned to the genus during the description process. In contrast, natural subdivisions of the genus have not always been obvious. The major disadvantage of accepting such a large, undivided genus is that it tends to mask a great deal of evolutionary diversity. For example, since Trichoptera are often used in biomonitoring programs (in an effort to monitor water quality), and identifications of taxa are usually made only to the genus level, the lumping of diverse lineages into a single genus diminishes the resolution of these efforts (DOHET 2002; RESH & UNZICKER 1975). Members of several distinctive lineages of *Chimarra* often co-occur in the same area and thus the broad definition of the genus and in our opinion, failure to formally recognize lower taxonomic groups, underestimates taxonomic and ecological diversity.

Historically, large assemblages comparable to the genus *Chimarra* were often split into smaller genera, based on distinctive characters shared by subsets of the taxa. This process began in the early 1800s when a flood of new species were being described and the generic infrastructure established by Linnaeus seemed inadequate for accommodating them. For Trichoptera, this process began much later than in many taxa, with the majority of the diversity for the order only being described after the middle of the 20th century, although many genera were established much earlier. One disadvantage to erecting new genera from within a broadly defined older genus is that the establishment of new genera may leave the original genus paraphyletic. The disadvantages of paraphyletic taxa were not properly appreciated until after the work of HENNIG (1950, 1966), and has led to the convention of modern taxonomy, which requires monophyly of taxa at all levels. A repercussion of this convention, often criticized by non-taxonomists, is that it results in a considerable amount of nomenclatural instability due to frequent changes in genus-species combinations. We have observed a recent preference in systematics toward establishing monophyletic genera, rather than arbitrarily splitting genera that are acknowledged to be monophyletic into additional taxa, thus avoiding the problem of nomenclatural instability when it is unnecessary. We agree with this philosophy, and for this reason, the focus in this paper is in trying to establish a useful subgeneric infrastructure for the genus *Chimarra*, rather than splitting the genus into additional genera. However, for *Chimarra*, subgeneric taxa probably have more or less the same level of taxonomic significance as genera in other taxa.

1.2. History of taxonomic effort in *Chimarra*

There are currently 3 genera in the philopotamid subfamily Chimarrinae: *Edidiehlia* (a monotypic genus known only from the holotype), *Chimarrhodella* and *Chimarra*, the latter with 4 recognized subgenera, *C.* (*Chimarra*), *C.* (*Curgia*), *C.* (*Otarrha*), and *C.* (*Chimarrita*). *Curgia*, *Otarrha*, and *Chimarrita* are confined to the New World.

Like many genera, *Chimarra* has a history of taxonomic shifts. The first attempt to consider relationships within *Chimarra*, on a world scale, was presented by ROSS (1956). The treatment was rather informal and the major focus of his work was on species of Philopotamidae in the subfamily Philopotaminae. Most species of *Chimarra* were yet to be described. Nevertheless, his discussion of character evolution in the genus has continued to be of value. Ross recognized various lineages within *Chimarra*, but synonymized all previous genus-group taxa in Chimarrinae within the genus *Chimarra*, except for several species considered to represent a distinct lineage, which he included in the genus *Protarra* Ross, 1956, now considered a synonym of *Chimarrhodella* Lestage, 1925. The genus *Chimarrhodella* has subsequently been revised (BLAHNIK & HOLZENTHAL 1992), and its sister taxon relationship to *Chimarra* confirmed. All other New World species were retained in the subgenus

Chimarra. Following the work of Ross, the subgeneric name of *Curgia* has continued to be used for a distinctive group of New World taxa. The name originally applied to a single Antillean species, *Curgia braconoides* Walker, 1860, and was synonymized under *Chimarra* by Ulmer (1905). *Curgia* was subsequently resurrected as a subgeneric name by Milne (1936), and modern workers have continued the usage. The subgenus was formally revised by Flint (1998), who recognized some 16 species groups within *Curgia*, widespread over Central and South Americas, and also extending into both the Greater and Lesser Antilles and into the southwestern part of the United States. The name *Chimarrhafra* Lestage, 1936 has also had some continued usage for some African taxa, either as a genus name (e.g., Jacquemart 1966) or as a subgenus name (Morse 1974). Blahnik (1998) reduced *Chimarrhafra* to a synonym of *Chimarra* (*Chimarra*) when treating New World species in the nominotypic subgenus. Blahnik's work also resulted in the recognition of 2 additional New World endemic subgenera, both created from taxa originally placed in the subgenus *Chimarra*.

All of the 4 currently recognized subgenera of *Chimarra* (*Chimarra*, *Curgia*, *Chimarrita*, and *Otarrha*) are present in the New World and the entire New World fauna has been the subject of relatively recent revisions (Lago & Harris 1987; Blahnik 1997; Blahnik 1998; Flint 1998; Blahnik 2002). New World species in *Chimarra* (*Chimarra*) were placed in 19 species groups, about half of them including only 1 or 2 species. Relationships among recognized species groups and subgenera of *Chimarra* remained unresolved. Species retained in the subgenus *Chimarra*, both New World and Old World, were hypothesized to reflect a monophyletic assemblage (Blahnik 1998). All Old World species are currently placed in the subgenus *Chimarra*. However, Blahnik (1998) recognized that this might not be a permanent solution, and that the subgenus *Chimarra* might eventually be split into additional subgenera to reflect the considerable diversity among Old World lineages. More recently, Blahnik et al. (2012) recognized 4 distinctive species groups in describing species of *Chimarra* from Vietnam. Of these, only the *tsudai* group, including about half of the Asian species, is well characterized (Blahnik et al. 2009). New World species of *Chimarra* (*Chimarra*) belong to the *digitata* group, a lineage also well represented in the fauna from Vietnam, and considered to be closely related to the type species for the genus. Part of the thrust of this work is to establish the relationships among the recognized subgenera, and to provide information relevant to the establishing of additional subgenera for Old World lineages currently placed in the subgenus *Chimarra*.

1.3. Utility of DNA barcode sequences for phylogenetics

For *Chimarra*, in June, 2013, 1171 sequences were available to us from the Barcode of Life Data Systems (BOLD) website (Ratnasingham & Hebert 2007), representing 205 species for a fragment of mitochondrial cytochrome c oxidase gene, subunit I (COI, the animal "barcode" fragment). We wished to explore the utility of the barcode data for establishing initial phylogenetic hypotheses for large genera, such as *Chimarra*. It has long been understood that single gene phylogenies are subject to error due to "gene tree/species tree" conflicts (Avise 1989) and potential introgression from hybridization. The COI has been criticized as being overly homoplastic for deep phylogenetic relationships (Kjer et al. 2001). Accepting all these limitations, we would like to know whether or not large-scale initial Trichoptera phylogenies generated from the huge amount of barcode data being generated are as "useless" as they are sometimes assumed to be. Even though "useless" is a subjective definition, we are qualified to discuss the utility of a barcode-generated phylogeny, because one of us (RB) has specialized on *Chimarra* and we find his discussion of the issue to be "useful".

Considerable effort has been extended to finding appropriate phylogenetic markers for Trichoptera. Kjer et al. (2001) concluded that the rRNA was reliable for the deepest nodes across the entire order, while the COI was rapidly saturated in all but the most closely related taxa. Rapid substitution rates are actually ideal for "species barcoding", as species identification from molecular markers requires variation, even among different populations of the same species. Zhou et al. (2007) took advantage of the properties of these individual markers in recommending that a combination of COI and the D2 region of the large subunit nuclear rRNA (28S) would be ideal for larval associations. Associating larvae with known adults requires that selected markers can each be used to generate a gene tree within a genus. Maddison (2012) noted that the D2 region of rRNA traced *Bembidion* (Coleoptera, Carabidae) species better than the barcode fragment, likely because as a member of a multigene family, nuclear rRNA is homogenized with gene flow. We have noticed that the D2 region of rRNA works very well for distinguishing Trichoptera species (Zhou et al. 2007; Geraci et al. 2010), but this observation may not apply to all insect groups. With this paper, we examine whether a combination of COI and rRNA can produce a reliable species-level phylogeny for Trichoptera by evaluating gene trees from both markers for congruence with the combined data as well as for congruence with species groups and subgenera previously established on a morphological basis. Since measures like bootstrap values cannot differentiate between gene histories and species histories, we assessed the utility of barcode generated phylogenies through congruence with phylogenies generated from an independent molecular dataset (28S), and compared the barcode phylogeny to a phylogeny from combined data. *Chimarra* has also been the subject of a great deal of recent morphological work, so one could also assess the utility of the barcode data by mapping morphological apomorphies onto barcode-generated phylogenies.

2. Materials and methods

Molecular data consisted of the COI barcode fragment, and approximately 1000 nucleotides of 28S rRNA, spanning the D1–D3 hyper-variable regions. The COI data were generated at the Biodiversity Institute of Ontario, University of Guelph, Canada, for the barcode initiative, and those sequences were downloaded from the BOLD website (http://www.boldsystems.org/). We pruned the COI data by eliminating fragments that were flagged in BOLD as contaminants, and included only fragments that were greater than 350 nucleotides long. Some of the taxa had sequences that were identical to other taxa; these duplicate sequences were eliminated in order to reduce computation time. The nuclear rRNA sequences were generated from these same individuals, using DNA left over from COI barcoding. Standard PCR methods were used, except that we included 400 mM Betaine in the PCR reaction mix. Primers for the 28S fragment were D1-3F CGAGTAGCGGCGAGCGAAACGGGA and D1-3shortR CGTGYRCGCTCTCAGTGCGT. Annealing temperature was 55°C. Sequences are available in GenBank, under accession numbers KM189833–KM189911.

The alignment of the COI sequences was trivial, as there were no insertions or deletions among translated amino acids. Ribosomal data were aligned by eye according to the structural protocols described in detail in KJER et al. (2009). Alignments are available on Kjer's website (http://rci.rutgers.edu/~insects/kjer.htm). Nucleotide data were analyzed with likelihood, using the program RaxML (STAMATAKIS 2006), under the –f a option with 100 rapid bootstrap searches. Partitions of the data were established through a modification of a site-specific rate partitioning scheme, first described in KJER et al. (2001), and elaborated upon in KJER & HONEYCUTT (2007). The nucleotides were binned into 5 partitions according to their estimated substitution rates (rather than by gene or codon positions).

Sampling strategy for the rRNA was not entirely systematic; first, we sequenced all of the species we could with the taxa, time and funds we had available and then made sure that all subgenera were represented, favoring taxa of phylogenetic ambiguity (such as *C. maldonadoi* and *C. usitatissima*). Since there were more COI sequences (750 unique haplotypes) than rRNA sequences (79), we had to devise a strategy for combining data. We analyzed data from separate genes, as well as from combined data. Two analyses of the COI were completed: one with all the COI haplotypes, and another including only species for which nuclear rRNA also existed, so that phylogenies could be directly compared without the influence of taxon sampling. Therefore, analyses consisted of all rRNA (79 taxa; Fig. 1), 79 COIs (Fig. 2), combined data (79 taxa; Fig. 3) and 750 COIs (Fig. ES1[1]).

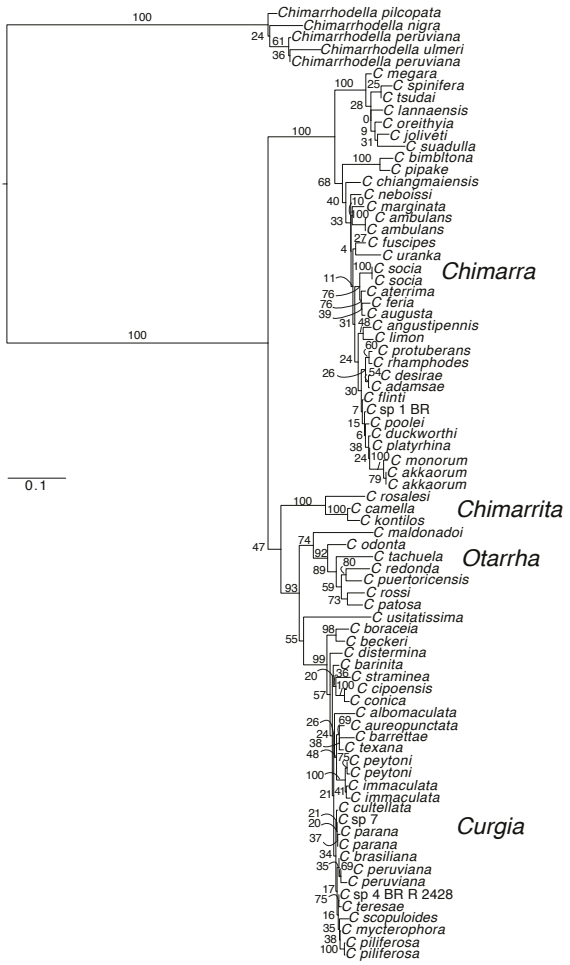

Fig. 1. *Chimarra* phylogeny generated from the ribosomal RNA data (28S rRNA, D1–D3) for 79 taxa, analyzed with RaxML. Numerals represent bootstrap support.

3. Results and discussion

3.1. Phylogeny

Figure 3 representing the analysis of the combined dataset shows that each of the subgenera, *Chimarra*, *Chimarrita*, *Otarrha*, and *Curgia* is monophyletic. In comparing the COI and rRNA trees (Figs. 1 and 2, respectively), it is immediately apparent that the nuclear rRNA has shorter branch lengths at every level. Of 1107 rRNA characters, only 348 are parsimony informative (310 within *Chimarra*), compared to 280 parsimony informative characters out of 654 for the COI (272 within *Chimarra*). Thus the datasets are close to the same size. The difference in branch lengths is due to an elevated substitution rate in the COI, relative to the slow rate in the rRNA. Further demonstration of this comes from comparing the nodes that each gene tree shares with the combined data tree. The rRNA data tree (Fig. 1) shares many nodes with the combined data tree (Fig. 3), including those at the deepest levels of the tree. This is in stark contrast with the distribution of congruence between the COI tree (Fig. 2)

[1] ES = Electronic Supplement, files listed at end of article.

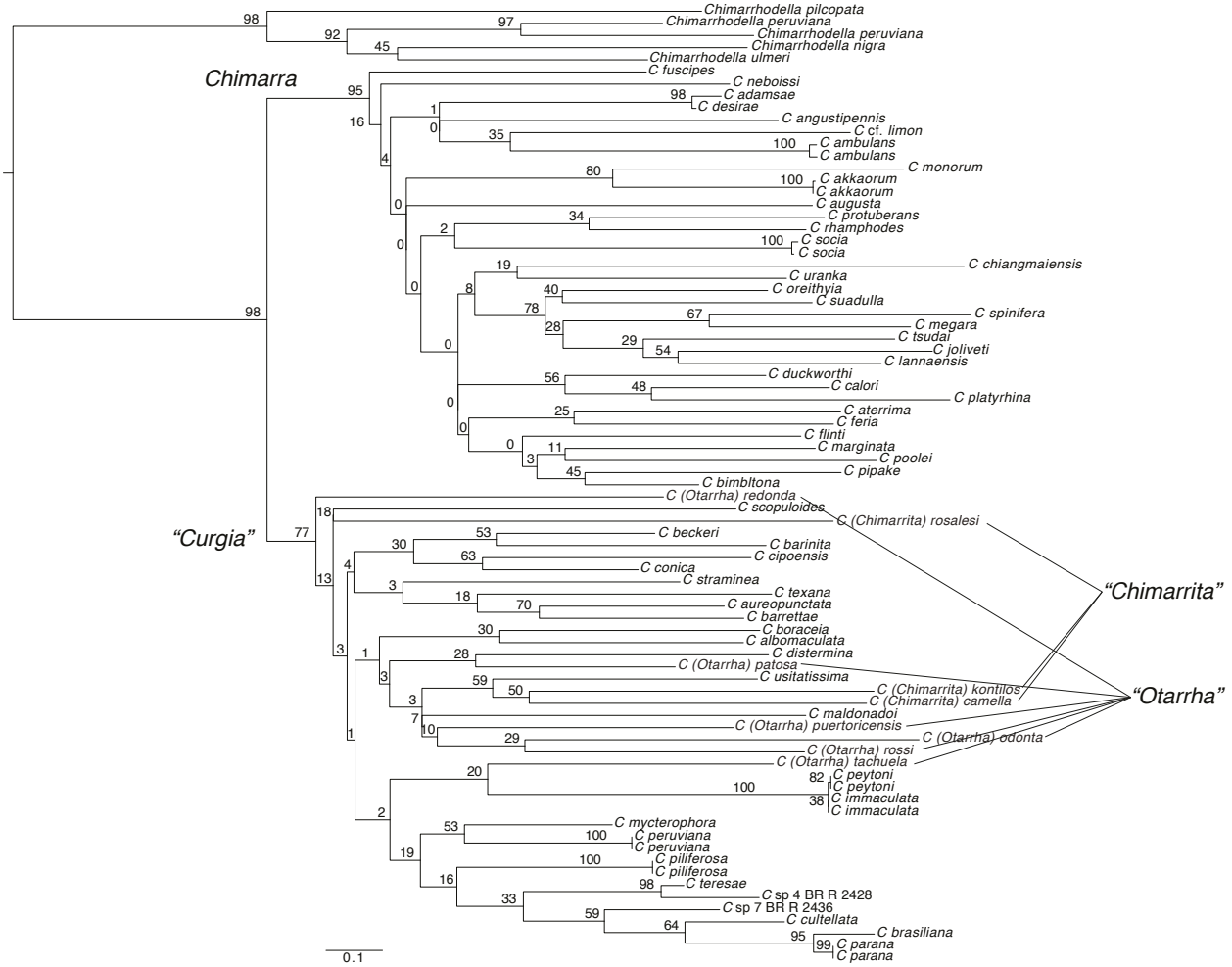

Fig. 2. *Chimarra* phylogeny generated from COI for 79 taxa, analyzed with RaxML. Numerals represent bootstrap support.

and the combined data, which is concentrated at the tips. Yet the COI does recover the deep ingroup node, as well as the *Chimarra* (*Chimarra*) node, and the node that supports the 3 entirely New World subgenera. We conclude that lack of congruence is stochastic; each strongly supported node from either marker (Figs. 1 and 2) is also found in the combined data, and weakly supported nodes from the COI are overturned by even a small number of rRNA characters. When we examine nodes shared with the combined data in both the rRNA tree, and the COI tree, bootstrap support increases in the combined data, indicating that the other gene was not strongly conflicting, but supportive of the results from the combined data even when the gene did not support a particular relationship by itself.

Not surprisingly, we find that adding another marker improves congruence with previously proposed species groups. Another long-established method to improve phylogenetic accuracy is to add more taxa. The question of whether barcode data can provide useful phylogenies depends on one's definition of "useful". We find it troubling that it is impossible to assess congruence from a single marker, and therefore, difficult to distinguish reality from error. Homoplasy that is particularly high among silent codon sites in the barcode fragment (Kjer et al. 2001) makes the recovery of the actual gene tree extremely difficult. If it is nearly impossible to recover "the gene tree" from a single gene, then it is also nearly impossible to evaluate gene tree/species tree conflicts. In this study, we have a number of means to assess gene performance and the influence of adding taxa, including bootstrap values, congruence with the rRNA, recovery of morphologically defined species groups, and logical geographic clusters. We find that adding taxa for the barcode fragment (Fig. ES1) helped with resolution in many cases. For example, while the COI analyzed in the small taxon set shows *Otarrha* scattered within *Curgia* (Fig. 2), both the combined data (Fig. 3) and the larger COI taxon set (Fig. ES1) recover *Otarrha* as monophyletic. Many of the previously proposed species groups were also recovered, although the *immaculata*, *laguna*, and *banksi* groups, which all have brustiate appendages on segment VII, were not recovered together in the barcode tree (Fig. ES1). A particularly good example of how fundamentally different the backbone tree from barcode sequences can be is given by the *primula* species group (Fig. ES1). The

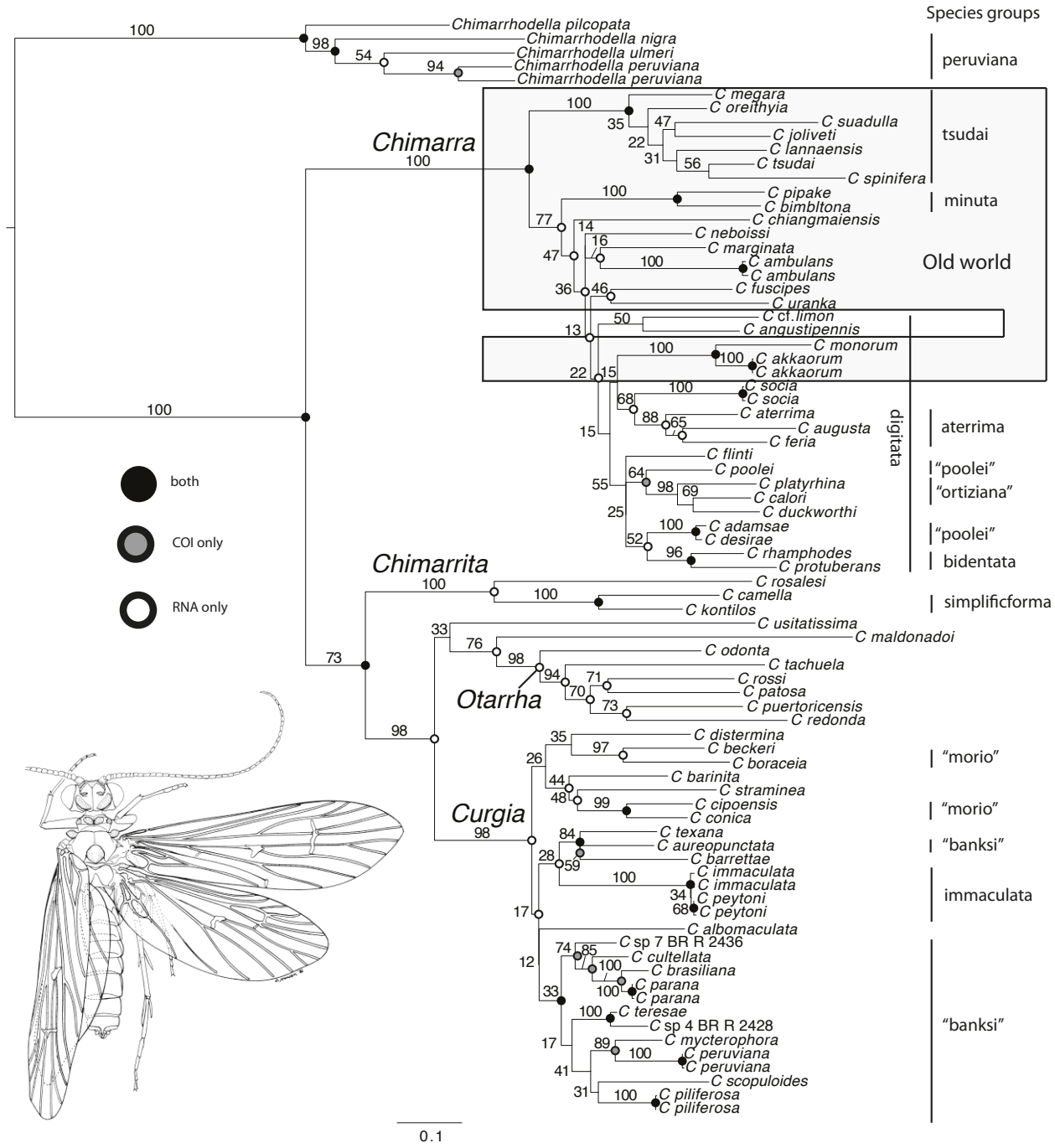

Fig. 3. *Chimarra* phylogeny generated from combined rRNA and COI data. Nodes marked with open circles are present in the rRNA tree. Nodes marked with grey dots are present in the COI tree. Nodes marked with black dots are present in both COI and rRNA trees. Old world taxa are boxed in grey. Inset: dorsal illustration of a female *Curgia* by Dan Hanson.

primula group is polyphyletic in the large barcode tree (Fig. ES1), arising from distant regions of the big tree, but their neighbors, *C. marginata* and *C. ambulans* form a monophyletic group in Fig. 3. If the combined data are better indicators of phylogeny, then the polyphyly of the *primula* species group undermines a huge portion of the barcode tree. Of course, if the "more taxa provides better results" is true, then we mistrust Fig. 3. We do not wish to rehash the "more taxa vs. more genes" debate here, because we have always felt that both are a good thing. However, the highest bootstrap support for any node separating genera in the *primula* group in the barcode tree is 4 (Fig. ES1). Since we advise collapsing nodes that are so weakly supported by bootstrap as to be essentially stochastic, we do not see that the barcode tree is in conflict. Using appropriate caution in evaluating branch support, we find that a dense taxon sample of the barcode fragment provides some information, but much of this information is confined to the tips of the tree, and indicated by high bootstrap support. We also note that some of the congru-

ent nodes, such as the three deepest nodes in *Chimarra* (Fig. 3) are also recovered by the COI alone. We suspect that there may be some pattern here, with non-silent 1st and 2nd codon positions providing reliable characters for the deepest nodes, and silent 1st and 3rd codon positions resolving the tips, while fouling intermediate nodes. We have not performed any real analysis to confirm this suspicion, but have seen it frequently enough in our work to suggest it as a possibility for further evaluation.

The rRNA offers several features that are similar to morphological characters that can be visualized in the alignment available in the supplementary materials and online (Fig. ES2). The grouping of *Chimarrita* with *Otarrha* and *Curgia*, along with *C. maldonadoi* Flint, 1964 and the *incertae sedis* taxon *C. usitatissima* Flint, 1971 is supported by the loss of a hairpin stem loop (stem-loop 30, Fig. ES2), and 4 unique nucleotides in the place of this missing stem that are clearly apomorphic. Stem-loop 30 is present in *Chimarrhodella* and all *Chimarra* (*Chimarra*), and also present in *Chimarra* n.sp. E, our only representative of the *C. georgensis* group ("*Chimarrhafra*") from Africa. Most of the data from this species were missing, so it was not included in the analysis, but the presence of stem loop 30 in *Chimarra* n.sp. E indicates that it belongs with the *Chimarra* (*Chimarra*) clade, or at least outside the exclusively New World subgenera. This same pattern is also supported by a 4 bp loss, just downstream of D2-1a, but *C. pipake* Malicky & Chantaramongkol, 1993 and *C. bimbltona* Malicky, 1979 (representing the *Chimarra* (*Chimarra*) *minuta* group of Blahnik et al. 2012) also lack these 4 nucleotides. We also find it interesting that the morphological outliers, *C. usitatissima* and *C. maldonadoi* have highly autapomorphic rRNAs (Fig. ES2).

Figure ES3 shows the geographic distribution of *Chimarra* used in this study and was generated automatically on the barcode database (BOLD). The geographic data on this database can be explored in greater detail on "Google Earth". By downloading the keyhole markup language (.kml) file on either the BOLD or Kjer website, and opening this file in the Google Earth application, locality and voucher information can be viewed in detail, frequently with images of the individual specimens used. Thus the supplementary materials for this paper are substantial, but could not be presented in print without an additional 6 pages of phylograms (Fig. ES1), two pages of aligned rRNA data (Fig. ES2), and countless pages of images and locality data (the .kml file). Many morphologists do not relate to the morphological features of nucleic acids, but the structural features of rRNA are clearly visible in Fig. ES2. The COI tree can be updated by anyone using the BOLD website by downloading these sequences, as more taxa are added to the database. And while it is difficult (and dull) to read tables of collection data in print, it can be enjoyable to "spin the globe" on Google Earth, and see patterns of distribution, right down to the bend in the river where individuals on our phylogenies were collected. These are exciting times for presenting phylogenies.

3.2. Taxonomic implications

Our phylogenetic results indicate that the previously defined subgeneric infrastructure for New World taxa is mostly satisfactory, with 2 exceptions.

(1) One lineage placed in the subgenus *Chimarrita*, the *C. maldonadoi* group (Blahnik 2002) appears closer to the subgenus *Otarrha* (Fig. 3). The *C. maldonadoi* group was only speculatively placed in the subgenus *Chimarrita* in the first place and thus molecular data indicating a different position is not surprising. Only 2 extant and obviously closely related species belong here, both from the Greater Antilles (Puerto Rico and Hispaniola). Barcode sequences taken from individuals from opposite ends of Puerto Rico that were both originally identified as *C. maldonadoi* provide another example of how the COI data can point to further research hypotheses; their barcodes appeared so divergent (14% uncorrected pairwise difference) that the possibility that they were different species was raised. Although we do not support the definition of species based on barcode divergence alone (Flint & Kjer 2011), subsequent examination of the genitalia revealed some morphological differences (Flint, pers. comm.) that leads us to believe that BOLD specimen 10OFCAD-307 is an additional cryptic species, yet to be named. Additionally, it appears that all 6 of the species from Dominican amber described by Wichard (1983a,b, 2007) also belong here. They were placed in the subgenus *Chimarrita* by Wichard (2007) primarily on the basis of characters used by Blahnik to define the *C. maldonadoi* group, and not the more numerous characters used to define other species of *Chimarra* (*Chimarrita*). Unfortunately, a number of these characters are probably plesiomorphic, and thus the possibility that the fossil species are thereby assigned to a paraphyletic assemblage cannot be eliminated. It is clear that they do not belong within either the subgenera *Curgia* or *Otarrha*, as currently defined, which represent the dominant elements in the modern fauna of the Greater Antilles (8 and 11 species, respectively). The somewhat unusual female genitalia of species in the *C. maldonadoi* group might be of some help in resolving this, if this also characterized the fossil species, but no females of fossil species have yet been described. Since the subgenera *Curgia* and *Otarrha* are not represented in the fossil fauna, the biogeographic implication is that the extant species now found there are likely derived from more recent immigrants to an ancestral fauna, thus contradicting some of the conclusions of Blahnik (1997, 2002), and making the retention of relict species from an ancestral biota even more significant.

(2) The other taxon that does not clearly fall in one of the subgenera is *C. usitatissima*, which has a sister species, *C. angularis* Blahnik, 2002. These 2 species are currently unplaced to subgenus. The unusual aspects of their morphology were noted by Blahnik (2002), who suggested they might have their closest affinity with or in the subgenus *Curgia*. With our phylogenetic results (Fig. 3), this seems to be the case.

However, the exact position of neither *C. usitatissima* nor *C. maldonadoi* is well supported in the current analysis. Both taxa appear to be relatively isolated and not embedded in the defined subgenera. Treating them as additional subgenera would be a possibility, but we decided that it was premature before their relationships are strongly supported and the merits of alternate taxonomic arrangements considered. It is clear, however, that the present placement of the *C. maldonadoi* group in *Chimarra* (*Chimarrita*) is unsupported. As a conservative treatment, consistent with analysis presented, we propose that all of the taxa now placed in the *C. maldonadoi* group of *Chimarra* (*Chimarrita*), including the 6 fossil taxa, be moved to a position of *incertae sedis* to subgenus within *Chimarra*, and that *C. usitatissima* and *C. angularis* also retain the designation of *incertae sedis*.

The strong support of monophyly of *Chimarra* (*Chimarra*) is something of a surprise, based on the considerable morphological diversity within the subgenus and relatively weak morphological character evidence uniting the entire clade (BLAHNIK 1997, 1998). In general, the relationships within the subgenus are consistent with the major groups currently proposed. Of particular interest is the clear separation of members of the *tsudai* group from the other sampled taxa, and the position of species in the *minuta* group as sister to remaining old world *Chimarra*, including the *marginata* group (including the *digitata* group of BLAHNIK et al. 2009, 2012). Unfortunately, no representatives of the *georgensis* group were included in the combined analysis. The *georgensis* group was considered by ROSS (1956) to be the most "primitive" lineage among Old World *Chimarra* and this was also the opinion of BLAHNIK (1997). Ross placed members of the *georgensis* group in *Chimarrhafra*, and also considered the Philippine genus *Vigarrha* Navás 1921, to be closely related. If they form a natural group, and given a subgeneric name, the name *Vigarrha* would have priority. Unfortunately, neither of these taxa are currently well characterized and *Vigarrha* is monotypic, based on a female specimen. Most of the species of the *georgensis* group are found in Africa, but a species in the group was recently reported from Vietnam (BLAHNIK et al. 2012). Additional taxa with primitive features are also known and were discussed by BLAHNIK et al. (2009), including *Chimarra noohi* Blahnik, et al. 2009, *C. furti* Mey, 1998, *Edidiehlia hiskia* Malicky, 1993, and *Chimarra uvana* Kimmins, 1957. There are undoubtedly others; species descriptions often lack the details to make assessments about relationships. The relationships of these taxa need to be considered before additional subgenera can be created. Despite this lack of complete sampling in the current analysis, it appears that the majority of species of *Chimarra* from Africa, Asia, Australia, North and South America fall within a defined lineage, which includes *Chimarra marginata*, the type species for the genus. This core of species would likely be retained in the nominate subgenus, should additional subgenera be created.

4. Conclusions

Phylogenies do not need to be perfect to be useful. However, we find the COI data alone fails to establish the monophyly of morphologically defined subgenera that were corroborated with combined molecular data. There is little doubt that some phylogenetic information can be obtained from the barcode data, and it is likely that clades shown in Fig. 2 that are supported by high bootstrap values are reliable, at least as a first hypothesis. A reader capable of evaluating bootstrap support, mentally collapsing nodes below some threshold, would find only information (not error) from the barcode sequences. The COI does recover *Chimarra* (*Chimarra*) as monophyletic in both taxon sets, and many of the groupings make geographic sense. Even with bootstrap support at less than 10%, we see clades from the 750 taxon COI dataset (Fig. ES1) that are entirely reasonable. Many of the species groups proposed by Blahnik and Flint are also recovered from the analysis of the larger taxon set for COI. On the other hand, it is impossible to distinguish "real" weakly supported groupings from false ones. Anybody working on *Chimarra* would find the COI hypotheses to be useful, even if only to generate hypotheses to test with more reliable data. However, this work should not be cited as finding that "the barcode fragment alone provides reliable phylogenies" as this is far from our conclusion. We find that the addition of a single PCR amplicon from nuclear rRNA (D1–3) provides enough data for the production of a reasonable phylogeny, as assessed by bootstrap values and congruence with morphology and geography.

With new developments in sequencing technology, there has been a shift of focus and attention towards genomics – the generation of huge datasets with millions of nucleotides for a smaller number of taxa. As these technologies get cheaper, it may be that many more taxa can be explored. However, there are many important evolutionary questions to be answered, particularly in alpha taxonomy and generic revisions, that may require data from hundreds or thousands of individual specimens. Taxonomic studies are essential in order to understand the larger scale patterns of species evolution, and form a critical foundation for any genomic and phylogenomic studies in the future. Organismal systematists form the link between our understanding of a species morphology (phenotype) and its DNA (genotype). We show that these questions can be addressed using a much less expensive, and simpler approach: reciprocal illumination from a combination of two markers and morphological data. Museum specimens exist for all described species, and are available for morphological work. We already have barcode data from over 45,000 individual Trichoptera, representing a significant proportion of the world's commonly encountered species. We conclude that a combination of nuclear rRNA and COI data provide a simple and inexpensive phylogeny for trichopteran

congeners. Each marker complements the other, and can quickly provide corroborative evidence for the monophyly of morphologically derived species groups.

5. Addendum

During final revisions of our paper, Wahlberg and Johanson generously provided us with a preview of their paper on *Chimarra* (Wahlberg & Johanson 2014). This paper used molecular data from mitochondrial COI, nuclear cadherin-like gene (CAD), and RNA polymerase II (Pol-II) in order to infer a phylogeny of *Chimarra*, and to estimate the age and radiation of the genus. Their phylogenetic results are similar to ours in that the new world subgenera, *Chimarrita*, *Otarrha*, and *Curgia*, form a monophyletic sister taxon to *Chimarra* s.str. They also recover taxa included in the Asian *tsudai* and *minuta* groups among the first splits among subgenus *Chimarra*.

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Electronic Supplement File

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File: kjer&al-chimarraphylogeny-asp2014-electronic-supplement.pdf – **Fig. ES1A,B.** COI based phylogeny with 750 unique terminals, analyzed with RaxML, in two parts A and B. Note that some nodes are supported by bootstrap values of "0". This is the result of RaxML performing fast bootstrap searches, followed by a more thorough tree search. The tree is presented from the slower search, and some of these nodes were not supported in the fast bootstrap search (resulting in zero, or very low values). This tree is available as a pdf on Kjer's website (http://rci.rutgers.edu/~insects/kjer.htm). The numbered arrows represent the order of the taxa as they appear in Fig. 3 (top to bottom). – **Fig. ES2.** Alignment of the rRNA, in the structural notation of KJER (1995). – **Fig. ES3.** Geographic distribution of *Chimarra* in the BOLD website. Circles with numerals are placed over the general area of the collection localities, which are available in greater detail in the .kml file, which can be generated on BOLD, or downloaded from Kjer's website. Numerals indicate the number of individuals collected from that particular region, with colors indicating quantity classes.