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Testing use of mitochondrial COI sequences for the identification and phylogenetic analysis of New Zealand caddisflies (Trichoptera)

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Abstract We tested the hypothesis that cytochrome *c* oxidase subunit 1 (COI) sequences would successfully discriminate recognised species of New Zealand caddisflies. We further examined whether phylogenetic analyses, based on the COI locus, could recover currently recognised superfamilies and suborders. COI sequences were obtained from 105 individuals representing 61 species and all 16 families of Trichoptera known from New Zealand. No sequence sharing was observed between members of different species, and congeneric species showed from 2.3 to 19.5% divergence. Sequence divergence among members of a species was typically low (mean = 0.7%; range 0.0–8.5%), but two species showed intraspecific divergences in excess of 2%. Phylogenetic reconstructions based on COI were largely congruent with previous conclusions based on morphology, although the sequence data did not support placement of the purse-cased caddisflies (Hydroptilidae) within the uncased caddisflies, and, in particular, the Rhyacophiloidea. We conclude that sequence variation in the COI gene locus is an effective tool for the identification of New Zealand caddisfly species, and can provide preliminary phylogenetic inferences. Further research is needed to ascertain the significance of the few instances of high intra-specific divergence and to determine if any instances of sequence sharing will be detected with larger sample sizes.

Keywords aquatic insects; Arthropoda; barcoding; mtDNA; phylogeny; systematics; taxonomy

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INTRODUCTION

The ability to accurately assess biological diversity is a cornerstone to most ecological research. For many taxonomic groups, species identifications can only be made by a few experts who often lack sufficient time to address requests for routine identifications. It can also be time-consuming to prepare and transfer specimens to the relevant taxonomic expert who may often be based overseas. Because of these limitations, a more widely accessible identification tool would be a major advance for ecologists, resource managers, and biologists at large. The use of molecular techniques such as DNA sequencing, offers one approach to simplify and speed up the routine diagnosis of species (Tautz et al. 2003). Mitochondrial DNA sequences from the cytochrome *c* oxidase subunit I (COI) gene locus have rapidly become the marker-of-choice for such

identifications, especially for animals (e.g., Hebert et al. 2003). Mitochondrial DNA sequences have been long used in phylogenetic research (e.g., DeSalle et al. 1987; Kjer et al. 2002; Johanson et al. 2009). However, the use of COI for species identification still requires further verification in New Zealand and internationally (e.g., Meier et al. 2006; Elias et al. 2007; Roe & Sperling 2007; Skevington et al. 2007; Trewick 2007).

The use of COI for species identification has been examined in several groups of arthropods including Arachnida (Barrett & Hebert 2005), Collembola (Hogg & Hebert 2004), Ephemeroptera (Ball et al. 2005), Odonata (Nolan et al. 2007), Coleoptera (Ahrens et al. 2007), Diptera (Skevington et al. 2007), Hymenoptera (Banks & Whitfield 2006), Lepidoptera (Hebert et al. 2003; Hajibabaei et al. 2006), and Phthiraptera (Johnson & Clayton 2003). But there remain some important gaps in coverage including Trichoptera (caddisflies; e.g., Pauls et al. 2009). Trichoptera is a taxonomically and ecologically diverse component of freshwater ecosystems (Winterbourn 2004), and their larvae are often used as a primary indicator of water quality (Resh 1993). Species-level assignments are often impossible for larvae (e.g., Wiggins 1996) and, similar to other aquatic arthropods, matching genders can be difficult (Chapman et al. 2002; Zhou et al. 2007). Accordingly, a molecular identification tool would greatly assist in providing accurate species designations (*sensu* Ahrens et al. 2007; Johanson 2007). New Zealand is an ideal location to test the feasibility of such an approach as its trichopteran fauna consists of approximately 240 species from 16 families, of which four are endemic to Australasia (Winterbourn 2004)

To assess the use of molecular techniques for trichopteran species identifications, we sequenced a portion of the COI gene for 61 species, including at least one representative of all 16 families. Furthermore, we used several phylogenetic reconstruction methods to determine if COI sequences might also be useful for inferring phylogeny at deeper levels.

MATERIALS AND METHODS

Larval and/or adult caddisflies were collected from 40 sites in New Zealand, mostly the North Island (Table 1). Larvae were killed by immersion in 100% ethanol and adults using ethyl acetate in a standard "killing jar" (Walker & Crosby 1988). These collections included representatives of 61 currently recognised species. A single leg was removed from specimens and placed in individual 1.5 ml microcentrifuge tubes with 95% ethanol. The remainder of the animal was mounted on a pin or stored in 95% ethanol and archived as part of the NIWA collection (National Institute of Water and Atmospheric Research Ltd, Hamilton, New Zealand). In other instances, larvae were used and the remainder stored in 95% ethanol. Tissue samples were transferred to the Canadian Centre for DNA Barcoding at the University of Guelph (Canada) for sequence analysis.

Genetic analysis

DNA was extracted from each sample using the NucleoSpin96 tissue kit (Machery-Nagel, Germany) following Hajibabaei et al. (2005). Polymerase chain reaction (PCR) amplification was carried out in a 50 µl reaction volume consisting of 2 µl of DNA, 1 × PCR buffer (Roche, Germany), 2.2 m*M* $MgCl_2$, 0.2 m*M* of each dNTP (Boehringer Mannheim, Germany), 1.0 µ*M* of each primer, and 1.0 unit of Taq DNA polymerase (Roche) on an Eppendorf Mastercycler gradient thermocycler. A 658 base pair fragment of the mitochondrial COI gene was amplified using the primers LCO1490 (5'-GGTCA ACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAAT CA-3') (Folmer et al. 1994). The thermal cycling conditions were: initial denaturation at 94°C for 60 s; followed by 40 cycles of denaturation at 94°C for 20 s; annealing at 50°C for 30 s; and extension at 72°C for 90 s; with a final extension at 72°C for 5 min. Sequencing was performed in both directions, using the same primers as those used for PCR amplification on an ABI 3730 automated sequencer (Applied Biosystems Inc., United States). Sequences were aligned using SEQUENCHER (Gene Codes v. 4.1.2) and verified as being derived from insect DNA using GenBank BLASTn searches. We used χ^2 tests, as implemented in PAUP* 4.0b10 (Swofford 2002), to determine whether the assumption of equal base frequencies among sequences was violated on: (1) all sites; (2) parsimony-informative sites only; and (3) with the third codon position only. Owing to computational restraints, we reduced individuals from the same morpho-species with identical sequences to a single representative for the phylogenetic analyses. We then estimated phylogenetic trees using Neighbour-joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and Bayesian methods as implemented in PAUP* 4.0b10 or MrBayes 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003).

A maximum likelihood phylogram was constructed using the GTR+I+G model with A = 0.4132, C = 0.1328, G = 0.0472, T = 0.4068, proportion of invariable sites = 0.3965, gamma distribution of 0.4775 (selected using the Akaike selection criteria in Modeltest 3.5; Posada & Crandall 1998). We used a heuristic search function with an as-is addition sequence.

We also estimated a phylogeny for the caddisflies using a Bayesian analysis in MrBayes 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The model used was the general time reversible model (GTR, Tavaré 1986) plus a proportion of invariable sites plus gamma (Rodríguez et al. 1990; Yang et al. 1994) and was selected using the Akaike selection criteria in MrModeltest 2.3 (J. A. A. Nylander unpubl. data). MrBayes estimated the model parameters from the data using one cold and three heated Markov chains. The Monte Carlo Markov chain length was 2 000 000 generations and we sampled the chain every 100 generations. We discarded the first 5000 samples as "burn-in" and thus estimated phylogeny and posterior probabilities from a consensus of the last 15 000 sampled trees, as the log-likelihood values for the cold chain had stopped increasing and were randomly fluctuating after approximately 10 000 generations (i.e., 100 sampled trees) indicating that stationarity had been reached (as suggested in the MrBayes manual, Ronquist et al. 2005).

Nonparametric bootstrap analysis (Felsenstein 1985) was used with 1000 pseudoreplicates (identical sequences were removed to decrease analysis time) on the MP, NJ and ML trees to assess support for nodes in the trees. We compared congruence among the phylogenies estimated by MP, NJ, ML and Bayesian analyses of the COI data by comparing tree-to-tree symmetric differences (Penny & Hendy 1985) using the tree-to-tree distances function in PAUP*. Phylogenies estimated by the different methods were compared in a pairwise manner with each other and to 1000 trees randomly generated under a Markovian model using the "generate trees" option in PAUP*. A Wilcoxon signed-ranks test (Templeton 1983) implemented in PAUP* was used to determine if significant differences existed between the trees.

All sequences were deposited in the project "Caddisflies of New Zealand" (NZCAD) in the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007; www.barcodeoflife.org; sequence pages NZCAD001-05–NZCAD140-05) and cross referenced to GenBank.

RESULTS

A 658 base pair fragment of the COI gene was recovered from 105 individuals. Alignment began at position 1513 of the *Drosophila yakuba* sequence (Folmer et al. 1994; GenBank accession No. X03240). No insertion, deletion or stop codon was detected in any sequence. Nucleotide composition averaged over all

Table 1 Mean pairwise distances (and range where applicable) among trichopteran taxa collected in New Zealand. For individuals within species, *n* = number of species which had two or more individuals; species within genus, *n* = number of genera with two or more species; species among genera, *n* = number of genera analysed within a superfamily; species among superfamilies, *n* = total number of species analysed; na = no comparisons available.

Superfamily	Individuals within species	*n*	Species within genus	*n*	Species among genera	*n*	Species among superfamilies	*n*
Sericostomatoidea	0.01 (0.00–0.08)	5	0.12 (0.04–0.15)	2	0.17 (0.03–0.23)	11	0.23 (0.18–0.27)	14
Limnephiloidea	0.01 (0.00–0.02)	3	0.04 (0.04–0.04)	1	0.13 (0.10–0.19)	4	0.22 (0.13–0.28)	4
Leptoceroidea	0.00 (0.00)	2	0.15 (0.14–0.20)	2	0.23 (0.21–0.24)	2	0.23 (0.18–0.28)	5
Hydropsychoidea	0.00 (0.00–0.03)	4	0.11 (0.08–0.14)	3	0.21 (0.12–0.28)	5	0.25 (0.21–0.29)	10
Philopotamoidea	0.00 (0.00–0.00)	2	na		0.16 (0.16–0.16)	2	0.22 (0.16–0.26)	2
Hydroptiloidea	na		na		0.23 (0.00)	2	0.25 (0.22–0.27)	2
Rhyacophiloidea	0.00 (0.00–0.00)	9	0.12 (0.02–0.17)	5	0.16 (0.12–0.19)	7	0.22 (0.18–0.28)	24

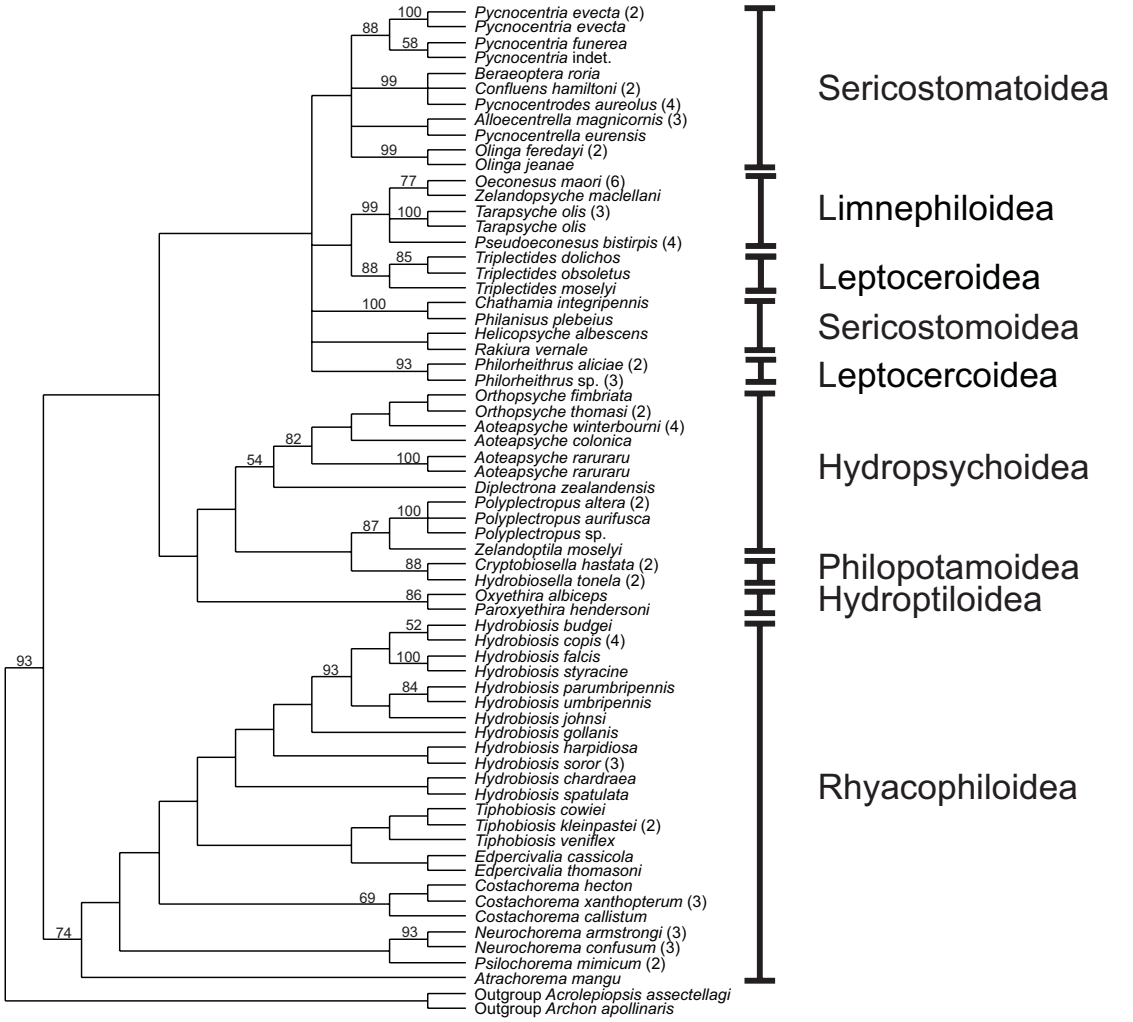

Fig. 1 Strict consensus tree of the six most parsimonious trees for 61 species of New Zealand caddisflies (superfamilies are indicated on the right). Numbers above branches indicate percentage nonparametric bootstrap support (>50%) from 1000 pseudoreplicates. The number of individuals with identical sequences is indicated in parentheses next to the taxonomic name.

taxa showed an A–T bias (A = 30%, T = 39%, C = 17%, G = 14%), and base frequencies were heterogeneous among sequences ($\chi^2_{189} = 223.64$, $P < 0.05$).

Twenty-two species were represented by two or more individuals allowing an analysis of within-species variation. Uncorrected p distances within these species for COI averaged 0.7% (range 0.0–8.5%; Table 1). Maximum divergence values in 20 of these species were less than 2%. However, two examples of deep intra-specific divergence were detected—individuals of *Pycnocentria evecta* (Conoesucidae) showed up to 8.5% divergence, whereas those of *Aoteapsyche rarururu* (Hydropsychidae) showed 2.7% divergence. When the values for these two species were excluded, within-species divergence for COI averaged 0.6%. In contrast, p distances between species within genera averaged 11.5% (range 2.3–19.5%), whereas species in different genera showed an average divergence of 21.7% (range 3.2–29.4%). The smallest distance between species in different genera (3.2%) involved the marine-affiliated species *Philanisus plebeius* and *Chathamia integripennis* (Chathamiidae).

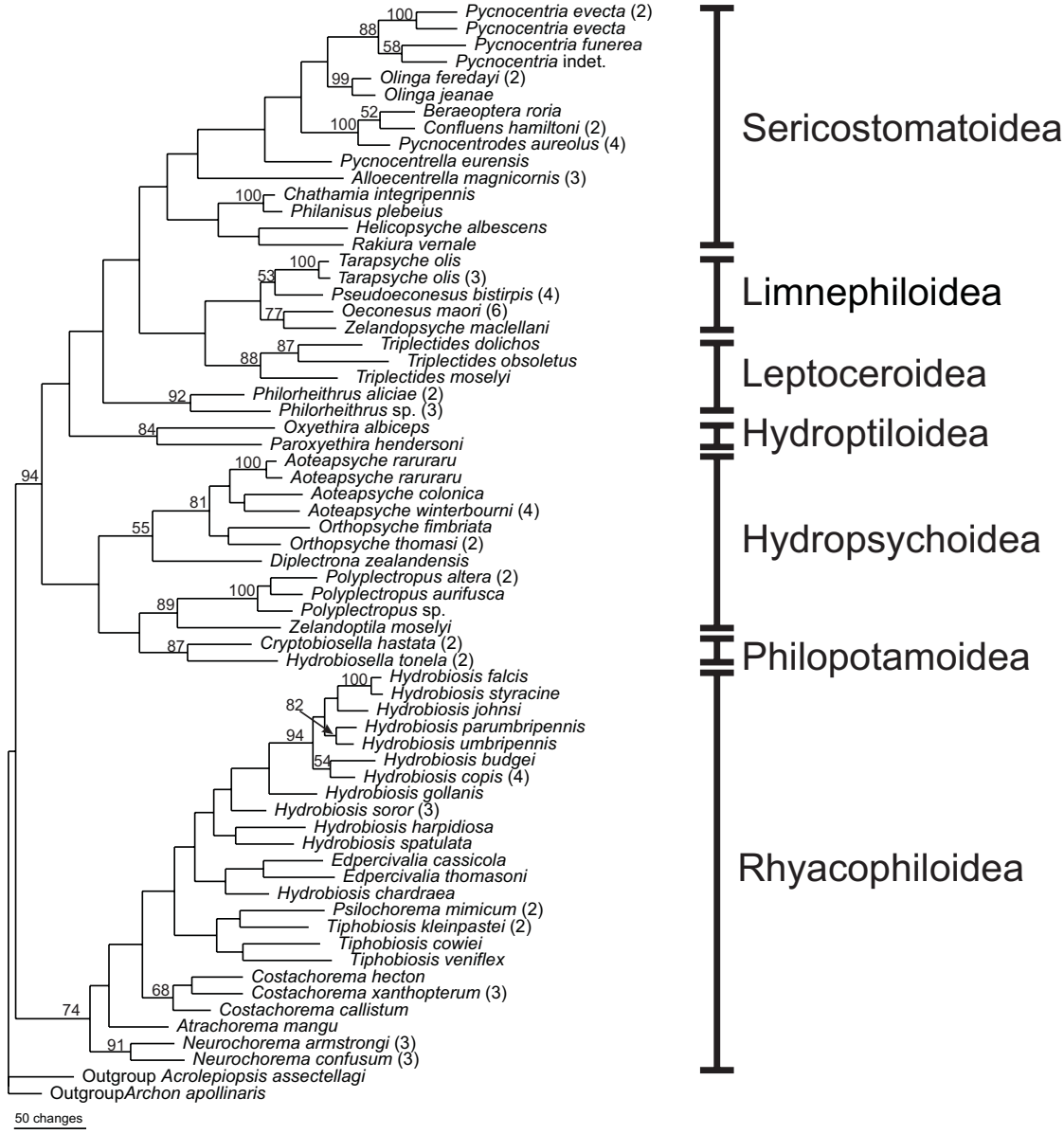

Fig. 2 Neighbour-joining tree for 61 species of New Zealand caddisflies (superfamilies are indicated on the right). Numbers above branches indicate percentage nonparametric bootstrap support (>50%) from 1000 pseudoreplicates. The number of individuals with identical sequences is indicated in parentheses next to the taxonomic name.

The analysis using maximum parsimony detected six most parsimonious trees from 351 parsimony informative characters, 20 variable but parsimony uninformative characters and 289 constant characters of tree length = 3775 and consistency index (excluding uninformative characters) = 0.18 and the retention index = 0.50. The strict consensus of the six most parsimonious trees shows the relationships among the nine superfamilies of caddisflies in New Zealand (Fig. 1). The single neighbour-joining tree had a tree length of 3810, a consistency index (excluding uninformative characters) of 0.19 and the

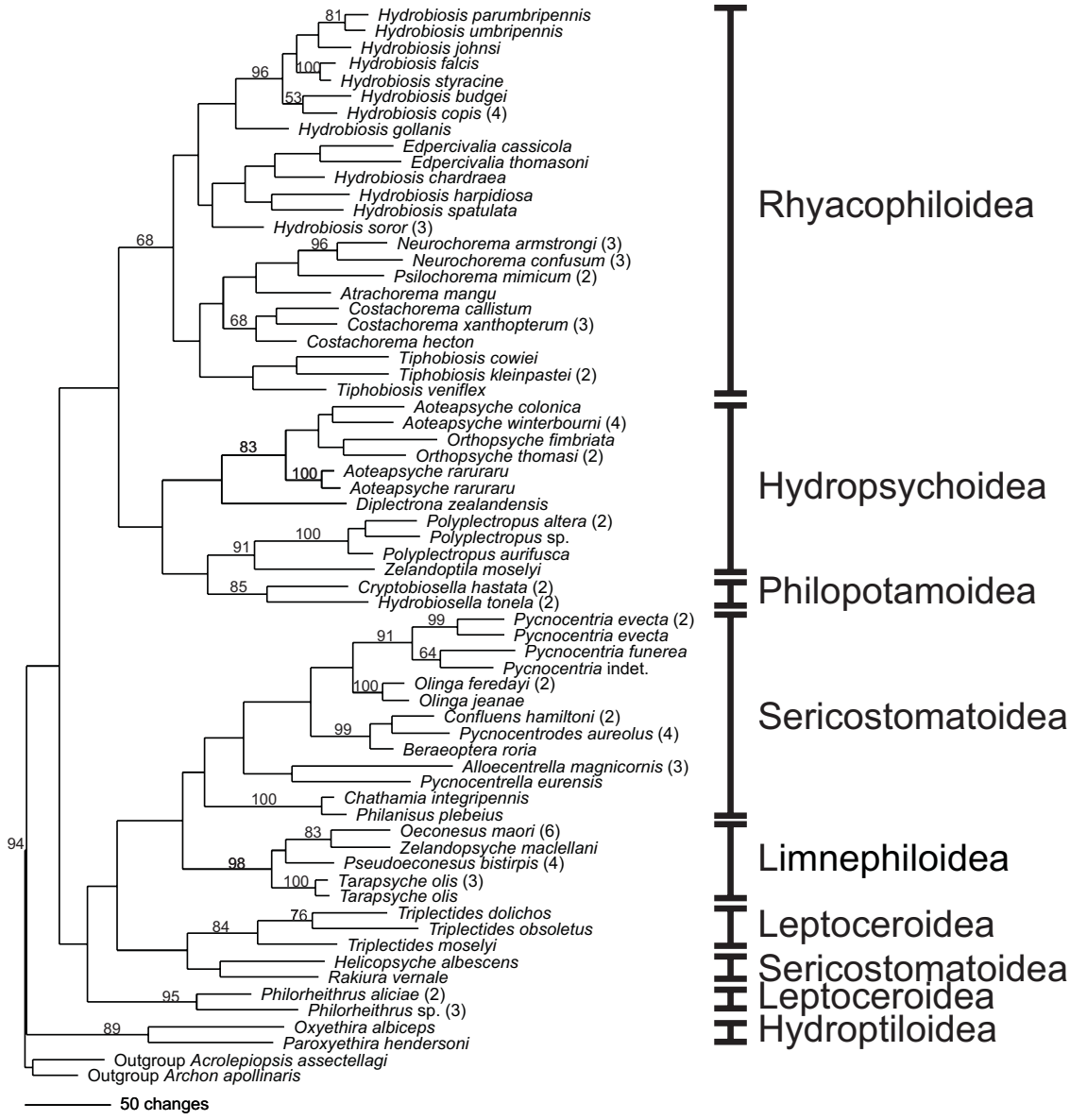

Fig. 3 Maximum likelihood tree for 61 species of New Zealand caddisflies (superfamilies are indicated on the right). Numbers above branches indicate percentage nonparametric bootstrap support (>50%) from 1000 pseudoreplicates. The number of individuals with identical sequences is indicated in parentheses next to the taxonomic name.

retention index of 0.49 (Fig. 2). Maximum likelihood analysis (Fig. 3) detected a single tree (log-likelihood score = –15074.8). The 50% majority rule consensus tree estimated from the Bayesian analysis was identical to that found using maximum likelihood, with the exception of one minor rearrangement for the placement of the *Triplectides* clade within the Sericostomatoidea (Bayesian tree not shown). The trees estimated from all four methods of analysis showed that families and genera, with the exception of *Orthopsyche* and *Aoteapsyche* (MP, ML, Bayesian), and *Edpercivalia* and *Hydrobiosis*

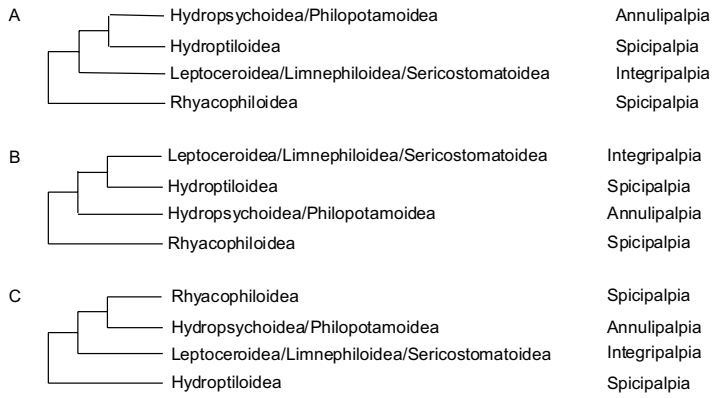

Fig. 4 Superfamily/suborder relationships estimated by: **A**, maximum parsimony; **B**, neighbour-joining; **C**, maximum likelihood and Bayesian algorithms. Superfamilies next to the branch tips are from Wiggins & Wichard (1989); suborders on the right are from Holzenthal et al. (2007).

(NJ, ML, Bayesian), were monophyletic (Fig. 1–3, Bayesian tree not shown).

The four methods of phylogenetic estimation detected trees that were significantly more similar to each other than they were to 1000 randomly generated trees (symmetric difference distances among the four methods: range 20–56, mean 52, $P < 0.001$). All four analyses resulted in the same four monophyletic groups, some of which did not correspond with currently recognised superfamilies. One monophyletic group included members of the superfamily Rhyacophiloidea, and another monophyletic group consisted of species from three superfamilies (Sericostomatoidea, Limnephiloidea, and Leptoceroidea). A third monophyletic group consisted of species from two superfamilies (Hydropsychoidea, Philopotamoidea), and a fourth was composed of the Hydroptiloidea. The estimation of relationships between these superfamilies differed depending on the method of analysis used, although maximum likelihood and Bayesian methods resulted in the same topology (Fig. 4). Sericostomatoidea (NJ, Bayesian and sometimes MP) and Limnephiloidea (all methods) were monophyletic groups within the Leptoceroidea/Limnephiloidea/Sericostomatoidea clade. Support for four main monophyletic clades was variable with percentage bootstrap support for 1000 replicates or Bayesian posterior probabilities = Hydropsychoidea/Philopotamoidea (<50 MP/<50 NJ/<50 ML/0.87 Bayesian posterior probability), Hydroptiloidea (86/84/89/1.00), Leptoceroidea/Limnephiloidea/Sericostomatoidea (<50/<50/<50/1.00) and Rhyacophiloidea (74/74/68/1.00) (Fig. 1–3, Bayesian tree not shown).

DISCUSSION

In general, sequence differences in COI revealed patterns of divergence among species that agreed with current alpha taxonomy developed solely through morphological analyses. Within-species divergences were very low in most instances (mean = 0.7%) in congruence with values recorded for other insect taxa such as Lepidoptera and Ephemeroptera (e.g., Hebert et al. 2003; Ball et al. 2005). However, *Pycnocentria evecta* (Conoesucidae) showed markedly higher genetic distances, suggesting that this taxon is a cryptic species complex.

In some instances, levels of divergence between species within a genus or between genera were low and fell in the range found for within-species comparisons. In three superfamilies (Sericostomatoidea, Limnephiloidea, Rhyacophiloidea), divergences between congeneric species overlapped with divergence values within species, and, in Sericostomatoidea, there was overlap in the divergence values found between species in different genera (e.g., Table 1). However, specimens were still assigned to their nominate species in all instances. The affected species in the Sericostomatoidea were both marine-affiliated and may have diverged relatively recently and hence are genetically similar, yet morphologically distinct—a pattern seen in other aquatic arthropods (e.g., Witt et al. 2003).

Levels of between-species divergence for some Rhyacophiloidea were also low (<3%). However, pairwise distances between species still exceeded that found for individuals within species, and for the nine species analysed within the Rhyacophiloidea, conspecific individuals had identical COI sequences.

Although no single divergence threshold value will enable recognition of all species, divergence values greater than 2% were usually indicative of different species. As a result, this value can be used as an approximate guideline for assessing species richness where more detailed analysis is not possible.

All phylogenetic analyses grouped the 61 species within the same four monophyletic clades. However, these clades did not correspond with the currently recognised classification of species within superfamilies (Holzenthal et al. 2007). This current placement of species within superfamilies produced both paraphyletic and polyphyletic groups within our phylogenies, suggesting that the present assignment of taxa to superfamilies may need reconsideration. We caution that this suggestion is based on only a single gene and a limited number of species (i.e., 61), and that the use of additional genes and individuals from further species may be required to fully resolve this issue. However, our results agree closely with a recent revision of caddisfly suborders (Holzenthal et al. 2007), although we found no support for placement of the Hydroptiloidea in Spicipalpia. All methods of analysis found strong support for monophyly of Rhyacophiloidea, and none found support for placement of the Hydroptiloidea as a sister taxon to Rhyacophiloidea, and thus did not support the inclusion of Hydroptilidea within Spicipalpia (Holzenthal et al. 2007).

Our estimation of the relationships among the superfamilies was not consistent across methods of phylogenetic estimations. Although ML and Bayesian methods estimated the same relationships among the four main clades, MP and NJ were both different. The inconsistency in the relationships estimated by the four methods reflects the low bootstrap support (<70%) and Bayesian posterior probabilities (<95%) for the relationships among the clades. Deeper relationships are known to be difficult to resolve with limited sequence data, in part because multiple substitutions can occur at a single nucleotide position (e.g., A – T – A), obscuring the true relationships (Banks & Whitfield 2006; Murphy et al. 2008).

In summary, we conclude that sequence variation in the barcode region of COI gene is effective for identifying individuals of New Zealand caddisflies to their nominate species. The use of COI sequences will enable previously unidentifiable larvae to be connected with their adult forms, thus aiding ecological studies (e.g., Zhou et al. 2007). Deep divergences within "species" may reflect cryptic species, whereas low divergences between some species pairs suggest the need for larger sample sizes, broader geographic coverage, and more comprehensive taxon sampling to ascertain if any species cannot be separated through barcode analysis. To further assess the utility of COI sequences for species identification of caddisflies, future research should target taxa with high levels of within-species divergence and those with low interspecific divergences.

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REFERENCES

Ahrens D, Monaghan MT, Vogler AP 2007. DNA-based taxonomy for associating adults and larvae in multi-species assemblages of chafers (Coleoptera: Scarabaeidae). Molecular Phylogenetics and Evolution 44: 436–449.

Ball SL, Hebert PDN, Burian SK, Webb JM 2005. Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. Journal of the North American Benthological Society 24: 508–524.

Banks JC, Whitfield JB 2006. Dissecting the ancient rapid radiation of microgastrine wasp genera using additional nuclear genes. Molecular Phylogenetics and Evolution 41: 690–703.

Barrett RDH, Hebert PDN 2005. Identifying spiders through DNA barcodes. Canadian Journal of Zoology 83: 481–491.

Chapman MA, Hogg ID, Schnabel KE, Stevens MI 2002. Synonymy of the New Zealand corophiid amphipod genus, *Chaetocorophium* Karaman, 1979, with *Paracorophium* Stebbing, 1899: morphological and genetic evidence. Journal of the Royal Society of New Zealand 32: 229–241.

DeSalle R, Freedman T, Prager EM, Wilson AC 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. Journal of Molecular Evolution 26: 157–164.

Elias M, Hill RI, Willmott KR, Dasmahapatra KK, Brower AVZ, Mallet J, Jiggins CD 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. Proceedings of the Royal Society of London Series B-Biological Sciences 274: 2881–2889.

Felsenstein J 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Hajibabaei M, deWaard JR, Ivanova NV, Ratnasingham S, Dooh RT, Kirk SL, Mackie PM, Hebert PDN 2005. Critical factors for the high volume assembly of DNA barcodes. Philosophical Transactions: Biological Sciences 362: 1959–1967.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN 2006. DNA barcodes distinguish species of tropical Lepidoptera. Proceedings of the National Academy of Sciences of the United States of America 103: 968–971.

Hebert PDN, Cywinska A, Ball SL, deWaard JR 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B-Biological Sciences 270: 313–322.

Hogg ID, Hebert PDN 2004. Biological identification of springtails (Collembola: Hexapoda) from the Canadian Arctic using mitochondrial DNA barcodes. Canadian Journal of Zoology 84: 749–754.

Holzenthal RW, Blahnik RJ, Prather AL, Kjer KM 2007. Order Trichoptera Kirby, 1813 (Insecta), caddisflies. Zootaxa 1668: 639–698.

Huelsenbeck JP, Ronquist F 2001. MR BAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754–755.

Johanson KA 2007. Association and description of males, females and larvae of two New Caledonian *Xanthochorema* species (Trichoptera: Hydrobiosidae) based on mitochondrial 16S and COI sequences. Entomological Science 10: 179–199.

Johanson KA, Kjer K, Malm T 2009. Testing the monophyly of the New Zealand and Australian endemic family Conoesucidae Ross based on combined molecular and morphological data (Insecta: Trichoptera: Sericostomatoidea). Zoologica Scripta 38: 563–573.

Johnson KP, Clayton DH 2003. Coevolutionary history of ecological replicates: comparing phylogenies of wing and body lice to columbiform hosts. In: Page RDM ed. Tangled trees. Phylogeny, cospeciation, and coevolution. University of Chicago Press, Chicago. Pp. 262–286.

Kjer KM, Blahnik RJ, Holzenthal RW 2002. Phylogeny of caddisflies (Insecta, Trichoptera). Zoologica Scripta 31: 83–91.

Meier R, Shiyang K, Vaidya G, Ng PKL 2006. DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. Systematic Biology 55: 715–728.

Murphy N, Banks JC, Whitfield JB, Austin AD 2008. Phylogeny of the parasitic microgastroid subfamilies (Hymenoptera: Braconidae) based on sequence data from seven genes, with an improved time estimate of the origin of the lineage. Molecular Phylogenetics and Evolution 47: 378–395.

Nolan L, Hogg ID, Sutherland DL, Stevens MI, Schnabel K 2007. Allozyme and mtDNA variability of the New Zealand damselflies *Xanthocnemis*, *Austrolestes,* and *Ischnura* (Odonata). New Zealand Journal of Zoology 34: 371–380.

Pauls SU, Theissinger K, Ujvarosi L, Balint M, Haase P 2009. Patterns of population structure in two closely related, partially sympatric caddisflies in Eastern Europe: historic introgression, limited dispersal and cryptic diversity. Journal of the North American Benthological Society 28: 517–536.

Penny D, Hendy MD 1985. The use of tree comparison metrics. Systematic Zoology 34: 75–82.

Posada D, Crandall KA 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Ratnasingham S, Hebert PDN 2007. BOLD: The barcode of life data system (www.barcodinglife.org). Molecular Ecology Notes 7: 355–364.

Resh VH 1993. Recent trends in the use of Trichoptera in water quality monitoring. In: Otto C ed. Proceedings of the 7th international Symposium on Trichoptera. The Hague, Dr W Junk. Pp. 315–319.

Rodríguez F, Oliver JF, Marín A, Medina JR 1990. The general stochastic model of nucleotide substitution. Journal of Theoretical Biology 142: 485–501.

Roe AD, Sperling FAH 2007. Patterns of evolution of mitochondrial cytochrome *c* oxidase I and II DNA and implications for DNA barcoding. Molecular Phylogenetics and Evolution 44: 325–345.

Ronquist F, Huelsenbeck JP 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Ronquist F, Huelsenbeck JP, van der Mark P 2005. MrBayes 3.1 manual. Retrieved 5 October 2009, from mrbayes.csit.fsu.edu/mb3.1_manual.pdf.

Skevington JH, Kehlmaier C, Stahls G 2007. DNA barcoding: Mixed results for big-headed flies (Diptera: Pipunculidae). Zootaxa 1423: 1–26.

Swofford DL 2002. PAUP*: phylogenetic analysis using parsimony. Sunderland, Massachusetts, Sinauer Associates Inc.

Tautz D, Arctander P, Minelli A, Thomas RH, Vogler AP 2003. A plea for DNA taxonomy. Trends in Ecology and Evolution 18: 70–74.

Tavaré L 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17: 57–86.

Templeton AR 1983 Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. Evolution 37: 221–244.

Trewick SA 2008. DNA barcoding is not enough: mismatch of taxonomy and genealogy in New Zealand grasshoppers (Orthoptera: Acrididae). Cladistics 24: 240–254.

Walker AK, Crosby TK 1988. The preparation and curation of insects. 2nd ed. DSIR Information Series 163. Mount Albert Research Centre, Auckland, New Zealand. 92 p.

Wiggins GB 1996. Larvae of the North American caddisfly genera (Trichoptera). 2nd ed. Toronto, University of Toronto Press. 457 p.

Wiggins GB, Wichard, W 1989. Phylogeny of pupation in Trichoptera, with proposals on the origin and higher classification of the order. Journal of the North American Benthological Society 8: 260–276.

Winterbourn M 2004. Stream invertebrates. In: Harding J, Mosely P, Pearson C, Sorrell B ed. Freshwaters of New Zealand. New Zealand Hydrological Society Inc. and New Zealand Limnological Society Inc. The Caxton Press, Christchurch. Pp. 16.1–16.14.

Witt JDS, Blinn DW, Hebert PDN 2003. The recent evolutionary origin of the phenotypically novel amphipod *Hyalella montezuma* offers an ecological explanation for morphological stasis in a closely allied species complex. Molecular Ecology 12: 405–413.

Yang Z, Goldman N, Friday A 1994. Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. Molecular Biology and Evolution 11: 316–324.

Zhou X, Kjer KM, Morse JC 2007. Associating larvae and adults of Chinese Hydropsychidae caddisflies (Insecta: Trichoptera) using DNA sequences. Journal of the North American Benthological Society 26: 719–742.