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Further use of molecular data in studying biogeographic patterns within the centipede genus *Craterostigmus*: the case for a monophyletic New Zealand species

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Abstract

A second species of the previously monotypic centipede genus *Craterostigmus* was recently established on the basis of New Zealand collections (*C. crabilli*) differing from the Tasmanian *C. tasmanianius* with respect to diagnostic characters in nuclear 18S and 28S rRNA, coupled with differences in body size, leg spinulation and internal anatomy. Analyses of molecular data resolved the New Zealand species as non-monophyletic because of the isolated phylogenetic position of a population from Lewis Pass on the South Island that had especially divergent cytochrome *c* oxidase subunit I (COI) sequences. Herein, previously missing 16S rRNA sequences for the Lewis Pass samples are added to the four-gene sample, together with newly collected specimens from South Island and Stewart Island. The more complete dataset retrieves both *C. crabilli* and *C. tasmanianus* as monophyletic, and the four-gene analysis dataset shows that Stewart Island and North Island populations fall outside a clade that unites most South Island samples. Despite its favoured role in DNA barcoding, COI performs more poorly than 18S, 28S or 16S rRNAs for identifying species of *Craterostigmus*.

Keywords: *Craterostigmus crabilli*, Craterostigmomorpha, COI, 16S rRNA, Lewis Pass, Stewart Island

1. Introduction

The centipede order Craterostigmomorpha, monotypic until recently, includes two species, *Craterostigmus tasmanianus* Pocock, 1902 in Tasmania, and *C. crabilli* Edgecombe & Giribet, 2008 in New Zealand. The New Zealand species can be differentiated from the type species in its internal anatomy (Prunescu & Prunescu 2006), body size, spinosity of particular leg podomeres, and can be easily diagnosed using molecular sequence data from the commonly sequenced genes 18S rRNA and 28S rRNA (Edgecombe & Giribet 2008). The phylogenetic/phylogeographic patterns of *C. crabilli* were recently investigated using four molecular markers and a broad geographic representation of the known localities for the species (Edgecombe & Giribet 2008).

Previous study concluded that nuclear ribosomal genes could be easily used as diagnostic molecular markers, showing only a few fixed changes and no apparent intraspecific variation, while mitochondrial markers showed informative variation for reconstructing within-species patterns. The mitochondrial ribosomal gene16S rRNA showed a pattern of North Island versus South Island vicariance not clearly recovered with the mitochondrial protein encoding cytochrome *c* oxidase subunit I. The latter gene furthermore failed to recover monophyly of each of the two species, and placed two specimens from Lewis Pass in the northern part of South Island completely outside *Craterostigmus*, instead resolving them amongst the outgroups. The failure in amplifying these two specimens for 16S rRNA prevented us from concluding whether this unusual position was due to accelerated evolution in cytochrome *c* oxidase subunit I, or a real phylogenetic pattern.

In this study, we build upon our previous work (Edgecombe & Giribet 2008) and add 16S rRNA sequence data for specimens from Lewis Pass, the locality that previously proved problematic. We also add several new specimens from New Zealand collected during a field trip in February 2008, including five from the South Island and one from Stewart Island, a land mass not represented in the previous study.

2. Materials and methods

New specimens were collected in February 2008 during a field trip to New Zealand by G. Giribet and S. Vélez, and include specimens from the Kahurangi N.P. (Flora Hut) and Ryans Creek Track on Stewart Island. We also added new data for the Lewis Pass specimens discussed by Edgecombe & Giribet (2008). Only specimens for which the mitochondrial genes were available were used in this study. Specimen distribution in New Zealand can be found in Edgecombe & Giribet (2008), with the addition of more specimens from the locality known as Flora Hut in the South Island, and from the northern part of Stewart Island. All specimens have been deposited at the Museum of Comparative Zoology, in the Department of Invertebrate Zoology (s. Appendix 1), and are stored at –80 ºC. Molecular data were obtained following the protocols and primers described by Edgecombe & Giribet (2008).

The analyses were restricted to the two informative regions of 18S rRNA and 28S rRNA plus the two mitochondrial genes 16S rRNA and cytochrome *c* oxidase subunit I (COI). Analyses were conducted with the new computer program POY v.4.0.2870 (Varón et al. 2008) under direct optimisation and using parsimony as the optimality criterion (Wheeler 1996, Wheeler et al. 2006) with the parameter set selected by Edgecombe & Giribet (2008) (indel opening cost = 3, indel extension cost = 1, base substitution = 2) (see De Laet 2005). Analyses consisted of a driven search (time = 1 hour) with ratchet (Nixon 1999) and tree fusing (Goloboff 1999). All partitions were analysed in combination. In addition, 16S rRNA and COI data sets were analysed independently and their implied alignments were used to generate trees with branch lengths proportional to the number of changes (under equal weights) using PAUP* (Swofford 2002). Nodal support was evaluated with parsimony jackknifing (Farris et al. 1996, Farris 1997).

3. Results

Analysis of the combined data set resulted in two trees of 2303 weighted steps. The search evaluated 11 independent repetitions with ratchet and fusing for 39 generations. The shortest

trees were found 13 times and differed only in the internal resolution of the Flora Hut specimens. Their strict consensus with jackknife values is presented in Fig. 1. Our combined data set shows monophyly of each *Craterostigmus* species, a result that was not found in Edgecombe & Giribet (2008) due to the divergence in COI sequence data in the Lewis Pass specimens, for which no 16S rRNA data were hitherto available (see 16S rRNA and COI trees in Figs 2–3). Interestingly, the Stewart Island specimen included in this analysis constitutes another lineage of *C. crabilli* not necessarily connected to any other lineage of South Island specimens. Its position varies, depending on the analyses or parameter sets explored (results not shown), but may appear as sister to all other *C. crabilli*, or sister to the South Island specimens. Specimens from the North Island, where the species is less abundant than in the South Island (most probably due to recent forest degradation), form a clade, even though they belong to two different mountain ranges (Figs 1–3). The South Island specimens appear in three distinct clades, (a) one including specimens from as far apart as the Catlins in the southernmost part of the island, Aoraki/Mt Cook and Arthur's Pass, (b) a second distinct lineage represented by the Lewis Pass specimens, and (c) a northern clade including the specimens from the Nelson Lakes and Flora Hut in the Kahurangi N.P.

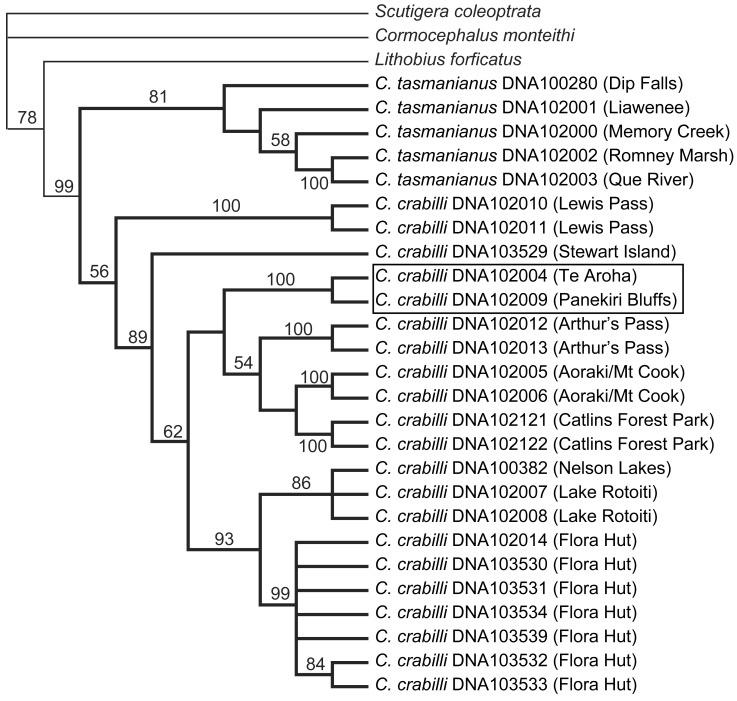

Fig. 1 Strict consensus of the two trees (length of 2302 weighted steps) obtained under parsimony direct optimisation for the combined analysis of all data. Numbers on branches are jackknife values. The square includes the North Island specimens.

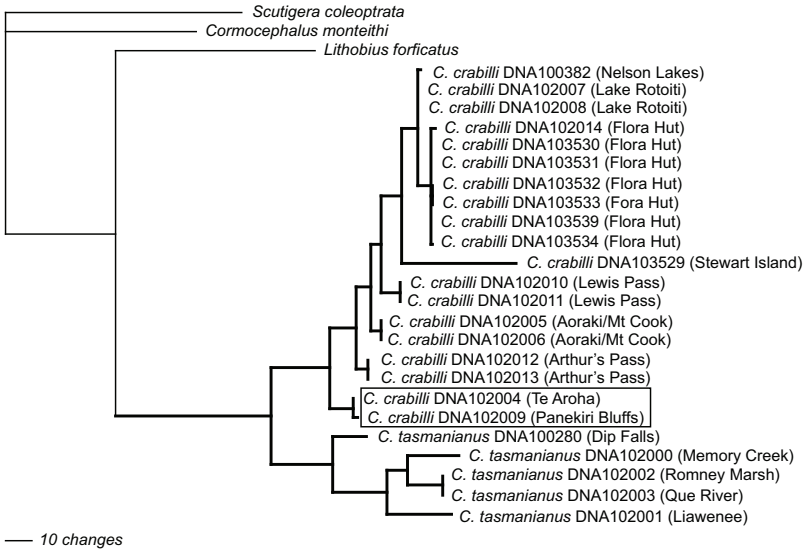

Fig. 2 One of two optimal trees at 698 weighted steps obtained under parsimony direct optimisation of the 16S rRNA data set. Topological differences occur only among the Flora Hut specimens. Branch lengths (under equal weights including indels as characters) were traced with PAUP* and are proportional to the number of changes.

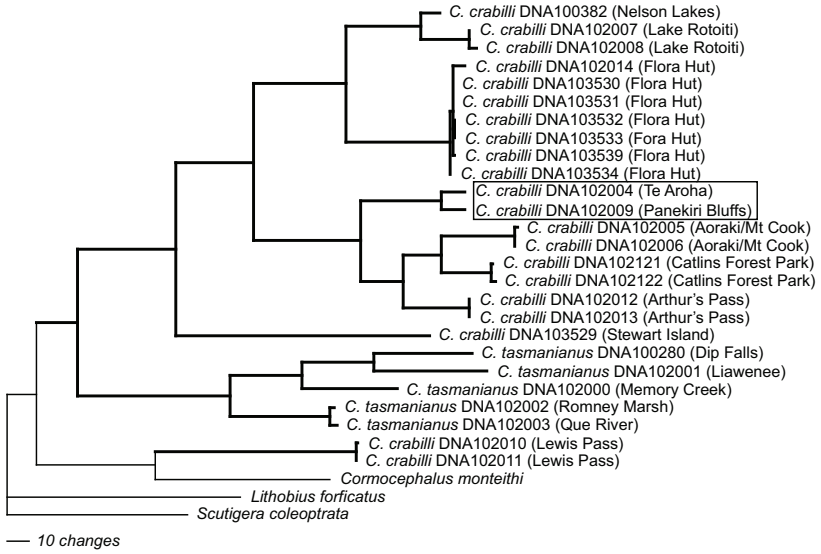

Fig. 3 One of 9 optimal trees at 1202 weighted steps obtained under parsimony direct optimisation of the COI data set. Topological differences occur only among the Flora Hut specimens. Branch lengths (under equal weights including indels as characters) were traced with PAUP* and are proportional to the number of changes.

4. Discussion

The results of the present study resolve the interrelationships of *Craterostigmus* with a better fit to biogeography than did a previous study (Edgecombe & Giribet 2008), which depicted *C. crabilli* as polyphyletic for the COI analysis as well as for combined analysis of all four genes. The four-gene analysis now resolves both *C. crabilli* and *C. tasmanianus* as monophyletic. This hypothesis of mutual monophyly conforms better with a vicariant, trans-Tasman explanation for speciation in *Craterostigmus* than did the previous results. An alternative trans-Tasman dispersal explanation would not be consistent with both species being monophyletic (one species should be expected to be paraphyletic with respect to the other).

Non-monophyly in the genes of two sister species could be explained by incomplete lineage sorting, which could lead to a discordance of gene trees and species trees and thus a lack of reciprocal monophyly (e.g. Avise et al. 1983), but this does not seem to be the case for COI, which places some haplotypes from Lewis Pass well before the divergence between the two species. This pattern strongly conflicts with the fixed nucleotide changes in the nuclear ribosomal genes and the reciprocal monophyly of the 16S rRNA haplotypes.

The addition of 16S rRNA sequence data for the Lewis Pass specimens proved to have a pivotal role in allying these samples with *C. crabilli*. The 16S tree (Fig. 2) depicts the Lewis Pass specimens within a South Island-Stewart Island clade, with the North Island haplotypes sister to the remaining *C. crabilli*, and *C. tasmanianus* in turn sister to *C. crabilli*. The more basal position of the Lewis Pass samples in the combined analysis (Fig. 1) reflects the continued tendency of the highly-divergent COI sequences to attract the Lewis Pass samples with outgroups (Fig. 3). Given the continued advocacy of COI as the standard for species identification in DNA barcoding initiatives, we point out that in the case of *Craterostigmus* this gene performs especially poorly for species identification. The more conserved nuclear ribosomal 18S and 28S rRNAs both allow for accurate identification of the Lewis Pass specimens as *C. crabilli*, as does 16S rRNA.

5. Acknowledgements

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Appendix 1 Specimen sampling with MCZ voucher numbers, locality data and GenBank accession numbers for the four loci sampled. New GenBank accession numbers are FJ550311-FJ550341.

	Voucher	Locality	Island	18S rRNA	28S rRNA	16S rRNA	COI
Scutigera coleoptrata				AF173238	AF173269	AF370859	DQ201426
Lithobius forficatus				EU024571	X90656	AF373608	AJ270997
Cormocephalus monteithi				AF173249	AF173280	AF370861	DQ201430
Craterostigmus tasmanianus	DNA100280	Dip Falls	Tasmania		EU024586	AF370860	AF37835
Craterostigmus tasmanianus	DNA102000	Memory Creek	Tasmania	EU024572	EU024587	EU024597	EU024611
Craterostigmus tasmanianus	DNA102001	Liawenee	Tasmania	EU024573	EU024588	EU024598	EU024612
Craterostigmus tasmanianus	DNA102002	Romney Marsh	Tasmania			EU024599	EU024613
Craterostigmus tasmanianus	DNA102003	Que River	Tasmania	EU024574	EU024589	EU024600	EU024614
Craterostigmus crabilli	DNA100382	Nelson Lakes NP	South Island, NZ	EU024575	AY288706	AY288718	EU024615
Craterostigmus crabilli	DNA102004	Mt Te Aroha	North Island, NZ	EU024575	EU024590	EU024602	EU024616
Craterostigmus crabilli	DNA102005	Aoraki/Mt Cook	South Island, NZ	EU024576	EU024591	EU024603	EU024617
Craterostigmus crabilli	DNA102006	Aoraki/Mt Cook	South Island, NZ			EU024604	EU024618
Craterostigmus crabilli	DNA102007	Lake Rotoiti	South Island, NZ			EU024605	EU024619
Craterostigmus crabilli	DNA102008	Lake Rotoiti	South Island, NZ			EU024606	EU024620
Craterostigmus crabilli	DNA102009	Panekiri Bluffs	North Island, NZ	EU024577	EU024592	EU024607	EU024621
Craterostigmus crabilli	DNA102010	Lewis Pass	South Island, NZ	EU024578	EU029985	FJ550326	EU024622
Craterostigmus crabilli	DNA102011	Lewis Pass	South Island, NZ	EU024579	FJ550318	FJ550327	EU024623
Craterostigmus crabilli	DNA102012	Arthur's Pass	South Island, NZ	EU024580	EU024593	EU024608	EU024624
Craterostigmus crabilli	DNA102013	Arthur's Pass	South Island, NZ	EU024581		EU024609	EU024625
Craterostigmus crabilli	DNA102014	Flora Hut	South Island, NZ	EU024582	EU024594	EU024610	EU024626
Craterostigmus crabilli	DNA102121	Catlins Forest Park	South Island, NZ	EU024584	EU024595		EU024627
Craterostigmus crabilli	DNA102122	Catlins Forest Park	South Island, NZ	EU024585	EU024596		EU024628
Craterostigmus crabilli	DNA103529	Stewart Island	South Island, NZ	FJ550311	FJ550319	FJ550328	FJ550335
Craterostigmus crabilli	DNA103530	Flora Hut	South Island, NZ	FJ550312	FJ550320	FJ550329	FJ550336
Craterostigmus crabilli	DNA103531	Flora Hut	South Island, NZ	FJ550313	FJ550321	FJ550330	FJ550337
Craterostigmus crabilli	DNA103532	Flora Hut	South Island, NZ	FJ550314	FJ550322	FJ550331	FJ550338
Craterostigmus crabilli	DNA103533	Flora Hut	South Island, NZ	FJ550315	FJ550323	FJ550332	FJ550339
Craterostigmus crabilli	DNA103534	Flora Hut	South Island, NZ	FJ550316	FJ550324	FJ550333	FJ550340
Craterostigmus crabilli	DNA103539	Flora Hut	South Island, NZ	FJ550317	FJ550325	FJ550334	FJ550341