Untangling lichens: a morphological and molecular study of the genus *Cladia* in Aotearoa / New Zealand.

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Introduction

Coral lichens (species of *Cladia*, *Pulchrocladia* and *Rexiella*) are conspicuous and attractive elements of Aotearoa grasslands, boggy or peaty habitats, rocky outcrops, and tree fern bases in forests. Recent molecular studies have found that *Cladia aggregata*, a species previously found in South and Central America, Australasia, Southeast Asia, and India, is now no longer recognized as being present in Australasia (Parnmen et al. 2012). Australasian specimens previously named as this species are now referred to multiple morphologically similar species, some newly named (Parnmen et al. 2013). For Aotearoa, a lack of morphological distinction has presented challenges in distinguishing the different species in the field or in herbarium collections which has implications for their conservation management, particularly as at least one species appears to be threatened. In addition, the new treatment for the genus has not been universally welcomed, particularly in Australia (Kantvilas 2019). This study aims to review all the species recorded for Aotearoa using molecular and morphological methods, identify useful distinguishing morphological traits and create a key to the species for field workers.

Methods

Fresh specimens of *Cladia* species were obtained from the Northland / Auckland regions, with supplementary collections from around Aotearoa and Rekohu. DNA was extracted using Qiagen DNeasy Plant Mini Kit, following the manufacturer's instructions. The nuclear ITS rDNA spacer region was amplified using using primer pairs ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). Phylogenetic reconstruction using maximum likelihood (ML) and Bayesian inference (BI) was performed using the novel sequences of the internal transcribed spacers (ITS1 and ITS2) and 5.8S nuclear ribosomal region of the fungal partner of *Cladia* with additional sequences from GenBank. Morphological and anatomical examination of fresh specimens and herbarium material was carried out in parallel.

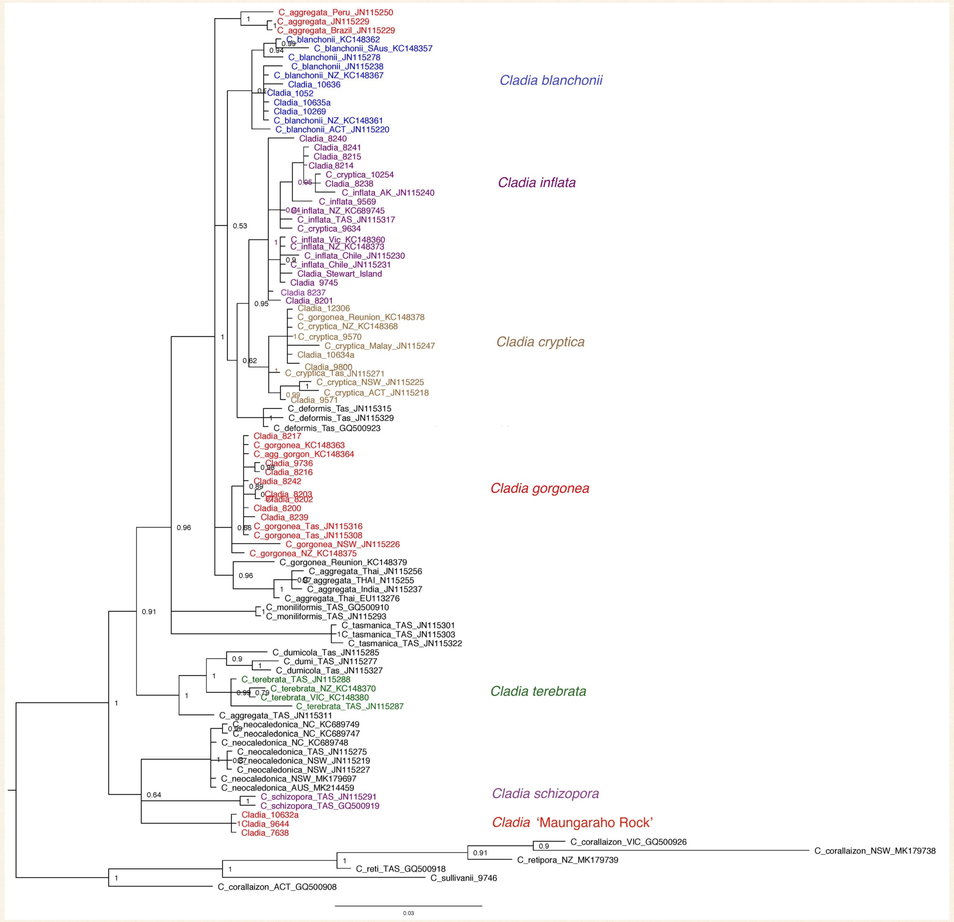

Figure 1, 50% majority rule Bayesian tree from MrBayes. Node labels are posterior probabilities

Results

ML and BI phylogenetic trees showed broad support for current species groupings, with collected specimens falling into monophyletic clades describing the species *C. cryptica*, *C. inflata*, *C. blanchonii*, *C. gorgonea*, and *C. terebrata* (Fig. 1). In addition, specimens from Maungaraho Rock in Northland (Fig. 2) were found to form another clade, highly divergent from the other species, and there is strong genetic support for it to be a separate species. This new clade belongs to a larger clade consisting of the sister taxa *C. schizopora* and *C. neocaledonica*. However, because the exact topology of this clade varies between analyses, the relationships among these three species remain unresolved.. The morphological and anatomical part of the study is ongoing, subtle differences have been found between all of the species, but results are not presented here.

Figure 2, *Cladia* specimen from Maungaraho Rock, Northland

Discussion

This research has largely confirmed the species recognized by Parnmen et al (2013), and has also identified a genetically and morphologically distinct taxon, which is currently being described as a new species. The subtle morphological differences between the different species are being used to develop an identification key for the genus.

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