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Identification of species in the *Cladia aggregata* group using DNA barcoding (Ascomycota: Lecanorales)

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Abstract

The DNA barcode approach is using a short genetic marker for rapid identification of a particular species. The internal transcribed spacer (ITS) region has been chosen as barcode marker for fungi. Here we tested the potential of ITS to identify distinct lineages in the *Cladia aggregata* complex, a group of lichenized fungi exhibiting remarkable morphological and chemical diversity. Our recent studies using multilocus DNA sequence data and coalescent-based species delimitation methods supported a 12 species delimitation scenario. In this study, we evaluated the ratio of the intra- and interspecific genetic distances of ITS in these 12 putative species. All 12 putative species showed a lower ratio of intraspecific variation than interspecific variation, supporting the hypothesis that these represent distinct lineages. Consequently, these lineages are here accepted at species level and three new species, viz. *Cladia blanchonii* Parnmen & Lumbsch, *C. cryptica* Parnmen & Lumbsch and *C. tasmanica* Parnmen & Lumbsch are described and the new combinations *Cladia gorgonea* (Eschw.) Parnmen & Lumbsch, *C. neocaledonica* (Räs.) Parnmen & Lumbsch, and *C. terebrata* (Laurer) Parnmen & Lumbsch proposed.

Keywords: Cladoniaceae, coalescence, cryptic species, DNA barcode, ITS, species delimitation

Introduction

DNA barcoding is an important tool for rapid species identification, accelerates biodiversity inventories, and helps in detection of cryptic species (Hebert *et al.* 2003, 2004; Hebert & Gregory 2005). For fungi, including lichenized fungi, the ITS rDNA region has been chosen as universal barcode (Schoch *et al.* 2012). In lichenized fungi, species delimitations remain difficult (Crespo & Lumbsch 2010; Crespo & Pérez-Ortega 2009; Lumbsch & Leavitt 2011). Phenotypical characters used for species delimitation are often not congruent with lineages identified in phylogenetic analyses of molecular data. This includes cases in which morphological characters, such as different reproductive modes (e.g., soredia vs. apothecia) have not been supported to distinguish species but appear to represent intraspecific plasticity (Articus *et al.* 2002; Buschbom & Mueller 2006; Cubero *et al.* 2004; Ott *et al.* 2004; Seymour *et al.* 2007; Tehler & Irestedt 2007; Tehler *et al.* 2009; Wirtz *et al.* 2008, 2012). Frequently, however, molecular studies reveal the presence of distinct lineages hidden under names of supposedly widely distributed species (Argüello *et al.* 2007; Crespo & Lumbsch 2010; Crespo & Perez-Ortega 2009; Divakar *et al.* 2005a, b, 2010, 2012; Elix *et al.* 2009; Hodkinson & Lendemer 2011; Leavitt *et al.* 2011a, b, c, 2012a, b, c, 2013a; Lumbsch & Leavitt 2011; Molina *et al.* 2004; Núñez-Zapata *et al.* 2011; Otálora *et al.* 2010; Parnmen *et al.* 2012; Wedin *et al.* 2009). Cases in which the phenotype-based species delimitation underestimates the diversity in a lineage are especially common in species complexes that have traditionally been regarded as difficult due to variability of characters and where authors accepted sometimes widely different species delimitations based on the interpretation of morphological and chemical variation.

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