

Two are better than one: Delimiting cryptic species within the lichen-forming genus *Peltigera* Section *Polydactylon* (Ascomycota, Lecanoromycetes) using DNA sequences from the fungal and cyanobacterial partners.

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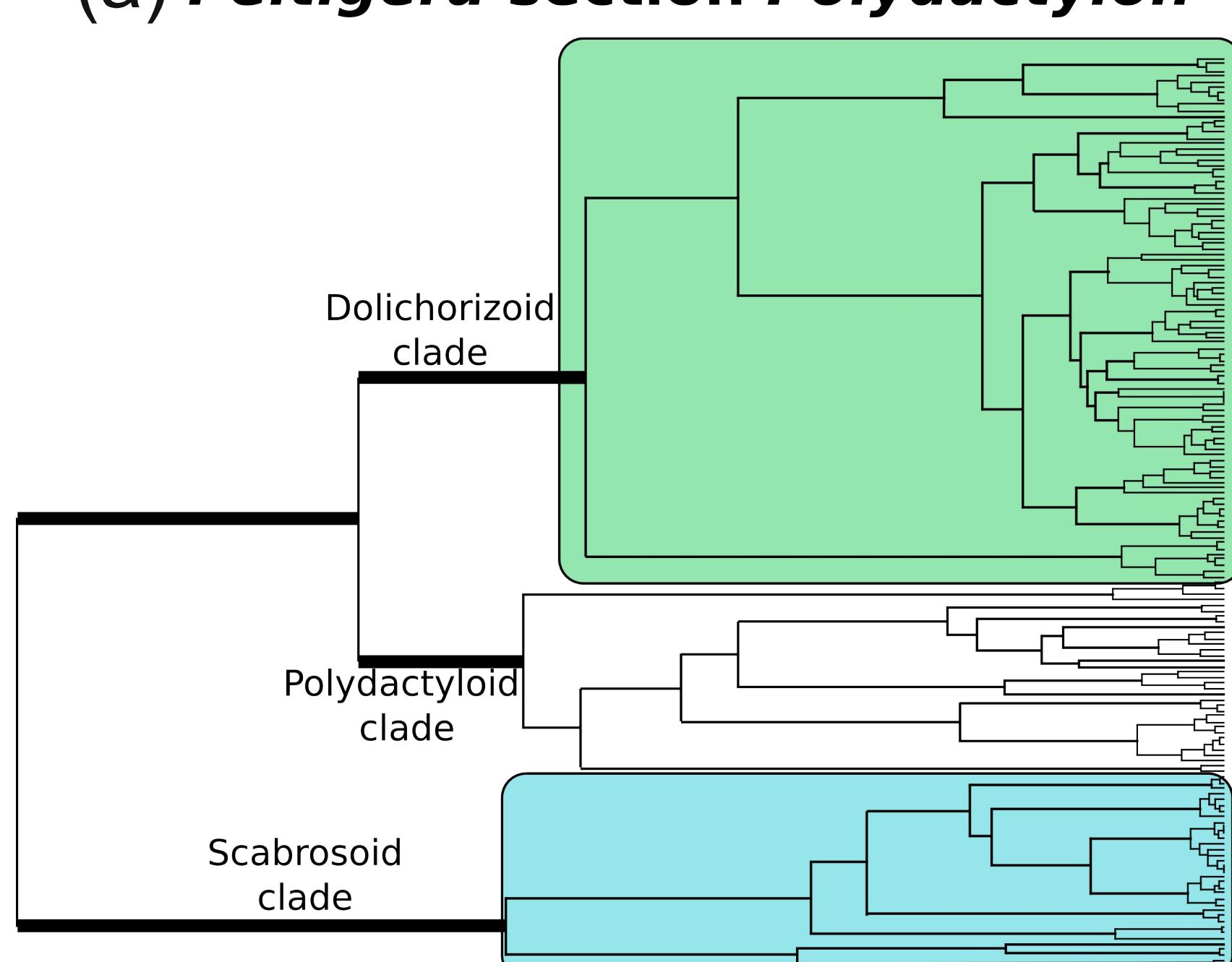
Introduction

Delimiting species is challenging in many groups of fungi because of the lack of diagnostic phenotypic traits, and difficulty to detect interbreeding, preventing an implementation of the morphological and biological species concepts. The genus *Peltigera*, section *Polydactylon* includes well-known described species, but is suspected to comprise many new species, not detected because of their similar morphology and due to poor sampling in many parts of the world. A recent radiation within this section further complicated the recognition of distinct species using phylogenetic methods.

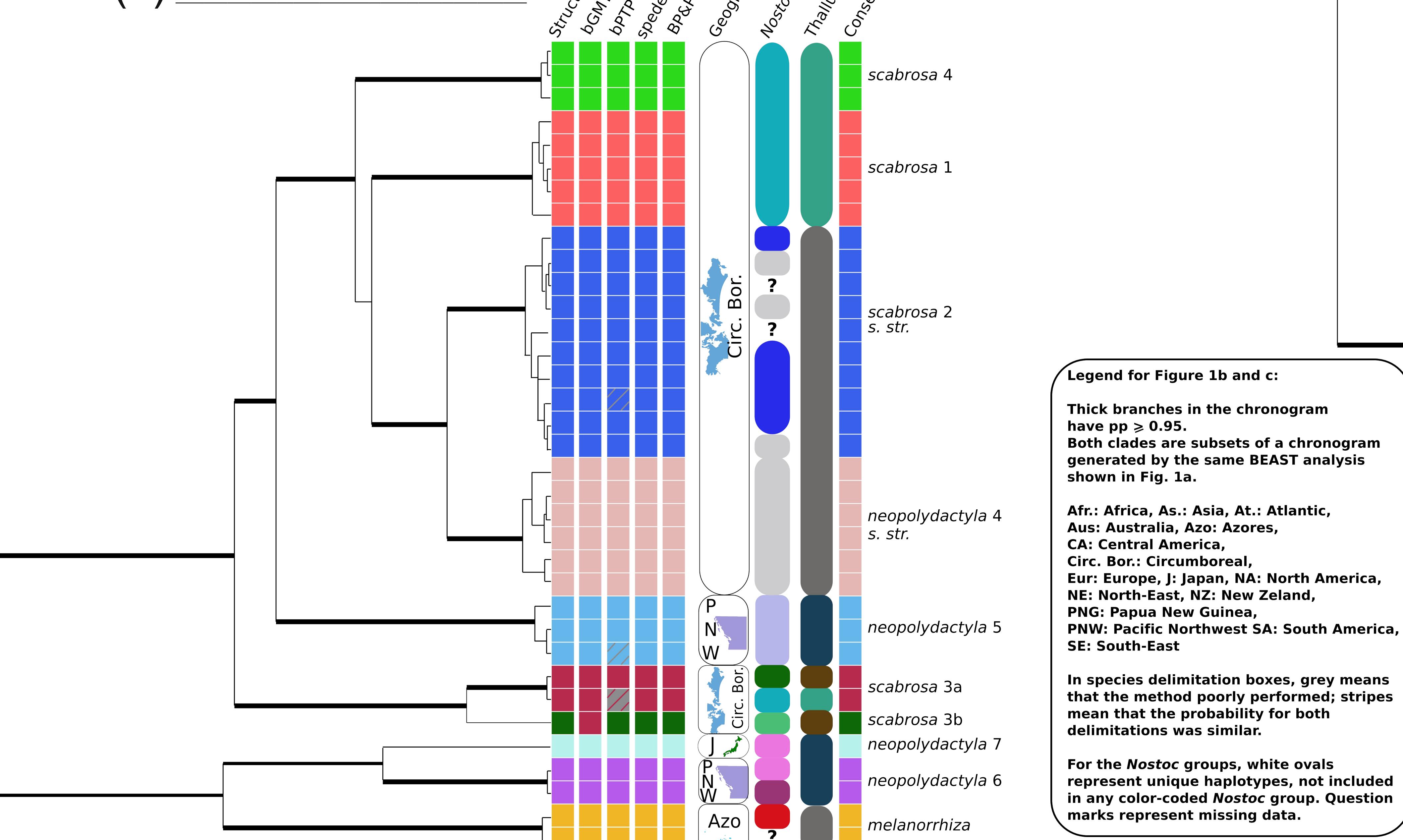
Material and methods

We sequenced eight loci (two nuclear ribosomal RNA loci, three protein-coding genes and three newly developed fast-evolving intergenic spacer regions) of the fungal partner from ca. 200 thalli selected across the world. We tested the boundaries of the fungal species using methods such as Structurama (1), bGMYC (2,3), bPTP (4), spedestEM (5) and BP&P (6,7). We also sequenced the *rbcLX* locus of the cyanobionts and recognized several distinct *Nostoc* lineages.

(a) *Peltigera* section *Polydactylon*



(b) Scabrosoid clade



(c) Dolichorhizoid clade

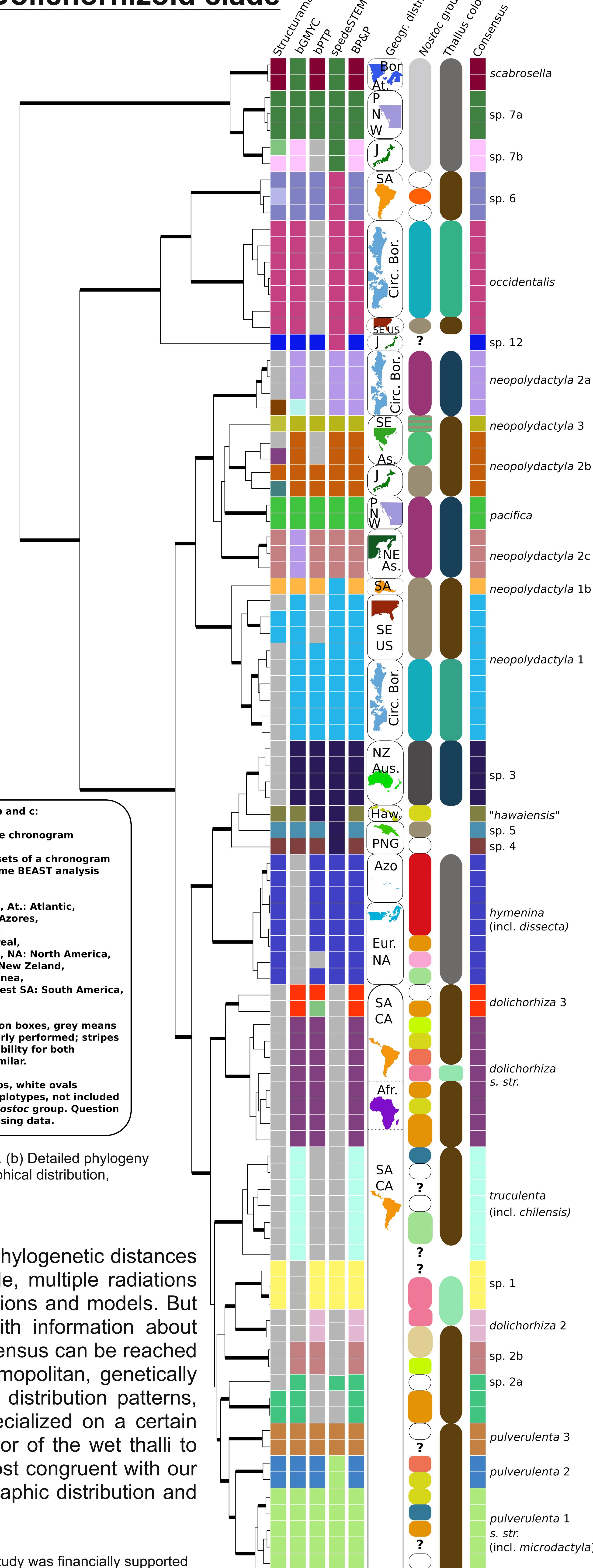


Figure 1. (a) Chronogram of *Peltigera* section *Polydactylon* generated with BEAST (8) on 8 loci (6553 characters) representing 164 taxa. (b) Detailed phylogeny of the Scabrosoid clade, and (c) the Dolichorhizoid clade, with results from five species delimitation methods, information about geographical distribution, patterns of *Nostoc* associations, general color of the thallus and a consensus on species delimitations.

Results and discussion

The results of the species delimitation methods converge well in the Scabrosoid clade where phylogenetic distances within delimited groups are distinctly smaller than among groups. In the Dolichorhizoid clade, multiple radiations complicate species delimitation, as each method delimits species based on different assumptions and models. But even in this complex clade, species delimitation are often congruent. When combined with information about geographic distribution and patterns of association with *Nostoc*, a biologically meaningful consensus can be reached for species delimitation within the Dolichorhizoid clade. Indeed, if morphospecies seem cosmopolitan, genetically defined species are not. The consensus species proposed here usually have very specific distribution patterns, which is an important asset for identifying them. Moreover, because many species are specialized on a certain *Nostoc* lineage, which often gives a specific color to the thallus, it is possible to use the color of the wet thalli to identify species within section *Polydactylon*. The species delimitation by BP&P seems the most congruent with our species concepts. Molecular data is a very powerful tool, but we also demonstrate that geographic distribution and the cyanobiont are very helpful in identifying lichen-forming fungal species!

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