Multilocus-based phylogeny and species recognition within the cosmopolitan *Peltigera neopolydactyla-dolichorhiza* complex

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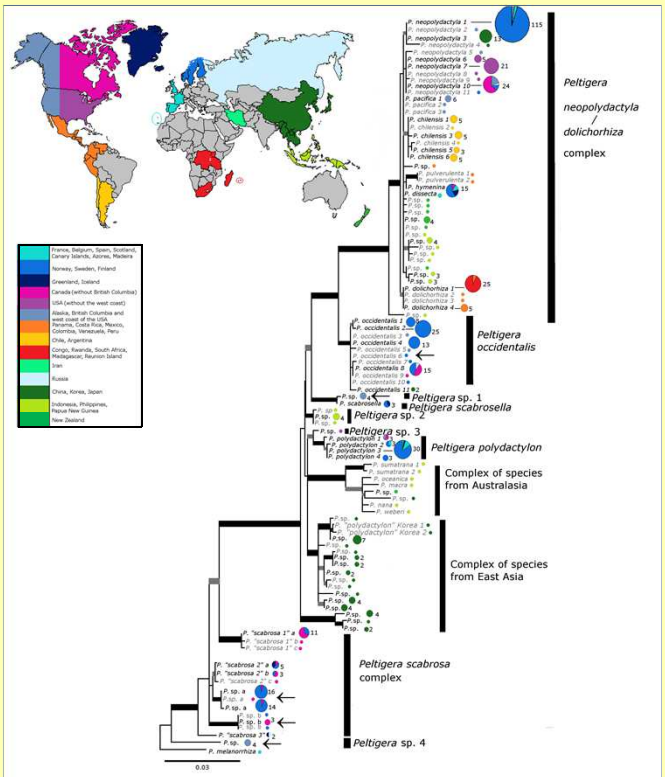

Figure 1. Phylogenetic relationships among 104 ITS-based haplotypes representing 525 specimens from *Peltigera* section *Polydactylon* using maximum likelihood as the optimization criterion. Thick black and grey internodes correspond to bootstrap values (BS) > 90% and 70%<BS<90%, respectively, based on 1000 pseudoreplicates. Pie charts associated with each haplotype represent the geographical collection sites for each specimem. Colors in the pie charts refer to the world map. The size of the pie chart is proportional to the number of identical sequences recovered for each haplotype, which is provided beside each pie chart. N = 1 when no number is shown for a specific pie chart. Arrows point to specimens that were identified as *P. neopolydactyla* or *P. dolichorhiza* but does not belong to this complex and might represent new species

Introduction

The *Peltigera neopolydactyla/dolichorhiza* complex is broadly distributed, growing in boreal and temperate regions from northern Norway to southern Chile, as well as in tropical mountains. Observed morphotype and chemotype variation within this complex suggested the presence of multiple undescribed species. This hypothesis was supported by a preliminary phylogeny using the ITS region, where *P. neopolydactyla* and *P. dolichorhiza* are not distinct monophyletic species, but rather form a complex assemblage of species with several well-known species, such as *P. hymenina*, nested within that species complex (Sérusiaux et al. 2009). We inferred the phylogeny of *Peltigera* section *Polydactylon* with a special focus on the *Peltigera neopolydactyla/dolichorhiza* complex to determine the full breadth of this species complex, and to assess if taxa from different parts of the worlds but with similar morphological features share a most recent common ancestor.

Materials and methods

We gathered material from herbaria and collected specimens of *Peltigera* in various countries. About 525 ITS sequences representing 104 distinct haplotypes were generated for representatives of *Peltigera* section *Polydactylon* (Fig.1). Maximum likelihood (ML) tree and bootstrap support (BS) were estimated using Rax-ML HPC (Stamatakis 2006) on CIPRES portal (Miller et al. 2010), with GTRGAMMA model and 1000 replicates on a data matrix containing a representative of each ITS haplotype and partitioned according to ITS1+ITS2 and 5.8S regions. Using the ITS tree we selected a representative of each broadly defined phylotype (see Fig. 1), for which three protein-coding loci: *RPB1*.1, β-tubulin and *EFT2*.1 (Elongation Factor 2 region 1, a new protein-coding marker developed as part of the AFTOL2 project) were sequenced (Table 1). ML search for the best tree and bootstrap analyses were conducted on each single locus for the combinability purpose (Mason-Gamer and Kellogg 1996) and on the combined data set using the same search parameters as for the ITS analyses. Each locus was partitioned according to the 1st, 2nd and 3rd position and non-coding regions(spliceosomal introns), if present. The root of the ingroup was inferred from the ML analyses on the extended dataset incorporating members of the *Horizontales* section as the outgroup (Miadlikowska and Lutzoni 2000).

Results and discussion

1. Topologies resulting from single locus analyses were in general congruent, however, a moderate conflict was detected in the placement of *Peltigera* sp. 1 (sister to *P. polydactylon* in the *EFT2*.1 tree *(*BS =75%) but closely related to *P. occidentalis* in the remaining trees) and *P. scabrosa* 3 *(*sister to *P. melanorrhiza* in the ITS tree (BS = 80%) but nested in the *P. scabrosa* complex in the remaining trees (Fig. 2). This topological incongruence may result from differences in taxon sampling among loci (Table 1) and will be further explored.

2. Each of the three protein coding loci provided equivalent or more resolution and support than the ITS locus (Fig. 2 and Table 1), which is highly variable within section *Polydactylon*, and required the exclusion of many ambiguously-aligned sites from our phylogenetic analyses at this stage of our project. The greatest proportion of significantly supported nodes across the tree resulted from β-tubulin alone (Table 1). *EFT2*.1 and *RPB1*.1 were also helpful especially in providing support for deeper nodes in the section and for the relationships within the *P. scabrosa* complex. However, none of these two genes, when alone, contributed substantially to resolving phylogenetic relationships in the *P. neopolydactyla/dolichorhiza* complex. The combined topology is very well resolved and most internodes are significantly supported (BS >90%; Fig. 3).

3. Many specimens identified as *P. neopolydactyla* and *P. dolichorhiza* are placed outside of this species complex (black arrows in Fig.1), such as individuals from Papua New Guinea described as *P. dolichorhiza* (Sérusiaux et al. 2009) or non-scabrous members of the *P. scabrosa* group, previously identified as *P. neopolydactyla*. As currently defined both species represent polyphyletic assemblages of taxa including several potentially undescribed species (Figs. 2 and 3).

4. In addition to the *P. neopolydactyla/dolichorhiza* complex, our phylogenies suggest the presence of putatively new species within the *P. scabrosa* complex, as well as several other undescribed species (including cryptic species) distributed across the section and occurring mostly in Australasia and Eastern Asia (Figs. 2 and 3).

5. The ITS haplotypes from Europe and North America are shared between the two continents most of the time. Several putative species seem to be restricted to the west coast of North America. Specimens from Central America (including Colombia and Venezuela) form a group with Africa, while they don't share haplotypes with Chile and Argentina. Australasian haplotypes are unique.

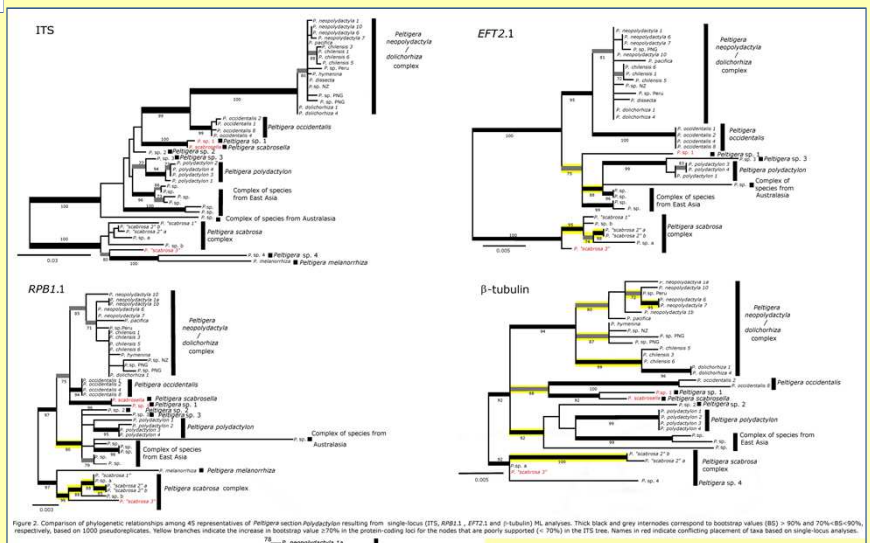

Figure 2. Comparison of phylogenetic relationships among 45 representatives of *Peltigera* section *Polydactylon* resulting from single-locus (ITS, *RPB1*.1 , *EFT2*.1 and β-tubulin) ML analyses. Thick black and grey internodes correspond to bootstrap values (BS) > 90% and 70%<BS<90%, respectively, based on 1000 pseudoreplicates. Yellow branches indicate the increase in bootstrap value ≥70% in the protein-coding loci for the nodes that are poorly supported (< 70%) in the ITS tree. Names in red indicate conflicting placement of taxa based on single-locus analyses.

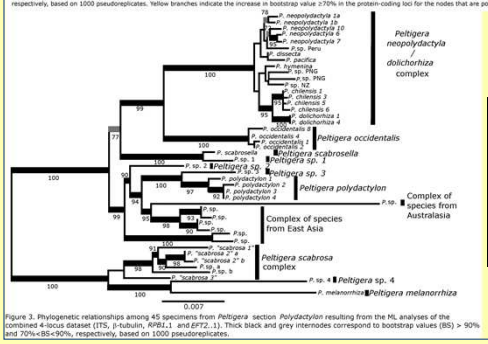

Figure 3. Phylogenetic relationships among 45 specimens from *Peltigera* section *Polydactylon* resulting from the ML analyses of the combined 4-locus dataset (ITS, β-tubulin, *RPB1*.1 and *EFT2*. 1). Thick black and grey internodes correspond to bootstrap values (BS) > 90% and 70%<BS<90%, respectively, based on 1000 pseudoreplicates.

Table 1. Comparison among data sets and resulting ML trees for the single-locus and combined 4-locus analyses.

	Total number of sites	Number of sites excluded	Number of sites included	Number of variable sites	Number of taxa	Number of nodes	Number of well-supported nodes	% of well-supported nodes
ITS	770	253	517	146	44	43	18	41,8
RPB1.1	679	0	679	65	41	40	17	42,5
EFT2.1	821	0	821	89	34	33	16	48,4
β-tubulin	544	0	544	139	33	32	22	68,7
Combined	2814	253	2561	439	45	44	30	68,1

Future directions

We will continue sequencing the four loci to assess geographic variability for the widespread putative species. We will explore and compare gene trees vs species trees methods and population genetic methods to delimitate species boundaries in the section *Polydactylon*.

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