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Molecular evidence indicates that subarctic willow communities in Scotland support a diversity of host-associated *Melampsora* rust taxa

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ABSTRACT

Rare and threatened subarctic willow scrub communities in the UK are the subject of ongoing conservation programmes, yet little is known about the diversity of fungal taxa that they support. Isolates of the rust genus *Melampsora* were sampled from 112 leaves of eight subarctic willow (*Salix*) taxa and their hybrids from twelve sites in the UK. In order to determine the number of *Melampsora* taxa present in the samples, isolates were sequenced for the Internal Transcribed Spacer (ITS) region of rDNA and data were subject to phylogenetic analysis. Maximum likelihood and Bayesian analysis indicated that the isolates fell into three strongly supported host-associated clades. Clade I contained only isolates from *Salix herbacea* and was distinguished morphologically by dense urediniospore echinulation and thin cell walls. Clade II contained isolates from *Salix arbuscula* and *Salix reticulata* only. These could not be distinguished morphologically from isolates in Clade III which were found on *Salix lapponum*, *Salix myrsinites*, *Salix myrsinifolia*, *Salix aurita*, *Salix lanata*, and their hybrids. Clade II was most distinct in ITS sequence, differing by 50 bases from Clades I and III, while the latter clades differed in sequence by only 24 bases on average. Clades I and III are likely to represent the previously recognised taxa *Melampsora alpina* Juel 1894 and *Melampsora epitea* Thüm. 1879 respectively, but Clade II has not apparently been described before. Significant differences in the intensity of infection by isolates of Clade III were found among different *Salix* species at a single site, suggesting either differences in resistance among *Salix* taxa, or the presence of further cryptic taxa within Clade III. The study illustrates the power of molecular phylogenetic analysis to reveal cryptic biodiversity within *Melampsora*, and suggests that conserving *Salix* host diversity within subarctic willow communities will ensure that a diversity of associated *Melampsora* taxa is maintained.

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Introduction

Background

Subarctic willow scrub communities in Scotland contain a range of dwarf *Salix* species and occur in base rich montane habitats at elevations between 600 and 900 m (JNCC 2009, 2012). They are among the most threatened plant communities in the UK, and are the subject of ongoing conservation and re-establishment programmes (Mardon 1991, 2003). Important components of the biodiversity within these communities are the mycorrhizal, endophytic, and parasitic fungi associated with the dwarf *Salix* species. Interactions with these fungi are likely to have a major impact on the fitness and population dynamics of the dwarf willows, and hence the long term persistence of the associated subarctic willow scrub communities (Milne *et al.* 2006). Particularly significant fungal interactions are those with leaf infecting rust pathogens of the genus *Melampsora* (Helfer 1992) that are widespread in subarctic willow communities. In some circumstances *Melampsora* may cause significant damage to foliage, with consequent effects on survival of the dwarf willows (Smith *et al.* 2004). However, more generally, *Melampsora* species will coexist with willows at endemic levels. In these situations the obligate nature of the interaction will mean that conservation of willow scrub communities will benefit the biodiversity of *Melampsora* rusts.

Despite their ecological importance within this threatened upland community, there is very little information available on the identity and diversity of *Melampsora* species present on subarctic willows, on their degree of host specialisation, or on their relationship with *Melampsora* taxa described from willows in other communities (Smith *et al.* 2004). To a large extent this is due to difficulties with morphological identification. There are few hyphal and urediniospore characters that can reliably be used to recognise and distinguish among *Melampsora* taxa (Helfer 1992). Moreover where confirmation of taxon identity requires determination of aecial hosts by artificial inoculation, this may be problematic (Pei *et al.* 1993).

In the absence of suitable morphological characters for delimiting *Melampsora* taxa present within the subarctic willow community, an alternative molecular approach for distinguishing and identifying taxa can be adopted (Smith *et al.* 2004; Pei *et al.* 2005; Feau *et al.* 2009). This involves collecting isolates from a range of hosts, and obtaining DNA sequence information from each isolate at phylogenetically informative loci. These data can be used in phylogenetic analysis to define molecular taxa which fall into well supported clades that are distinct from other such clades (Taylor *et al.* 2000; Blaxter *et al.* 2005). The morphological characteristics, host ranges and ecological attributes, such as virulence on hosts, of these molecular taxa can then be assessed to determine their status (Bennett *et al.* 2011). While this approach may not detect all the biological species present in a sample of isolates, it can nevertheless provide an estimate of the minimum number of taxa present, and significantly improve our understanding of the levels and nature of *Melampsora* biodiversity within the community.

Aims

The principal objective of this research was to assess the range, identity, and host specificity of *Melampsora* taxa infecting *Salix* species in the subarctic willow scrub communities of Scotland. *Melampsora* infected leaves were sampled from eight species of subarctic willow and their hybrids located across eleven different native sites in Scotland and one in England. Morphological identification was attempted for each *Melampsora* sample on the basis of sorus structure, hyphal and urediniospore characters using existing keys. Samples were sequenced at the Internal Transcribed Spacer (ITS) locus which has previously proved informative for distinguishing among *Melampsora* species (Smith *et al.* 2004; Pei *et al.* 2005; Feau *et al.* 2009). Phylogenetic analysis was used to identify well supported clades likely to represent reproductively isolated taxa, and the degree to which these taxa are associated with particular host taxa was assessed. The sequence information was also used in conjunction with existing ITS database sequences for *Melampsora* to relate the clades to previously described *Melampsora* taxa. Finally at a site containing multiple host species the distribution of infection intensities on different *Salix* species was measured to establish whether the subarctic *Salix* species at this site differ in their susceptibility to *Melampsora*.

Materials and methods

Sample collection

Leaves infected with *Melampsora* were collected in the U.K. from natural populations of eight *Salix* species and their naturally occurring hybrids which are members of the European Union protected community 4080, subarctic *Salix* spp. scrub (JNCC 2012). Collections were made at 12 different subarctic willow scrub sites throughout Scotland and Northern England (Fig 1) in the summers of 2002 and 2003 (Table 1). Sampling was designed to obtain a good representation of each *Salix* species, and relative frequencies of samples from different taxa do not reflect their frequencies in the field. Infected leaves were placed in silica gel and labelled with the site and *Salix* taxon from which the leaves were taken. In total 112 infected leaf samples were collected from *Salix herbacea* (9), *Salix lapponum* (50), *Salix arbuscula* (7), *Salix reticulata* (2), *Salix aurita* (1), *Salix lanata* (7), and *Salix myrsinites* (7), *Salix myrsinifolia* (2) and hybrids most similar morphologically to *S. lapponum* (7), *S. lanata* (1), *S. myrsinites* (14), *S. aurita* (1), and *S. myrsinifolia* (4) (Table 1).

Morphological identification

Silica dried leaves were examined using a Wild 420 macroscope fitted with an Optimas image analysis system. The key characters for identification of *Melampsora*, as described in Helfer (1992), were investigated. Presence and size of sori were determined and micrographs taken. Small samples were scraped off the leaf surface and suspended in a small drop of lactophenol-cotton blue on a microscope slide. These