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Recent occurrence of *Ripartites metrodii* in Scotland: DNA barcoding, phylogenetic reconstruction and implications for conservation

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Abstract

Ripartites metrodii, a saprotrophic basidiomycete, has seldom been recorded in Britain (two records currently available on the Global Biodiversity Information Facility (GBIF), both from England, and none on the NBN Atlas database). The 2005 Checklist of the British & Irish Basidiomycota (CBIB) recognised only a single *Ripartites* species, *R. tricholoma*, following a widely held view that this was a 'very polymorphic' species. *R. metrodii* was cited only as one of several synonyms variously recognised at species level elsewhere in Europe. The FRDBI database currently follows CBIB. There have been five previous Scottish collections reported as *R. tricholoma*, but none of these appear to correspond to *R. metrodii* as now understood.

Here we report on the recent occurrence of *R. metrodii* in a coniferous forest in the Scottish Highlands, present its barcoding sequence, and discuss the implications for conservation of this species. Morphological and molecular analyses, including DNA barcoding and phylogenetic reconstruction, confirmed the identity of the specimens. Our material fitted into a well supported cluster treated as *R. metrodii* in the UNITE database and distinctly separate from a cluster treated as *R. tricholoma*. This supports the view that they should be treated as two different species, albeit with the names provisionally assigned until such time as sequences are obtained for type material. The findings also underscore the importance of mature conifer plantations, traditionally considered low in

biodiversity, in supporting valuable fungal communities. These plantations, while often overlooked, contribute significantly to the conservation of ectomycorrhizal and saprotrophic fungi, highlighting the need for their inclusion in biodiversity management plans.

Introduction

Fungi are an important component of forest ecosystems; they are involved in complex multivariate interactions in soil and litter and are crucial for overall ecosystem functioning (Krivtsov *et al.*, 2004).

Ripartites metrodii is a saprotrophic basidiomycete rarely recorded in the UK. At the time of writing, there appear to be only two UK records (both from England) on the GBIF database (<https://www.gbif.org/species/2531578>). The NBN database contains no records for this species. The Checklist of the British & Irish Basidiomycota (Legon & Henrici, 2005) and the FRDBI database include this species, as well as *R. helomorphus*, within *R. tricholoma*. The FRDBI contains only

five records of *R. tricholoma* in Scotland, none of them of *R. metrodii* as now understood. Furthermore, none were from the Scottish Highlands (the record of Dennis from 1974 is of *R. helomorphus* on Ness island).

Here we report on the recent occurrence of *R. metrodii* in the Scottish Highlands and discuss implications for conservation of this species.

Materials and Methods

Basidiomes of *R. metrodii* were observed on 6 September 2024 in Craigvinean Forest (grid reference NO007419). The site belongs to the Forestry Commission and is a mature coniferous plantation. The forest floor is covered by bryophytes and ericoid shrubs with occasional birch saplings.

Two basidiomes were sampled for subsequent microscopic examination and DNA barcoding. In the laboratory both basidiomes were examined using a dissecting microscope. Spore prints were obtained and the colour recorded. Standard preparations were then made using water,



Fig. 1. Appearance and key microscopic characteristics of the *Ripartites metrodii* specimen from Craigvinean Forest. Upper panels: macro characters (specimen appearance, cross-section and spore print colour), a standard laboratory slide is provided for the scale. Note the absence of hairs on the cap margin. Lower panels: micro characters - gill edge showing basidia and the absence of cheilocystidia (left), spores (measuring 4–5 μm) under magnification 400 $\times$ (middle), and spores under magnification 1000 $\times$ (right). Images © Vladimir Krivtsov.

Melzers and cotton blue reagents, and subsequently examined using a high-power compound microscope. The appearance and key characters of the sampled material are shown in Figure 1.

One of the specimens was submitted to the IBERS Genomics Facility at Aberystwyth University for DNA amplification and Sanger sequencing of the ITS barcode region. The sequence for our collection (GenBank accession number PQ724458) was compared with sequences held in the UNITE database (Abarenkov *et al.*, 2024) to determine the best match Species Hypothesis (see below). A maximum likelihood phylogenetic tree (Figure 2) was inferred using these sequences to examine the placement of our collection and visualise its relationships with closely related taxa. A sequence for *Paralepista*

flaccida was incorporated as an outgroup.

Results and Discussion

The nucleotide sequence obtained from our Scottish collection was identical to corresponding sequences labelled as *R. metrodii* on GenBank. Both the macro- and micro-characters of sampled specimens (Figure 1) showed a good fit to the descriptions of *R. metrodii* available in the literature. The ornamented non-amyloid sub-globose spores measured approximately 4–5 µm and revealed a moderate response to cotton blue. Although this cyanophilous reaction is not unknown (see Vizzini *et al.*, 2024 and references therein), we found no mention of it in the majority of the consulted entries (Breitenbach & Kränzlin, 1991; Bon, 2007; Læssøe & Petersen, 2019). This should be borne in mind whilst identifying this

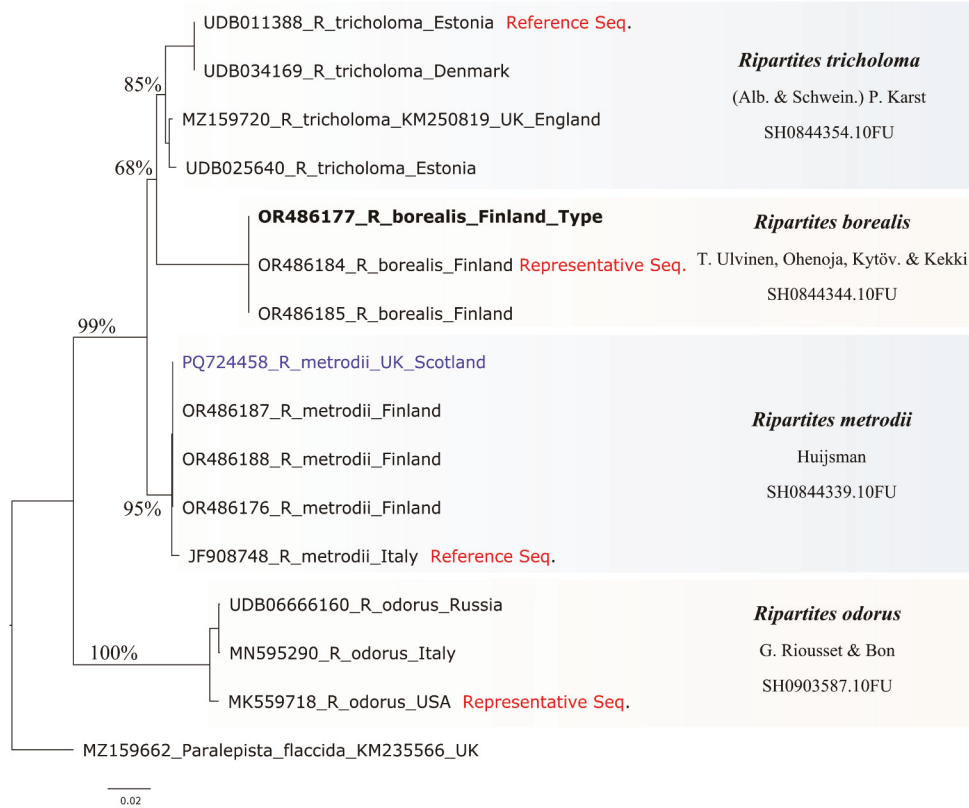


Fig. 2. Phylogenetic tree of selected *Ripartites* species. Bootstrap values (shown next to key horizontal branches) provide an assessment of confidence for each clade, with values of 70% or more often regarded as good support. They are calculated by repeating the process of generating a phylogenetic tree on a resampled set of data and counting how many times a branch is observed. The horizontal branch length indicates the amount of genetic change over time. The scale bar at the bottom of the figure shows the length of branch that represents a genetic change of 0.02 where the units are the number of nucleotide changes divided by the length of the sequence. The *Ripartites* species included in the tree are those for which genetic sequences were available in the UNITE database at the time of writing.

fungus, as confusion with *Lepista* (which also has ornamented cyanophilous spores) needs to be avoided. Pegler and Young (1974) provide a comprehensive treatment of *Ripartites* and *Lepista* spore shapes using scanning electron microscopy; however, the cyanophilous reaction of *Ripartites* spores does not appear to be mentioned there explicitly either.

Recent molecular studies (Vizzini *et al.*, 2024) place *Ripartites* into the *Paralepistaceae* family within the *Tricholomatineae* suborder of the order *Agaricales*. Species concepts within *Ripartites* vary significantly between authorities with the number of recognised European species ranging from seven in the GBIF database and five or possibly six in Fungi of Temperate Europe (Læssøe & Petersen, 2019) down to three in Flora Agaricina Neerlandica 3 (Bas *et al.*, 1995) with just one, *R. tricholoma*, currently listed in the Checklist of the British & Irish Basidiomycota (Legon & Henrici 2005). Apart from GBIF, none of the above recognise *R. metrodii* as a separate species. The same is true of Funga Nordica (Knudsen & Vesterholt, 2012). However, this species is recognised by Moser (1983), Courtécuisse and Duhem (1995) and Bon (2007). It should also be noted that Buczacki and Wilkinson (1989) list *R. metrodii* as a European species, but note its rarity and question whether it actually occurs in Britain. However, an earlier article of Pegler and Young (1974) recognised both *R. tricholoma* and *R. metrodii* as two separate species occurring in the British Isles and provided a key for their identification. In general, therefore, there is no consensus among authorities on this matter.

The UNITE database (<https://unite.ut.ee> accessed January 2025) is a publicly accessible resource within which fungus DNA sequences (ITS region) are curated and stored. Sequences are clustered into groups approximating to species level with each group assigned a unique identifying label called a Species Hypothesis (SH). Within each group a sequence is selected by the computer program to provide a Representative Sequence for the corresponding SH. In cases where expert knowledge on a particular taxon is available these choices are designated as Reference Sequences.

Named ITS sequences have been published for four genetically distinct *Ripartites* species and include UNITE reference sequences for the two of particular relevance to this paper: *R. tricholoma* and *R. metrodii*. There is no record of an ex-type collection sequence for either species but a

sequence derived from the holotype is reported for the recently described *R. borealis* (Kekki, 2023).

A maximum likelihood phylogenetic tree (Figure 2) was derived from selected *Ripartites* sequences held in the UNITE and GenBank databases with the inclusion of our collection from Scotland together with a sequence for *Paralepista flaccida* as an outgroup. The collection from Scotland clearly falls within the *R. metrodii* clade based on sequences from Finland and Italy. This is *R. metrodii* sensu Kekki (e.g. OR486187) and Osmundson *et al.* (2013) (JF908748) which, in the current absence of an ex-type sequence, seems to be the best available candidate to represent *R. metrodii* in the original sense, at least for now.

Bon (2007) states that *R. metrodii* is rare in Britain. That may, however, be linked to the fact that its identification is not straightforward and that the species is missing from popular field guides (e.g. Phillips 1981). Furthermore, fungal fruiting is subject to a multitude of complex interactions, and some species persist without fruiting for many years (Krivtsov *et al.*, 2003).

It should also be noted that some of the prominent books treat *R. metrodii* as part of *R. tricholoma* (e.g. Knudsen & Vesterholt, 2012). However, the phylogenetic reconstruction presented here, places *R. tricholoma* and *R. metrodii* in separate clades, indicating that separation of these species on a molecular basis is significant (Figure 2).

Further studies should focus on the characterisation of *R. metrodii* population(s) and its/their interactions with other members of the biological community (*sensu* Krivtsov *et al.* 2004, 2006).

Coniferous plantations are generally regarded as of poor biodiversity value, but there is evidence that plantation forests can provide valuable habitats and contribute significantly to biodiversity conservation (Brockerhoff *et al.*, 2008). In particular, studies in Britain have shown that mature coniferous plantations can have high species richness of ectomycorrhizal and saprotrophic fungi and are therefore important for their conservation (Humphrey *et al.*, 2000).

The occurrence of *R. metrodii* within this mature plantation is important for its conservation. This should be taken into account whilst designing land use plans and environmental management actions. A recent discovery of *Chamonixia caespitosa* in a spruce plantation in Wales (which was subsequently

felled) emphasised the difficulties related to conservation of rare fungi associated with non-native trees (Hobart, 2024). Our study contributes further evidence for the conservation potential of coniferous plantations, which should be carefully examined on a case-by-case basis and weighed against any commercial benefits.

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