Evidence for an interesting association between *Cortinarius pratensis* (Section *Dermocybe*) and Sand Sedge, *Carex arenaria*

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For the overwhelming majority of *Cortinarius* species there is either direct evidence, or a strong indication from their habitat, that they form ectomycorrhizal associations with woody plants. The small number of grassland *Cortinarius* species were considered to be an exception until associations were discovered between them and Bearberry, *Arctostaphylos uva-ursi* (referred to by Watling, 1988), Mountain Avens, *Dryas octopetala* (Harrington, 1996) and Common Rockrose *Helianthemum nummularium* (reviewed by Henrici, 2011).

In 2009 (and again in 2011), TL collected specimens of a *Cortinarius*, section *Dermocybe*, from sand dunes at Blakeney Point (TF9946) in north Norfolk, where none of these vascular plant associates are present (Figs 1 and 2). Through a published note by Peter Roberts (2009) he became aware of the discovery that Irish specimens assigned to *C. cinnamomeus* had been unambiguously shown to be associated with two species of sedge, Pill Sedge, *Carex pilulifera* and Glaucous Sedge, *Carex flacca* (Harrington & Mitchell, 2002). As Sand Sedge, *Carex arenaria* is common at Blakeney Point, and was growing adjacent to the fungi collected, the possibility was considered that a mycorrhiza had formed between the two.

Identification of the *Cortinarius* species

Attempts to name the Blakeney Point fungus were first made using *Funga Nordica* (Høiland 2012). *C. cinnamomeus* and *C. croceus* were rejected as 4 out of 15 spores measured were over 9.5 µm long, but *C. croceoconus* fitted well except for the habitat ('among mosses, often *Sphagnum*, on moist soil with *Picea* and *Pinus*, rarely under

Fig. 1. *Cortinarius pratensis* growing with Sand Sedge on fixed dunes at Blakeney Point, Norfolk. December 1st 2011. Sand Sedge is visible lower left. Photo © Tony Leech.

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doi:10.1016/j.fldmyc.2017.07.004

birch'). However, in the *Checklist of the British and Irish Basidiomycota* (Legon & Henrici, 2005) it is noted that *C. croceoconus* is not authentically British. Despite this, there are fifteen records for *C. croceoconus* on FRDBI (Nov 2016), relating to 14 separate collections. One of these, from chalk grassland at Redhills Pasture, Arnside, Westmorland (vc 69: SD4577), was made by John & Sheila Weir who kindly sent notes, photographs and dried material. These specimens also occurred in the absence of trees but in the likely presence of *Carex* species.

Cortinarius pratensis was initially excluded by the yellow gills of the young specimens collected (according to Høiland (2012) they should be orange). However, the spore size and habitat given for *C. pratensis* matched the Blakeney specimen well as it was noted that it can occur 'on dry, sandy soil ... often without any woody plants'. Indeed, it is the only species in this section stated in *Funga Nordica* to occur in the absence of trees. There are 14 records for *C. pratensis* on FRDBI (Nov 2016), relating to 13 collections; five of which mention grassland without reference to a tree species.

At the suggestion of Alick Henrici, Klaus Høiland, Norwegian author of the key in *Funga Nordica*, was approached for an opinion. From notes and photographs (but without seeing material) he considered that the specimens should be named *C. pratensis*. In 2015, an apparently identical *Cortinarius* species was found by TL, again with *Carex arenaria*, at Burnham Overy dunes (TF858458) on the north Norfolk coast.

The opportunity arose for AE to sequence the ITS region, the official barcode region of fungi (Schoch *et al.*, 2012), of the nuclear DNA of the 2011 Blakeney Point collection (GenBank No. KY857923). Subsequently, John Weir's Arnside specimen was also sequenced. To establish the identity of a species from its ITS region nucleotide sequence it is necessary to compare this with the corresponding sequence determined from its type collection. Sequences from type specimens of *C. cinnamomeus*, *C. croceus* and *C. croceoconus* were published by Niskanen (2014) and that of *C. pratensis* communicated to us during the course of the study by Jean-Michel Bellanger.

Investigation of mycorrhizal association

In October 2014, Philip Amies collected a *Cortinarius* species on sand dunes at Holme

Fig. 2. Specimens of *Cortinarius pratensis* collected at Blakeney Point. Maximum cap diameter: 36 mm. Spores 8.5–11.0 x 5.0–6.2 µm, average 9.3 x 5.7 µm. Photo © Tony Leech.

(TF7144) in west Norfolk. At TL's suggestion he also collected rhizomes of the adjacent *Carex arenaria* and supplied both to AE who extracted DNA from 'nodules' on the sedge rhizomes and determined the corresponding nucleotide sequence.

Results and discussion

The nucleotide sequence of the ITS region of the collections made at Blakeney Point, in Norfolk, Arnside in Westmorland (as *C. croceoconus*) and in Ireland (GenBank no. AY082608 as *C. cinnamomeus* by Harrington & Mitchell, 2002) were identical with the holotype of *C. pratensis*, confirming that they are all the same species (Bellanger, pers. comm.). The sequence obtained by AE from the Holme collection differed from this in three nucleotides (0.74%). Two of these differences were located at the very beginning of the sequence where some editing errors may occur, so in the opinion of Bellanger (pers. comm.) it is highly likely that this too is *C. pratensis*.

The sequences obtained from the Holme *Cortinarius* specimen (GenBank No. KY857924) and the adjacent *Carex arenaria* rhizome were identical, indicating that fungal DNA was present in the sedge. It is an assumption, but a very likely one, that a mycorrhiza has formed between the two species. Harrington & Mitchell (2002) were able to demonstrate microscopically that the association between *Cortinarius pratensis* (as *C. cinnamomeus*) and *Carex pilulifera* and *C. flacca*, was ectomycorrhizal in nature in the case they studied. A significant number of arctic and alpine tundra herbaceous plants have been reported to form ectomycorrhizal associations (listed by Gardes & Dahlberg, 1996), including *Kobresia myosuroides*, a member of the *Cyperaceae* (sedge family).

With the re-determination of the Irish *Cortinarius* collections first reported as forming a mycorrhiza with a sedge, it is now the case that only *C. pratensis* has so far been found to form such an association. Sand Sedge, *Carex arenaria* can be now be added to Pill Sedge, *Carex pilulifera* and Glaucous Sedge, *Carex flacca* as known vascular plant partners in this association.

Our study also showed that *C. pratensis*, like *C. croceoconus*, can have yellow gills when young (Fig. 3).

Current status of *C. pratensis* and *C. croceoconus* in Britain

Prior to this study Kew had no confidently named collections of either of these species. As described above we now know for sure that *C. pratensis*

Fig. 3. Section through young *Cortinarius pratensis*, showing yellow gills and grey flesh. Blakeney Point, Dec. 2011. Photo © Tony Leech.

occurs in Ireland, in Westmorland and in Norfolk. Tuula Niskanen at Kew considers that it is still not possible to identify either species with confidence without sequencing. The following summarises the state of play:

C. pratensis: The only collections previously held at Kew were three from semi-improved grassland in Wales in 2004 and 2005 and one from grassland in Somerset in 2008 all identified by Peter Roberts with a 'cf.' (= 'compare' = 'looks as if it might be'). These could do with DNA confirmation, but only risk being wrong should *C. pratensis* as now understood turn out to be a species complex. Roy Watling has also recorded *C. pratensis* in grassland in Shetland in 1992 and 2001 (material preserved?). Most other British records are dubious as they mention an associated tree species (there is a suggestion in *Funga Nordica* that it might sometimes associate with *Salix*). Anyone finding a *Dermocybe* in open country should look for a *Carex* nearby (or even a *Juncus*?) and preserve both in the hope of further amplifying the present meagre knowledge of its host range.

C. croceoconus: The only material preserved at Kew is one Scottish collection made by Tuula Niskanen herself in pine/birch woodland in the Black Wood of Rannoch in 2015. This also has a 'cf.' which won't be removed until she has sequenced it. At the time CBIB was compiled there were nine distinct records on FRDBI. It was known to be a northern conifer associate which ruled out four of them, and with no material known to back up the rest it was excluded. Two recent records from pine plantations in northern Scotland seem further plausible candidates for establishing this species as British if the material has survived, but for the time being it remains excluded.

Acknowledgements

The initial DNA rxtraction and sequencing required to identify the fungus were carried out at the John Innes Centre, Norwich by visiting school students during a practical session of the Year 10 Science Camp, an educational outreach scheme which provides an experience of life as a scientist at Norwich Bioscience Institutes. Funding for the scheme was provided by the John Innes Centre, The Institute of Food Research, The Genome Analysis Centre and The Sainsbury Laboratory.

We are very grateful to Alick Henrici, Klaus Høiland and Jean-Michel Bellanger for their helpful comments and especially to Tuula Niskanen for additional information and much guidance in presentation of this work. We thank Philip Amies for collecting material and John and Sheila Weir for making their notes and material available.

Dried material has been deposited at Kew. Accession Nos. K(M)234090 (Holme), K(M)234091 (Blakeney).

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