DNA-confirmed records of *Cortinarius epipurrus* and *C. hirtus*

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I (TL) have long been fascinated and daunted in equal measure by the genus *Cortinarius* but the acquisition of Geoffrey Kibby and Mario Tortelli's new guide to the genus (2021) gave me greater confidence and I looked forward to road-testing it last autumn. The opportunity came with two public forays, and I recorded *C. epipurrus* and *C. decipientoides* with a little more certainty than usual. The latter turned out to be wrong but nevertheless led to an interesting conclusion.

Members of the Norfolk Fungus Study Group have been developing their skills of DNA extraction for barcode sequencing and kindly processed my specimens. Until recently this might have been a pointless exercise but the large-scale study spearheaded by mycologists at Kew has clarified synonyms of *Cortinarius* species through barcoding of type specimens (Liimatainen *et al*., 2020).

Cortinarius epipurrus

A pair of webcaps was collected by Jodie Humphrey from a hedgerow with mature oak trees, although just how close they were to the trees was not noted. **Location**: Little Plumstead, Norfolk (TG312114). **Cap**: convex with flattish tops, 30–50 mm diameter, Cinnamon[1] at centre; Fulvous at fluted margins with sparse white veil. **Gills**: distant, adnexed and Fulvous. **Stipe**: 70 x 7-10 mm; buff, darkening to Hazel towards clavate base; white veil over lower half; ring absent. **KOH**: black. **Spores**: ellipsoid, warted; (7.5) 8–9 x 5–6 µm.

My first tentative identification using Kibby & Tortelli (2021) was of *C. subbalaustinus* although no birch trees, its stated partner, were recalled in the hedgerow. However, the ITS barcode sequence was a 100% match for those of *C. epipurrus* (GenBank # MZ088098.1. Col. Lebanon; and GenBank # MT935030.1. Col. Italy), *C. pallescens* (GenBank # MT935267.1. Holotype. Col. France) and *C. pseudosafranopes* (GenBank # MT935350.1. Holotype. col. France). These have been shown to be synonyms, with *C. epipurrus* the preferred name (Liimatainen *et al*., 2020).

So where had I gone wrong? At *Telamonia* Key F, couplet 19! When fresh, the specimens were quite bright orange-brown but became duller with time although always brighter than the photograph in Kibby & Tortelli (2021). However, logically, the phrase 'or host trees differ' should have kept me on the straight and narrow.

No records for *C. epipurrus* exist on the Fungal Records Database of Britain and Ireland (FRDBI) but Kibby & Tortelli (2021) state that several collections have been made in England.

Cortinarius hirtus

Several fruitbodies of a small brown webcap were collected by Donna Welch in a damp area under mixed *Pinus sylvestris* and *Betula*. **Location**: Briston, Norfolk (TG061307). **Cap**: convex with prominent obtuse umbo, 15-19 mm diameter, Cigar Brown[1] at centre to Snuff Brown at margin; surface fibrillose with cream-coloured veil at margin. **Gills**: More or less distant, adnate with tooth, Snuff Brown (mature). **Stipe**: 45-50 x 3.5mm; red-brown with slightly banded off-white veil over lower part, bruising reddish at base. **Spores**: elongate-ellipsoid, warted, (11) 12–12.5 x 6–7.5 µm.

A provisional identification of *C. decipientoides* (spores 9.0-13 x 6.0-7.0 µm) was chosen over *C. hirtus* (spores 9.0–11.0(-12) x 5.0–6.0 µm) because of the larger spores; both species show reddening of the stipe when bruised. However, the DNA sequence for the ITS barcode region showed a 100% match to that of type specimens of *C. hirtus* (GenBank # MT935140.1. Holotype. Col. Czech Republic), *C. querculus* (GenBank # MT935366.1. Holotype. Col. France GenBank), *C. similigenus* (GenBank # MT935435.1.

1. Colours with uppercase initials refer to the Colour Identification Chart, Flora of British Fungi, (1969).

Holotype. Col. France) and *C. substemmatus* (GenBank #MT935528). These have all been shown to be synonyms, with *C. hirtus* the preferred name (Liimatainen *et al*., 2020). It would therefore appear that the range of spore dimensions is greater than that given by Kibby & Tortelli (2021). This publication states that *C. hirtus* occurs in southern England and in Scotland, and is probably widespread but uncommon, although, again, no records exist on FRDBI (May 2023).

Discussion

We have at least shown that the final statement in the abstract to Liimatainen *et al*., (2020) that "*identification of species based on ITS barcodes becomes an easy task even for non-experts of the genus*" is true.

It is likely that species with many synonyms are intrinsically more variable, *C. epipurrus* has four and *C. hirtus* six (Liimatainen *et al*., 2020). No field guide can be expected to describe all variants but as knowledge improves, the range of variation can be better circumscribed. This may take longer with rare species. A case in point is our finding of *Amanita olivaceogrisea* with brown edges to its gills and rust-spotting on the volva. As these features were not noted by Geoffrey Kibby (2014), he encouraged us to sequence our specimen. The results agreed 100% with eleven sequences of the ITS region for *A. olivaceogrisea*.

Cortinarius epipurrus, Little Plumstead, Norfolk. 9 Oct. 2022. Photograph © Tony Leech.

Methods

DNA extraction was performed using Bento Lab's dipstick DNA extraction kit and methodology.

The internal transcribed spacer (ITS) region was amplified using the primer pair ITS1F and ITS4. Thermocycling was done with a mini16 thermal cycler (miniPCR bio, Boston, USA), using the following protocol: 4 minutes at 94˚C, then 35 cycles: of 94˚C for 30 seconds, 53˚C for 30 seconds, 72˚C for 45 seconds followed by a final extension step for 5 min at 72°C. 7 µl of PCR products were visualised with a blueGel electrophoresis unit on a 1.5% agarose gel stained with Gelgreen DNA stain in TBE buffer.

Sequencing was performed by the Institute of Biological, Environmental & Rural Sciences (IBERS) of the University of Aberystwyth.

Nucleotide traces were checked manually for quality and errors in FinchTV v1.4.0 (Yang *et al*., 2017). *C. epipurrus* was trimmed to a length of 553 bp and *C. hirtus* was trimmed to 536 bp. Approximate taxonomic affiliations were determined using a nucleotide BLAST search in GenBank. Sequences were aligned using the MAFFT v7.490 algorithm within AliView v1.28 (Larsson, 2014) and phylogenies were calculated using the FastTree algorithm and visualised in FigTree.

Acknowledgements

We very gratefully acknowledge the advice and support of Brian Douglas (formerly of the Royal Botanic Gardens, Kew) and of Sam Rowe (Earlham Institute, Norwich) in setting up the fungus DNA sampling facility at the Ted Ellis Trust (Wheatfen, Norfolk). Grant aid for equipment, materials and sequencing was provided by the British Mycological Society and the Darwin Tree of Life project.

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Cortinarius hirtus, Briston, Norfolk, 18 Oct. 2022. Photograph © Tony Leech.

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Martin Gregory obituary

Frequent forayers will be sad to hear that Martin died at the end of 2022. He was a regular at all kinds of forays for several decades and especially enjoyed our overseas meetings.

He lived near Danbury Common in north Essex and was a volunteer warden on the nature reserve, helping with management and recording. He was also recorder for fungi for the Essex Field Club.

Martin was a keen gardener and looked after the large garden at his home. He would not have called himself an expert but was a very good all-round naturalist. His main interest was in the myxomycetes and made a fair-sized collection, beautifully labelled. As an engineer he was not surprisingly meticulous over detail and produced the neatest and most detailed record sheets at forays. On a walking trip to the Himalayers, in Nepal, he collected bark samples which yielded many rare species of corticolous myxomycetes, expanding our knowledge of their distribution. His British collections are with the Essex Field Club and his overseas material will come to me, and then be deposited in the herbarium of the Royal Botanic Garden, Edinburgh. His enthusiasm and cheerfulness will be greatly missed at forays.

Bruce Ing