

## The curious case of the *Coprinellus* that turned out to be a *Psathyrella*

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As Sherlock Holmes knew well, a new clue when investigated thoroughly can cast remarkable light on older findings and reveal things that were previously missed. We describe here an example that we puzzled over during the British Mycological Society's 2022 Spring Meeting at Cranfield, the clue (as is often the case these days) being a DNA sequence. The taxonomy behind the detective story we describe below was fully and clearly published in Update 11 of the Checklist of British and Irish Basidiomycota in 2023 but we thought the following account would be of interest to readers, if only to illustrate that attending BMS fungal recording meetings provides a valuable and enjoyable opportunity to discuss and explore such issues with fellow field mycologists!

YM looked at a tiny dark-spored agaric growing on deer dung found by MJ in East Norfolk, north west of Norwich. The deer dung was collected on 1 January 2022 from Houghen, St. Faith's Common, grid reference TG178172, and incubated to see what might emerge. The fruitbodies fruiting from 12th

January were very tiny, the caps a maximum of 2 mm high (Fig. 1) making microscopy a little difficult.

However, the microscopic characters (Fig. 2) were clear and, with sphaerocysts in the veil and lageniform pileocystidia (setules) in the cap, similar lageniform cheilocystidia, caulocystidia and very small spores, it keyed out in the Coprinoid keys (Uljé, 2005; Nagy *et al.*, 2012) to *Coprinellus parvulus*, a species described in Uljé & Keizer (2003) as *Coprinus parvulus* and combined in *Coprinellus* in Házi *et al.* (2011). DJS looked at the dried Norfolk collection, found the same characters and agreed that it was this species. It had not been reported as found in Britain. Study of additional collections from Norfolk by YM and DJS and a re-read of the original Uljé & Keizer (2003) paper did show that this species was somewhat different from other setulose *Coprinellus* species. Differences included few widely spaced lamellae, small lageniform cystidia and other cells that were much smaller than generally found in *Coprinellus*, as we had noted in our collection.



Fig. 1. Fruitbodies: A, B, growing on dung © Mark Joy; C, dried herbarium specimen © Derek Schafer.

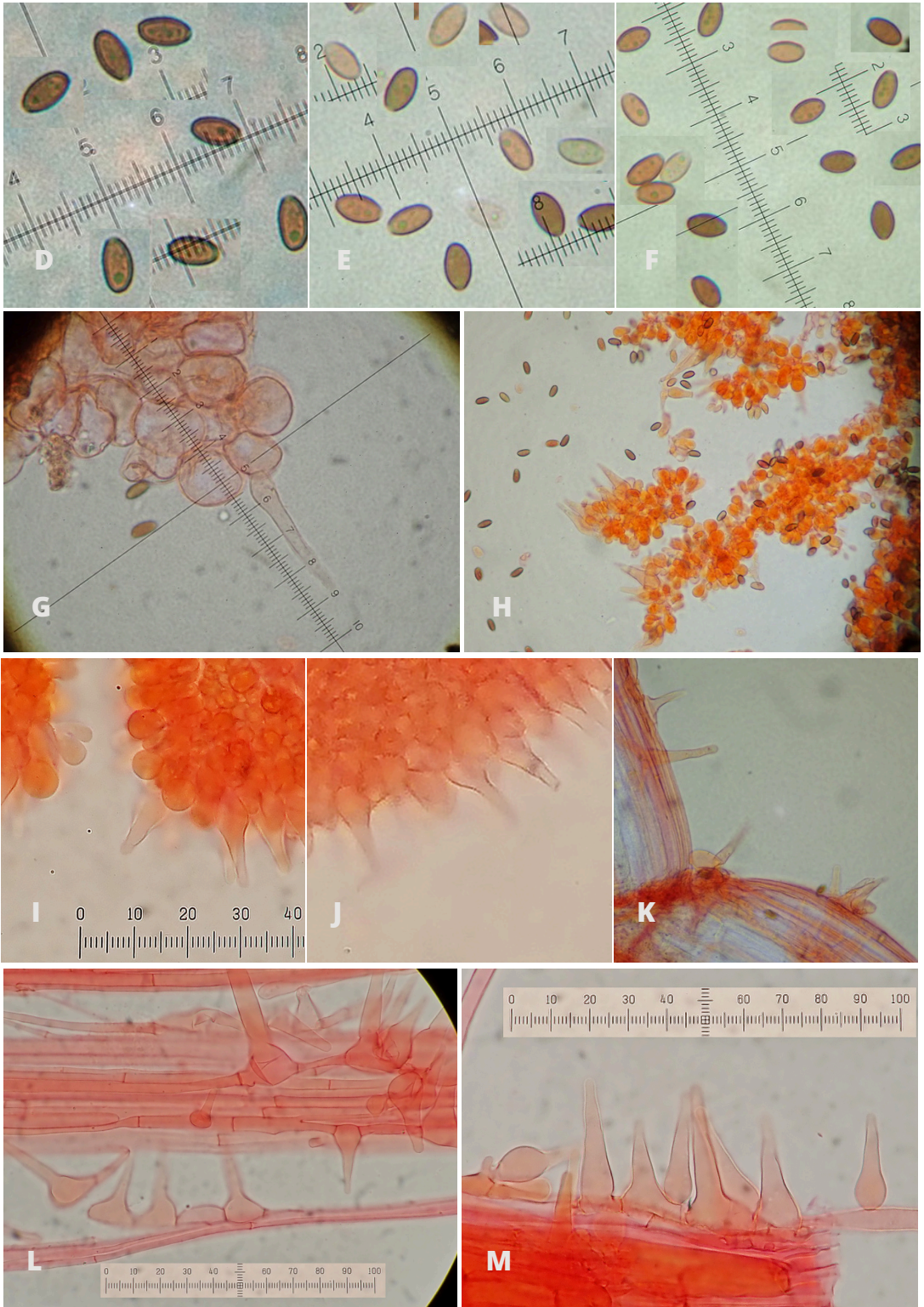


Fig. 2. D, E, F, Spores; G, veil cells and pileocystidia on cap; H, I, J, cheilocystidia; K, L, M, caulocystidia. Scale division 1  $\mu$ m. when present. © Yvonne Mynett: D, E, F, G, H, K; Derek Schafer: I, J, L, M.

So, *Coprinellus parvulus* new to Britain would be an exciting find but clearly would benefit from a DNA sequence to fully justify that status. Nagy *et al.* (2012) had included the holotype in their study of setulose *Coprinellus* species but their ITS and  $\beta$ -tubulin sequences were too noisy to be included in their phylogenetic analysis and there were no other sequences of *Coprinellus parvulus* on GenBank. Sequencing our collection would provide useful information, although we expected there to be no matching sequences of this species.

YM was part of a DNA barcoding group in Norfolk that produced an ITS sequence for the collection. The result was conveyed to us when we were at the BMS meeting at Cranfield in 2022. Brian Douglas, who had a copy of this ITS sequence, told us, when asked, that it was *Psathyrella tenuicula*. YM responded with "I didn't send any Psathyrellas to be sequenced!"

Dash! Another misidentification! Where had we gone wrong?

*Psathyrella tenuicula* was described by Karsten in 1879 as *Psathyra tenuicula* and transferred to *Psathyrella* following type studies by Örstadius and Huhtinen (1996). We seized DJS's copy of the 2005 checklist of British and Irish Basidiomycota (CBIB), finding *Psathyra tenuicula* listed as a *nomen dubium*, included in "British Basidiomycetae" by Rea (1922) but not in Kits van Waveren's *Psathyrella* monograph (Kits van Waveren, 1985). Where else might we look to cast some light?

The details on GenBank of the published sequences that matched the Norfolk material included a reference to a paper by Larsson & Örstadius (2008). DJS had previously looked at this paper several times and it was the paper that combined *Psathyrella conopilus* into *Parasola* and *Psathyrella marcescibilis* into *Coprinopsis*. Searching now for *P. tenuicula*, we found that the paper had a full description of *Psathyrella tenuicula* placing it in a *Psathyrella* section there called "*Cystopsathyra*". This section also included *P. sphaerocystis*, a P.D. Orton species described

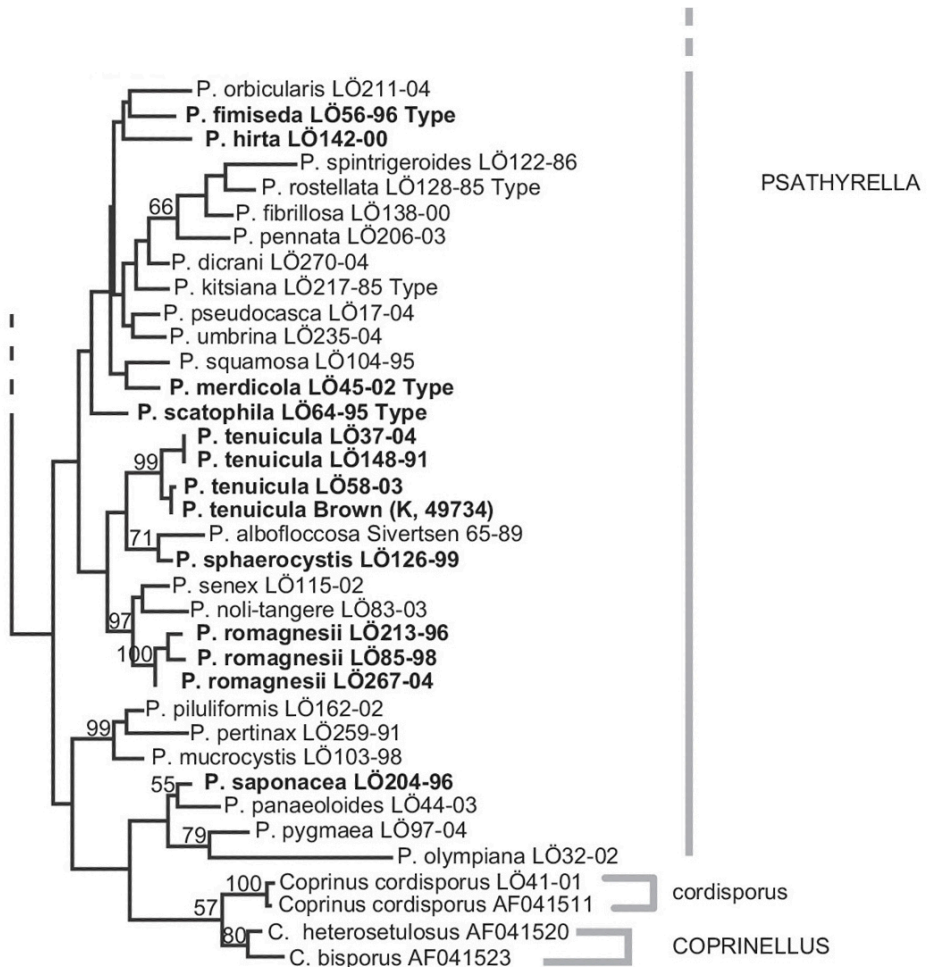


Fig. 3. Extract of part of phylogram (p.1170) from Larsson & Örstadius (2008).

from Britain in 1964 that differs from *P. tenuicula* by having broader spores and lacking pubescent cap and stipe. All of this was supported by molecular phylogeny. Where did this leave *Coprinellus parvulus*?

To our surprise, we found that the paper had considered this and provided cogent arguments for *Coprinellus parvulus* being a later synonym of *Psathyrella tenuicula*. DJS had not noticed this in earlier reading and it had apparently not been picked up by Species Fungorum or other papers on *Coprinellus*. CBIB in 2005, we discovered later, was also unaware that *Psathyra tenuicula* had been updated to *Psathyrella tenuicula* in the paper by Örstadius and Huhtinen (1996). The fourth update of CBIB (2009) had subsequently taken the 2008 paper into account with the following comment:

“Now with a modern interpretation and combination in *Psathyrella*. Listed as British by Rea (1922) and (consequently?) by Larsson & Örstadius [Mycol. Res. 112(10): 1165-1185 (2008)], but without voucher material.”

So, accepting that the Norfolk collection is *Psathyrella tenuicula*, is it the first British record? Checking through the records in the Fungal Records Database of Britain & Ireland (FRDBI), we found none listed. However, looking again at the Larsson & Örstadius (2008) paper, we saw that one of the collections of *Psathyrella tenuicula* that they had sequenced was labelled with the voucher references “Brown K49734 or Brown K, 49734”. Not obvious as a Kew number, which would have been “K(M)49734”, but worth further investigation?



Fig. 4. GenBank phylogram of Norfolk *Psathyrella tenuicula* collection based on ITS sequences.

Table 1. Sequences and specimens used in this study.

Taxon name	Country	Year	Collector/ Identifier	Voucher#	Sequence locus ITS
Uncultured fungus clone	Poland			Clone RM_44	MK796503.1
<i>Psathyrella tenuicula</i>	Sweden		Leif Örstadius	LO148-91	DQ389705.1
<i>Psathyrella tenuicula</i>	France	2016	Pierre Ivaldi and Francis Fouchier		KX384666.1
<i>Psathyrella tenuicula</i>	Sweden		Leif Örstadius	LO37-04	DQ389704.1
<i>Psathyrella tenuicula</i>	Sweden		Leif Örstadius	LO58-03	DQ389706.1
<i>Psathyrella tenuicula</i> (as <i>P. cf. sphaerocystis</i> )	England	1997	Ted Brown (C), Nick Legon (I)	K(M)49734	DQ389707.1
<b><i>Psathyrella tenuicula</i></b>	<b>England</b>	<b>2022</b>	<b>Yvonne Mynett</b>	<b>NFSG_20220101_01YM</b>	<b>OR999124.1</b>
<i>Psathyrella tenuicula</i>	Germany	2013	Andreas Melzer	AM1598	MK045710.1
Uncultured fungus clone	Lithuania			Clone 3993_2399	MW215932.1

Back to FRDBI! This was a Ted Brown collection, K(M)49734 from Burnham Beeches in 1997, identified by Nick Legon as *Psathyrella* cf. *sphaerocystis* but shown by the 2008 paper to be *P. tenuicula*. This was the reason why the 2008 paper considered *P. tenuicula* as British, not its earlier listing by Rea. So *P. tenuicula* is British from this find at Burnham Beeches. Nick Legon expressed some doubts about K(M)49734 labelling it as *P.* cf. *sphaerocystis* and was not aware of the more recent *P. tenuicula* publications.

Finally, before we relegate the Norfolk collection to just the second such record, there is another issue to consider. Larsson & Örstadius (2008) treated *P. tenuicula* in a broad sense because the phylogenetic clade, reproduced above (Fig. 3) from their 2008 paper, divided into two branches. These were recognised as potentially reflecting a complex of at least two species. However, a full resolution of the complex would need to consider previously described species such as *Psathyrella minima*, *P. berolinense*, *P. coprinoides* and *P. granulosa* and find characters to distinguish what the sequencing reveals. Larsson & Örstadius referred to *P. tenuicula* in this broad sense. The complex was also indicated in the major analysis of Psathyrellaceae by Wächter & Melzer (2020). The paper recognised two clades in the phylograms, labelling them /*tenuicula* A and /*tenuicula* B.

Our phylogram is shown in Fig. 4, the Norfolk collection being NFSG\_20220101\_01YM and the details of the material sequenced set out in Table 1. The sequence of the Norfolk collection nests in clade /*tenuicula* B, along with the Kew collection. So, the Norfolk record does seem to be the second of both *Psathyrella tenuicula* in the broad sense and of whatever name attaches to clade /*tenuicula* B if the complex is resolved into two species by further study. If, on the other hand, there are more than two species found to make up the complex, the Norfolk collection might still be the first of one of them!

We await with interest the results from anyone prepared to take on the challenge of sorting out the complex. Such is the joy(?) of biological recording in the DNA age!

### Acknowledgements

We convey our grateful thanks for help in this study to: Norfolk DNA Group; Brian Douglas at RBG Kew; Darwin Tree of Life Barcoding the Broads Project; and Sam Rowe at the Earlham Institute, Norwich. We are also grateful to Alick Henrici, Martyn Ainsworth at Kew and Leif Örstadius for providing many helpful comments.

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