

Ovary smuts in seed capsules of British chickweeds

A. Martyn Ainsworth¹, Ben Blades¹, Alexandra Dombrowski^{1, 2},
Kare Liimatainen¹, Laura M. Suz¹ & Roseina Woods¹



Fig. 1. Smut-free examples of chickweed species known to host ovary smuts in Britain: (left) *Cerastium diffusum* (E. Sussex, Normans' Bay, coastal shingle, May 2024) and (right) *Moenchia erecta* (W. Sussex, Watersfield, short turf, May 2018). Photographs © A. Martyn Ainsworth.

Introducing the hosts: *Cerastium* and *Moenchia* (Caryophyllaceae)

White-flowered and weedy, the British members of *Cerastium* and *Moenchia* are small and inconspicuous plants which can look very similar (Fig. 1). They are classified in the family Caryophyllaceae alongside the more familiar and colourfulampions, catchflies and pinks. Collectively, they are known as chickweeds and mouse-ear chickweeds, the latter often shortened to mouse-ears, and several are widespread and very common annual plants. They usually start flowering in early spring and have swollen seed capsules by May (Fig. 2). The developing seeds require a plentiful supply of nutrients which are funnelled from elsewhere in the plant. This presents an opportunity for ovary-infecting smuts to redirect those nutrients into the production of dense masses of dark purplish-brown smut spores within capsules that remain seedless. The development of infected capsules appears quite normal on the outside, thus concealing the internal diversion of resources into teliospore production. Infected capsules are, however, more detectable when they are fully mature and open at the apex. Peering through a hand lens into the toothed apical openings of infected mature

capsules, it should be possible to distinguish the mass of dark brown spores that lies within from the naturally shadowy interior of a healthy seed-bearing capsule. That's the theory, but where have such smutty chickweed capsules been found in Britain?

First British collection of a *Cerastium* ovary smut and its taxonomic placement

After searching among the dried specimens preserved in the British fungal collections at RBG Kew, Spooner & Legon (2006) reported finding a single collection (K-M000106945) of smut-infected *Cerastium* capsules made in May 1902 by F.J. Chittenden. The host was *Cerastium glomeratum* Sticky Mouse-ear which had been found in Rainsford End, Chelmsford (vc19 North Essex), probably in OS grid square TL6807 or TL6907. Knowing that this annual plant was common throughout the country, Spooner & Legon (2006) noted that its capsule smut was "evidently very rare or perhaps overlooked in Britain". Another four years without any further discoveries prompted Natural England, the government's adviser for the natural environment, to accept that, after 108 years without any records at all, it was safe to assume that this smut fungus was extinct in England (Anon., 2010). Before moving

on to the more recent finds of this species, we should mention the four names under which the Essex specimen has appeared in print and why.

As noted in Spooner & Legon (2006), the collection had originally been filed at Kew, along with many other specimens of diseased di- and monocotyledonous host plants, under the name *Ustilago violacea*. However, in recognition of accumulating evidence that this name had been applied to numerous distinct and host restricted species, some of whose earlier names were being retrieved from synonymy, it entered the British & Irish checklist as *U. duriaeana* (CBIB; Legon & Henrici, 2005). This name, which was accepted in Vánky's (1994) monograph of European smut fungi, was introduced for a species originally found inside *C. glomeratum* capsules in N. Africa (Tulasne & Tulasne 1847). The CBIB authors retained *U. duriaeana* as a true smut under the heading "Ustilaginomycetes" (subphylum Ustilaginomycotina). In stark contrast, *U. violacea* s.str., along with several other anther-infecting smuts segregated from it, were moved to *Microbotryum* to join the rust fungi under the heading "Urediniomycetes" (subphylum Pucciniomycotina). This reflected a major DNA-supported realisation that morphologically recognisable smuts were the products of convergent evolution. Some species within their ranks were outwardly concealing a very rusty ancestry! One year after the checklist was printed, however, Spooner & Legon (2006) stopped using

the name *U. duriaeana* and switched to *Microbotryum duriaeana*, recognising that this was yet another smut that should be classified in the rust subphylum. Later that same year, the Essex specimen appeared under a fourth name in the first CBIB update (Anon., 2006). Swiftly following the erection of the genus *Haradaea* by Denchev (Denchev *et al.*, 2006), *H. duriaeana*, the generic type, was adopted as the checklist's new accepted name for the Essex specimen. This change was duly reflected in the recuration of the collection within the Kew fungarium and, furthermore, the name *H. duriaeana* was taken up by the BMS for the smut whose newly minted English name was Chickweed Seedsmut (BMS, 2024). However, this most recent taxonomic move deserves further scrutiny.

Denchev *et al.* (2006) erected *Haradaea* to accommodate the seed-destroying smuts on *Caryophyllaceae* based, at least in part, on the phylogenetic tree published in Almaraz *et al.* (2002). However, this ecological group of fungi was only represented by two sequences in Almaraz *et al.*'s tree: one was derived from *Arenaria* capsules and the other from *Cerastium*. Although this pair clustered together in the tree, they occupied an outlying position. Cautioning that this placement indicated that the *Cerastium* ovary smut's taxonomic relationships were "still uncertain", Almaraz *et al.* left the species within *Ustilago*. It was not long before Denchev (2006), followed by Lutz *et al.* (2008), acknowledged that Almaraz *et*



Fig. 2. Seeds squashed from mature capsules of (left) *Cerastium diffusum* (E. Sussex, Normans' Bay, May 2024) and (right) *Moenchia erecta* (E. Sussex, Rye Harbour, May 2015). Photographs © A. Martyn Ainsworth.

al. had sequenced fungal contaminants instead of the target ovary smuts. Nevertheless, further species were recombined in *Haradaea*, albeit on increasingly shaky grounds. As more published sequences accumulated, however, a taxonomic choice had to be made: to retain *Haradaea* for a monophyletic group of ovary smuts on *Caryophyllaceae*, which would necessitate the splitting of *Microbotryum* into many new and relatively small genera (complicated option), or, to add *Haradaea* to the synonymy of a monophyletic *Microbotryum* (simpler option). As a result, MycoBank, Species Fungorum, Vánky (2012), Kemler *et al.* (2020) and Denchev *et al.* (2023) all opted to synonymise *Haradaea* with a broadly circumscribed anther- and ovary-infecting *Microbotryum*. For now, at least, *Haradaea* has been abandoned, even by its own author. This decision is now reflected in CBIB Update 13, which includes an entry for *Microbotryum duriaeaeum*, thus marking a reversion to the taxonomy adopted in Spooner & Legon (2006).

Subsequent British collections on *Cerastium*

Microbotryum duriaeaeum was recorded on *Cerastium glomeratum* as a new addition to the Welsh funga in 2013 (as *Haradaea*, in Woods *et al.*, 2018) and was re-found in England, after a gap of 117 years, in 2019. It is now known from three sites in England and two in Wales and the list of host plants now extends to: *C. diffusum* Sea Mouse-ear, *C. glomeratum* and *C. semidecandrum* Little Mouse-ear. Thus far, AMA's own searches have been restricted to Sussex (where he lives) where coastal sites were deliberately targeted in the hope of finding several *Cerastium* species growing in close proximity. The searches involved peering into a seemingly endless supply of chickweed capsules for signs of smut over a period of a few hours at each site visited, only to be rewarded with, if anything, just one tiny patch of infected host plants per visit. This smut will certainly have been overlooked in the past, due to a lack of deliberate searching, nevertheless it still seems to be rather uncommon, at least along the East Sussex coast. AMA found no infected plants of *C. glomeratum*, but did find some very small patches of infected *C. diffusum* and *C. semidecandrum* among some large populations of uninfected plants. Interestingly, although all sites visited had at least three *Cerastium* species present, no more than one of these was found to be infected at each site. It is generally accepted that these infections are systemic (e.g. Vánky, 2012) and this was borne out by AMA's failure to find

any healthy seed-bearing capsules on infected plants. This is clearly bad news for the individual host plants, but it is invaluable knowledge for the field mycologist trying to select good material for DNA sequencing. Having found an infected plant, one should try to sample some of its immature capsules as these will still be densely packed with smut spores on arrival back at the lab. Capsules that are collected when mature, open and releasing spores, on the other hand, are quite likely to lose most of their dusty contents in transit.

All the known post-1902 British collections are listed below:

England. East Sussex (vc14): Normans' Bay, on *C. diffusum* on consolidated coastal shingle, TQ694059, 14 May 2024, coll. A.M. Ainsworth (K-M001442720, GenBank PV124721, Fig. 3). Rye Harbour, southeast of Camber Castle, on *C. semidecandrum* in parched turf overlying shingle ridges, TQ92311831, 21 Apr. 2019, coll. A.M. Ainsworth (K-M000263027, GenBank PV124720, Fig. 3). *Ibid.*, north of Camber Castle, on same host and in same habitat as in 2019, TQ921188, 27 May 2024, coll. A.M. Ainsworth (K-M001442722). Tide Mills, on *C. diffusum* on consolidated coastal shingle, TQ459001, 20 May 2024, coll. A.M. Ainsworth (K-M001442721). **Wales.** All on *C. glomeratum*. **Cardiganshire** (vc46): Llanrhystud, in poached coastal grassland between clumps of *Ulex*, SN534702, 11 Jun. 2016, coll. A.O. Chater (K-M000205685, GenBank PV124719) and seen here again in May 2018 (host erroneously listed as *C. diffusum* in Woods *et al.*, 2024). **Montgomeryshire** (vc47): Ffridd Faldwyn, by hedge under grazed *Ulex*, SO21609671, 2 Jun. 2013, coll. A. Jones (K-M000263026, GenBank PV124718).

British collections on *Moenchia erecta*

In contrast to the situation in *Cerastium*, there is only one British species of *Moenchia*: the uncommon spring-flowering annual, *M. erecta* Upright Chickweed. The printed checklist entry for *Ustilago duriaeaeana* includes Wales in its distribution details (Legon & Henrici, 2005). This is presumably a reference to the two Welsh collections on *Moenchia* found in 1997 and 1998 at Ffridd Faldwyn in Montgomeryshire (see below) which are now preserved at Kew. The checklist authors thought these two smut collections were "possibly" conspecific with the Essex specimen on *Cerastium*. This possibility was explored further in Spooner & Legon (2006), who concluded that *Moenchia* might represent a new host for



Fig. 3. *Microbotryum duriaeanum* spores squashed from capsules of (upper left) *Cerastium diffusum* (K-M001442720, E. Sussex, May 2024) and (below) *C. semidecandrum* (K-M000263027, E. Sussex, Apr. 2019) and spores of *M. moenchiae-manticae* in capsules of (upper right) *Moenchia erecta* (K-M000197779, E. Sussex, May 2015). Photographs © A. Martyn Ainsworth.

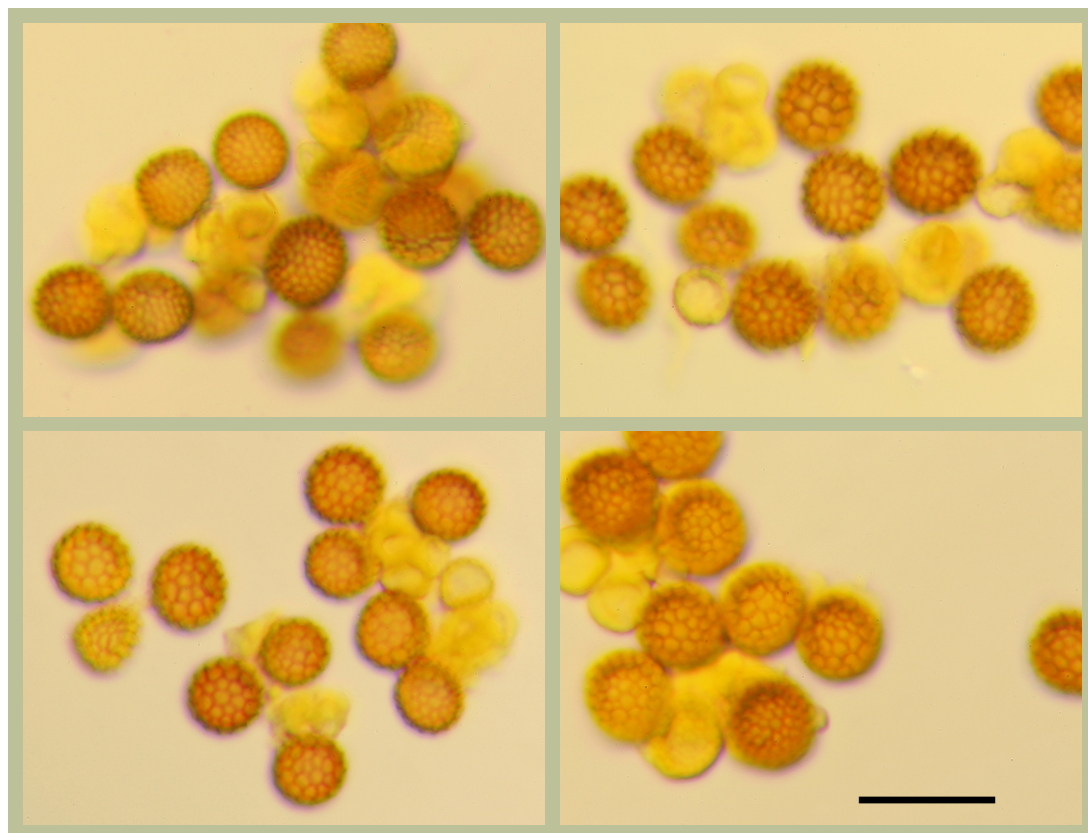


Fig. 4. Teliospores of *Microbotryum* species from infected chickweed ovaries in Melzer's reagent showing reticulate ornamentation. One micrograph shows *M. moenchiae-manticae* spores (can you spot it?) and the others show those of *M. duriaeanum* (answer in the text). Scale bar (for all four) represents 20 μ m. Micrographs © A. Martyn Ainsworth.

Microbotryum duriaeanum since the only known ovary smut on *Moenchia*, namely *Microbotryum moenchiae-manticae*, recorded on the non-British *Moenchia mantica*, had larger spores than they had seen in the Welsh material. On the contrary, after visiting Kew in 2010 to examine the morphology of the material collected in Wales in 1998, Denchev *et al.* (2011) concluded that its spores did indeed match those of the distinct species *Haradadea moenchiae-manticae*, a species which would be new to Britain. Their opinion was followed in the fifth CBIB update and *H. moenchiae-manticae* was duly added to the British and Irish list (Anon., 2011). Woods *et al.* (2018) drew attention to the remarkable coincidence of *Cerastium* and *Moenchia* ovary smuts, described as “two extremely rare fungi”, being found at the same site (Ffridd Faldwyn) “so close together”. This prompted them to resurrect the taxonomic uncertainties that had been expressed earlier by Spooner & Legon (2006) and they concluded that further work was required as the two smuts “may prove to be conspecific” and “a result is still

awaited”. Interestingly, this curious coincidence was repeated during the current study when infected ovaries of *Cerastium* and *Moenchia* were recorded (by AMA) at the same site (Rye Harbour) in southern England. Despite such improbable coincidences, the ovary smuts on these two genera continue to be recognised as distinct species in Britain and Ireland (Woods *et al.*, 2018), an opinion supported by DNA barcode evidence (see Fig. 5).

All the known British collections are listed below:

England. East Sussex (vc14): Rye Harbour, south of Camber Castle, on host in thin soil overlying consolidated shingle, TQ92231808, 4 May 2015, coll. A.M. Ainsworth (K-M000197779, GenBank PV124716, Fig. 3). **South Hampshire** (vc11): New Forest, Bull Hill, near car park, on host in tightly grazed dry grass heath, SZ34209796, 11 May 2015, coll. A. Lucas & A.M. Ainsworth (K-M000197978, GenBank PV124717). **Wales. Montgomeryshire** (vc47): Ffridd

Faldwyn, SO29, 2 Jun. 1997, coll. A. Jones (T.F. Preece 5617) (K-M000106050, specimen not found). *Ibid*, SO216968, 15 May 1998, coll. A. Jones (T.F. Preece 6356) (K-M000106303), ITS barcode MN657198 published in Kemler *et al.* (2020).

Morphological study

Teliospores of *Microbotryum duriaeanum* and *M. moenchiae-manticae* are globose to ovoid or short ellipsoid with reticulate ornamentation and they have similar size ranges (Fig. 4). Vánky (2012) gives measurements of $12\text{--}17 \times 11\text{--}15 \mu\text{m}$ for the former and $12\text{--}15\text{--}(17) \times 11\text{--}15.5 \mu\text{m}$ for the latter. He indicates that there might be a slight difference in the number of meshes per spore diameter: 4–7(–8) in the former and 6–9 in the latter, although the ranges show considerable overlap. Ten spores measured at $\times 1000$ magnification (in Melzer's reagent and including ornament) from sequenced *M. duriaeanum* K-M000263026 (ex *C. glomeratum*,

Montgomeryshire) were in the range $12.8\text{--}16 \times 12.2\text{--}15 \mu\text{m}$ with 5–6 meshes per diameter. Corresponding values for sequenced *M. moenchiae-manticae* K-M000197978 (ex *M. erecta*, South Hampshire) were in the range $12.8\text{--}16 \times 12.2\text{--}14.1 \mu\text{m}$ with 6–7 meshes per diameter. Examining spores from several collections did not increase confidence in the discriminatory power of these morphological characters. Looking at Fig. 4, for example, the smallest mesh size (largest number of meshes per diameter) is seen in the upper left micrograph which is of *M. duriaeanum* (K-M001442721 on *C. diffusum*). The other micrographs show *M. duriaeanum* (K-M000205685 on *C. glomeratum*) at upper right and (K-M001442722 on *C. semidecandrum*) at lower left. The lower right micrograph is the odd one out and shows spores from K-M000197978 on *M. erecta*. Clearly, the results of our morphological study were not very taxonomically helpful and prompted us to switch to a molecular barcode-based approach.

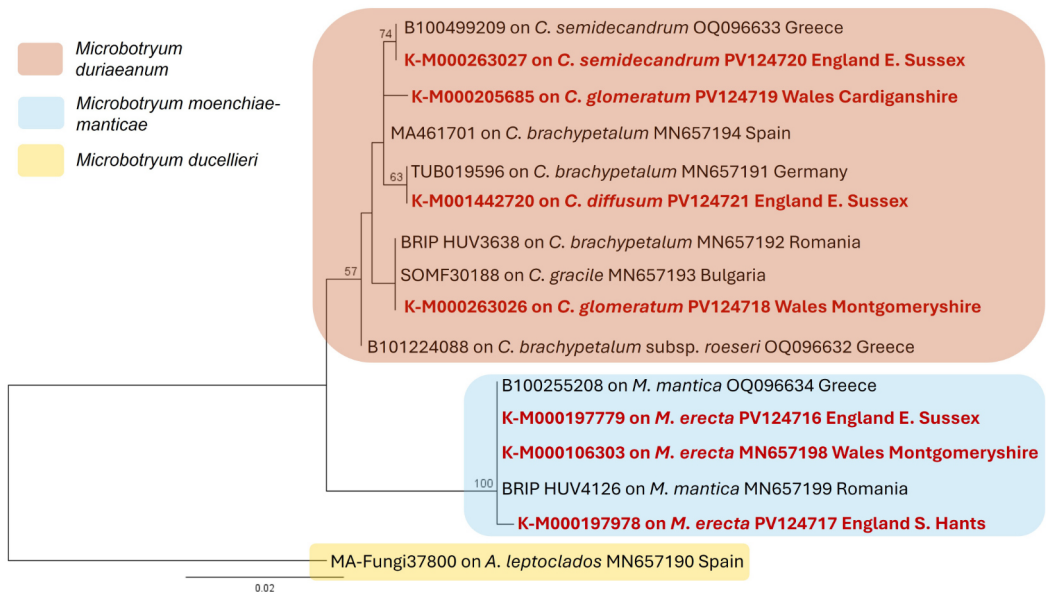


Fig. 5. Maximum likelihood phylogram showing ITS sequences of three *Microbotryum* species (shaded boxes) which infect ovaries of *Caryophyllaceae*. Sequences are labelled respectively with their fungarium accession number or collector's code, host plant name (*Arenaria*, *Cerastium* and *Moenchia*), GenBank accession number and geographic source. Sequences derived from British collections are shown in red. Nodes are labelled to indicate branch support (shown as a maximum likelihood bootstrap percentage) where this exceeds 50%. Scale bar indicates the number of substitutions per site.

DNA sequencing and analysis

We attempted to sequence the full nuclear ribosomal internal transcribed spacer regions (ITS) of eight of the British collections listed above using previously published protocols (Dentinger & Suz, 2014; Liimatainen & Ainsworth, 2018; Wainhouse *et al.*, 2024). We obtained sequences from seven of these collections: two from infected *Moenchia erecta* and five from *Cerastium* spp., all of which were sourced from material preserved in Kew. A sequence (MN657198) derived from one of these specimens (K-M000106303) and matching ours was published in Kemler *et al.* (2020). This left us with six newly generated and unpublished ITS sequences which were deposited in GenBank (accession numbers PV124716–PV124721).

Our sequences were aligned using MUSCLE 5.1 and a maximum likelihood phylogram (Fig. 5) was generated using RAxML 7.2.8. with the nucleotide substitution model GTRGAMMA and branch support estimated with 1000 rapid bootstrap replicates in Geneious version 2024.0. The phylogram includes ten additional *Microbotryum* sequences downloaded from GenBank including MN657190, which was selected as the outgroup. This was generated from a specimen labelled as *Microbotryum ducellieri*, an ovary smut found on *Arenaria leptoclados* and one which has yet to be recorded in Britain or Ireland. Downloaded sequences whose codes begin with MN are from Kemler *et al.* (2020) and those beginning with OQ are from Denchev *et al.* (2023). The ITS sequences from ovary smuts of *Cerastium* and *Moenchia* collected in England and Wales formed two distinct clusters which corresponded with their host plant genus (Fig. 5). Although there are no publicly available sequences from type specimens of *Microbotryum duriaeaeum* or *M. moenchieae-manticae*, all the British sequences clustered with existing sequences bearing one of these two labels. The *M. moenchieae-manticae* cluster is well supported (bootstrap support value 100) whereas the corresponding support for *M. duriaeaeum* is lower (57). Future studies, including broader taxon sampling and sequencing of additional gene regions could increase support for these clusters. Based on the available molecular evidence therefore, British material found on *Cerastium* should continue to be determined as *M. duriaeaeum* while that on *Moenchia* should continue to be assigned to *M. moenchieae-manticae*. Although they are rarely recorded and, based on our very limited field survey data, are quite possibly genuinely rare in Britain, our analysis

confirmed that both species do coexist at some sites, e.g. in the Rye Harbour area in England and at Ffridd Faldwyn in Wales.

Conclusion

This study provides further evidence that *Microbotryum duriaeaeum* and *M. moenchieae-manticae* are both extant in England and Wales and should dispel any residual doubts arising from the taxonomic concerns expressed in Spooner & Legon (2006) and Woods *et al.* (2018).

Acknowledgements

Thanks to A.O. Chater and A. Jones for depositing their chickweed ovary smut collections in RBG, Kew and making them available for our study. BB, AD, and RW gratefully acknowledge sequencing support from the Darwin Tree of Life project (<https://www.darwintreeoflife.org/>).

References

- Almaraz, T., Roux, C., Maumont, S. & Durrieu, G. (2002). Phylogenetic relationships among smut fungi parasitizing dicotyledons based on ITS sequence analysis. *Mycological Research* 106(5): 541–548.
- Anon. (2006). CBIB update 1, winter 2006. Available at: <https://fungi.myspecies.info/content/checklists>
- Anon. (2010). *Lost life: England's lost and threatened species, (NE233)*. Natural England, Peterborough. Available at: <https://publications.naturalengland.org.uk/publication/32023>
- Anon. (2011). CBIB update 5, spring 2011. Available at: <https://fungi.myspecies.info/content/checklists>
- BMS (2024). *Recommended English names for fungi*. Accessed 27 Sept. 2024. <https://www.britmycolsoc.org.uk/resources/english-names>
- Denchev, C.M. (2006). *Haradaea afromontana*, comb. nov. (*Microbotryaceae*). *Mycologia Balcanica* 3: 216.
- Denchev, C.M., Denchev, T.T., Spooner, B.M. & Helfer S. (2011) [2010]. New records of smut fungi. 3. *Mycotaxon* 114: 225–230.
- Denchev, C.M., Moore, R.T. & Shin, H-D. (2006). A reappraisal of the genus *Bauhinus* (*Microbotryaceae*). *Mycologia Balcanica* 3: 71–75.
- Denchev, T.T., Denchev, C.M., Begerow D. & Kemler M. (2023). New records of *Anthracoidea pseudofetidae* (*Anthracoideaceae*) from Russia, and *Microbotryum* (*Microbotryaceae*) from Greece and Morocco. *Anales del Jardín Botánico de Madrid* 80(2): e140. <https://doi.org/10.3989/ajbm.584>

Dentinger, B.T.M. & Suz, L.M. (2014). What's for dinner? Undescribed species of porcini in a commercial packet. *PeerJ* 2:e570. <https://doi.org/10.7717/peerj.570>

Kemler, M., Denchev, T.T., Denchev, C.M., Begerow, D., Piątek, M. & Lutz, M. (2020). Host preference and sorus location correlate with parasite phylogeny in the smut fungal genus *Microbotryum* (Basidiomycota, Microbotryales). *Mycological Progress* 19: 481–493.

Legon, N.W. & Henrici, A. (2005). *Checklist of the British & Irish Basidiomycota*. RBG Kew, London.

Liimatainen, K. & Ainsworth, A.M. (2018). Fifteen *Cortinarius* species associated with *Helianthemum* in Great Britain: Results of a DNA-based analysis. *Field Mycology* 19(4): 119–135.

Lutz, M., Piątek, M., Kemler, M., Chlebicki, A. & Oberwinkler, F. (2008). Anther smuts of *Caryophyllaceae*: Molecular analyses reveal further new species. *Mycological Research* 112(11): 1280–1296.

Spooner, B.M. & Legon, N.W. (2006). Additions and amendments to the list of British smut fungi. *Mycologist* 20(3): 90–96.

Tulasne, L.-R. & Tulasne, C. (1847). Sur les Ustilaginées comparées aux Urédinées *Annales des sciences naturelles, sér. 3, partie botanique* 7: 12–126.

Vánky, K. (1994). *European smut fungi*. Gustav Fischer, Stuttgart.

Vánky, K. (2012). *Smut fungi of the world*. American Phytopathological Society, St Paul, Minnesota.

Wainhouse, M., Detheridge, A.P., Griffith, G.W., Dombrowski, A., Woods, R. & Ainsworth, A.M. (2024). Above- and below-ground detection of *Phellodon secretus*, a tooth fungus new to Britain. *Field Mycology* 25(2): 51–58.

Woods, R.G., Chater, A.O., Smith, P.A., Stringer, R.N. & Evans, D.A. (2018). *Smut and allied fungi of Wales. A guide, Red Data List and census catalogue*. A.O. Chater, Aberystwyth. www.aber.ac.uk/waxcap/downloads/

Woods, R.G., Chater, A.O., Stringer, R.N., Evans, D.A. & Smith, P.A. (2024). *Towards a handlist of microfungus parasites of vascular plants from Britain and Ireland and a census catalogue for Wales*. A.O. Chater, Aberystwyth. www.aber.ac.uk/waxcap/downloads/

¹ Royal Botanic Gardens, Kew. TW9 3AE.

² Department of Biological and Environmental Sciences, Gothenburg Global Biodiversity Centre, University of Gothenburg, Box 463, 405 30 Göteborg, Sweden.

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

Vladimir Krivtsov^{1,2,3} & David Harries⁴

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