

R. K. GREVILLE'S FUNGUS NAMED "ORANGE *SCLEROTIUM*" IS SHOWN TO BE A MEMBER OF THE BOLETALES (FUNGI: BASIDIOMYCOTINA)

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Morphological and molecular data show the orange sclerotia depicted in Plate 101 of R. K. Greville's *Scottish Cryptogamic Flora* to be a species of *Penttilamyces* (Coniophoraceae: Boletales), which is described here as new to science.

Keywords. Boletales, bryophilous, new species, R. K. Greville, Scotland.

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Introduction

Sclerotia are the vegetatively produced resting structures of various fungi. Considered by the early classical authors to be specific entities in themselves, they were given binomials and placed in what is today considered a polyphyletic genus: *Sclerotium* Tode. In early mycological research, connections were not made between *Sclerotium* and any sexual stage of a taxon. However, since the classical (early post-Linnean) period, many connections between the two have been established. Recent research has shown that sclerotia function as sexual structures in various, often unrelated fungi in genera ranging from the basidiomycetous to the ascomycetous. Examples in the Basidiomycotina include *Athelia* Pers. (Atheliales) (Julich, 1972), *Collybia* (Fr.) Staude (Agaricales) (Hansen & Knudsen, 1992), *Sistotrema* Pers. (Sistotrematales) (Eriksson *et al.*, 1984) and, in the clavarioid fungi, *Typhula* (Pers.) Fr. (Corner, 1950). In the micro-fungi, examples are found in the Sclerotiniaceae (Helotiales) (Dennis, 1968) and Venturiaceae, specifically the single species of the bizarre genus *Lasiobotrys* Kunze, which relies for reproduction on aerial dispersal of its sclerotia (Dennis, 1968). Even some of the Myxogastrales (slime moulds) produce sclerotia (Alexopolous *et al.*, 1996).

Sclerotia vary in size and morphology, ranging from the lentil- or apple pip-like structures of *Collybia* to small aggregates of cells found in cultures of *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo (Watling & Moore, 1994). They may be smooth or slightly pubescent, regular in outline or nodulose, pale or dark, and orange to brown and even black. In *Typhula* the interlocking hyphae on the surface, when viewed under a low-power microscope, have been shown to be diagnostic because they make different patterns depending on the species.

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Warcup & Talbot (1962), using techniques more familiar to those studying glomeraceous chlamydospores or examining nematode cysts, found sclerotia to be common in soil collected in Australian wheat fields, and more widespread than first thought. There is every reason to suspect, when such techniques are applied, that sclerotia of several kinds will be found in most, if not all, soil ecosystems. Culture of the sclerotia that Warcup & Talbot (1962) isolated from their soil samples produced sporomes of genera such as *Ceratobasidium* D.P.Rogers (Botryobasidiales) and *Omphalina* Quél. *sensu lato* (Agaricales), and these authors were even able to describe new genera and species based on their findings.

Research on sclerotia has focused on the pathogenic members of the Helotiales (Ascomycotina), with one family name even reflecting the formation of sclerotia: Sclerotiniaceae. Many species of this family are the causal organisms of diseases. With the concentration of research on this family by plant pathologists, it seems to have been forgotten that on applying the International Code of Nomenclature (Turland *et al.*, 2018), the type of the genus *Sclerotium* is, in fact, *Sclerotium semen* Tode. This is the anamorph of *Typhula variabilis* Reiss, and therefore the generic name applies to a basidiomycete and not to an ascomycete. Thus, the large volume of work that is available in the literature is not applicable to sclerotia in their strictest sense but applies to the ascomycetous components. This has left many 'species' in limbo, even though they may have distinctive characters, such as being bright orange in the case of the fungus here discussed.

This state of uncertainty remained much the same for many years, but recently a brightly coloured sclerotial structure was recognised, growing between the podetia of lichens at various sites in Canada (Thorn *et al.*, 1998). Thorn and colleagues found that their sclerotia did not belong to the ascomycetous fungi but rather, to their great surprise, to a basidiomycete. They also found through anatomical and cultural analysis that the structures represented the sclerotial stage of a species of *Leucogyrophana* Pouzar (Hygrophoropsidaceae: Boletales) as then circumscribed, and from molecular analysis that they belonged to a genus within the Coniophoraceae, which they newly described as *Penttilamyces* Zmitr., Kalinovskaya & Myosnikov.

The Leucogyrophana group

Ginns (1968, 1971) examined all the then-known species of *Leucogyrophana*, recognising eight species, to which was added *L. lichenicola* Thorn, Malloch & Ginns (Thorn *et al.*, 1998). Since the recognition of that latest species, *Leucogyrophana* has been subdivided, and the independent genus *Penttilamyces* has been segregated from it based on the findings of molecular phylogenetic studies (Zmitrovich *et al.*, 2019).

Penttilamyces has been found to belong to the Coniophoraceae, an entirely separate family to that containing the type species of *Leucogyrophana* (Hygrophoropsidaceae). Included in the new genus, *Penttilamyces*, are other former members of the genus

Leucogyrophana, namely *L. olivascens* (Berk. & M.A.Curtis) Ginns & Weresub and *L. romellii* Ginns. Of these, *Leucogyrophana olivascens* forms sclerotia but they are dark brown, differing dramatically from the brightly coloured ones of *L. lichenicola*. Finally, in *Leucogyrophana sensu* Ginns & Weresub, *L. pinastri* (Fr.) Ginns & Weresub also possesses sclerotia, but in that species they are quite different in morphology, especially in being much more elongate (Ginns & Weresub, 1976). This character, coupled with the morphology of the hymenophore, indicates that there might be an additional grouping within *Leucogyrophana*. Indeed, molecular studies (Jarosch & Besl, 2001) have found it to represent a third genus, namely *Hydnomerulius* Jarosch & Besl in the paxiloid fungi (Paxillaceae; Boletales).

It is clear that *Leucogyrophana*, as conceived by Ginns (1968, 1971), is polyphyletic and contains three genera in three totally different families, although all in the Boletales. *Leucogyrophana lichenicola* is now placed in *Penttilamyces*, which until this taxon was recognised, consisted exclusively of species associated with conifers. Recently, *Leucogyrophana lichenicola* was found in a blanket bog at the base of decaying stems of what was probably *Cladonia ciliata* Stirt. in Caithness, Scotland (P.D. Crittenden s.n., 26 vi 2013, E [barcode E00661348]).

Greville's fungus

In his *Scottish Cryptogamic Flora* (1823–1828), R. K. Greville described and illustrated in colour seven members of *Sclerotium*: four dark-coloured species, all basidiomycetous, namely *S. durum* Pers. (= *Typhulaceae*), *S. quercinum* Pers. (= *Typhulaceae*), *S. scutellatum* Alb. & Schwein (= *Macrotyphula phacorhiza* (Reichard) Olariaga, Huhtinen, Læssøe, J.H.Petersen & K.Hansen) and *S. semen* (= *Typhula variabilis* Reiss); a white taxon, *S. aegerita* Hoffm., which has been shown to be the anamorph of the basidiomycete *Bulbillomyces farinosus* (Bres.) Jülich; a pink taxon, *S. persicolor* Schumach; and a bright-orange *Sclerotium* that is the subject of this paper (Figure 1).

The bright-orange *Sclerotium* had been collected, by Greville's "scientific and highly esteemed friend" Dr W. C. Trevelyan, from *Trichostomum* Bruch growing at the summit of Beinn Resipol ("Ben-Reishapol"), Loch Sunart, Ardnamurchan, in the Scottish Highlands. Greville (1824a), in his *Scottish Cryptogamic Flora*, cited it as a synonym of *Sclerotium subterraneum* Tode, under the name *S. muscorum* Pers., an early epithet also used by de Candolle (1807). However, in *Flora Edinensis* (Greville, 1824b), he followed Persoon (1801) in using, for the same fungus, the name *Sclerotium subterraneum*, but the original description given by Persoon would appear to be based on a fungus with slightly different features. Greville (1824a, 1824b) probably included more than one species under this name, because he described *Sclerotium subterraneum*, in addition to being found in the mountains, also growing nearer sea level up tree bases and "on the lower, half-rotten stems of mosses". In *Flora Edinensis* (Greville, 1824b), he mentions that "when growing on roots or tree-bases it is somewhat larger and of a paler colour and white within". J. Hedger (Dundonell, personal

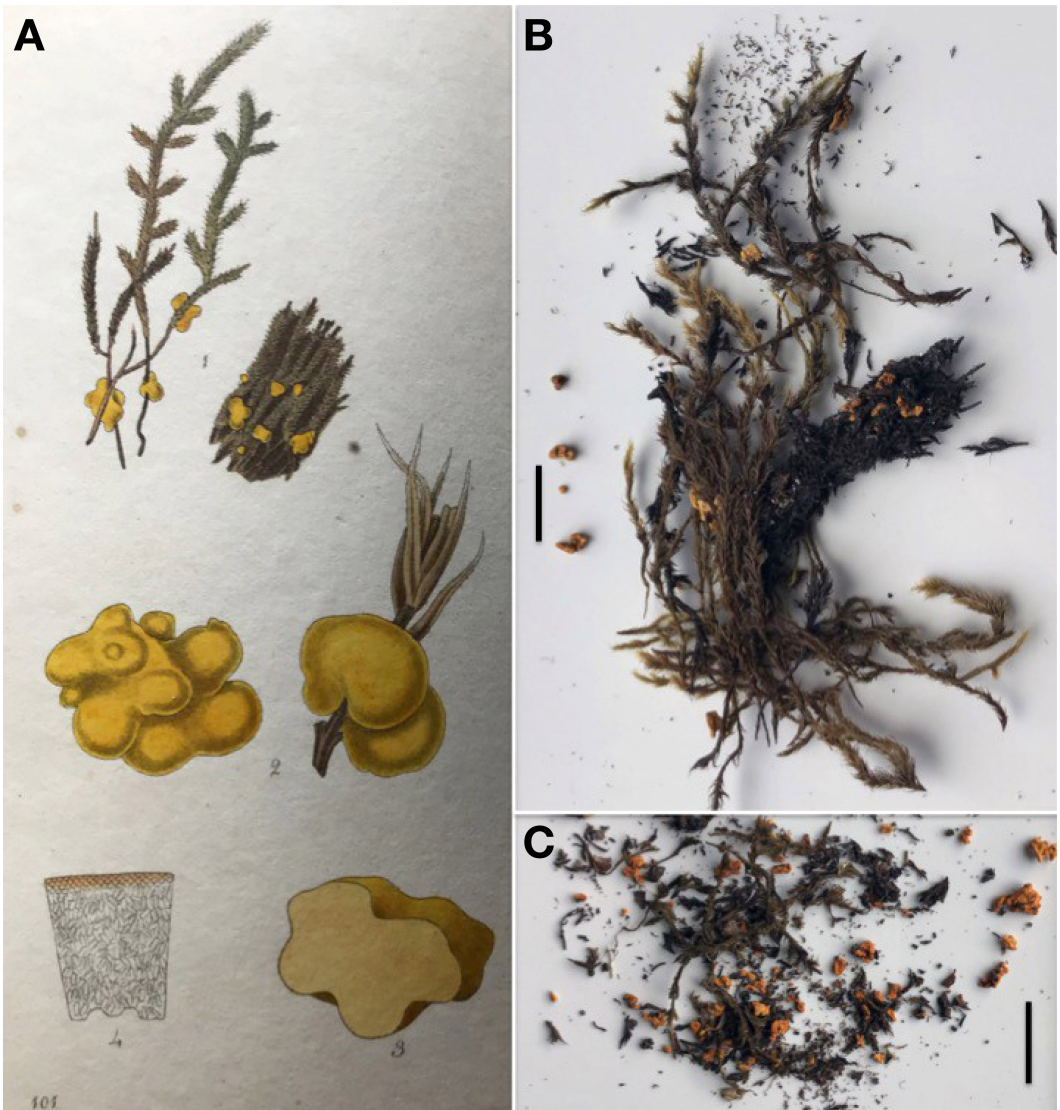


Figure 1. A, Colour illustration from R. K. Greville's *Scottish Cryptogamic Flora* (1824a), Plate 101; B, a specimen of Henderson 9540 (E); C, a specimen of P. D. Orton s.n. (E). Scale bar for B and C: 10 mm. Photographs: R. Watling.

communication, 1982) described a collection, made by J. Rishbeth, that was growing up the base of a *Sequoiadendron* J. Buchholz previously killed by *Armillaria* (Fr.) Staude; this was a pale-yellow sclerotium that was subsequently found to be the sclerotia of *Collybia cookei* (Bres.) J.D. Arnold. Material of *Sclerotium subterraneum* cited in Greville's *Flora Edinensis* has been traced in the Herbarium of the Royal Botanic Garden Edinburgh (E) and is more

in keeping with Hedger's collection than Trevelyan's Scottish collections, the latter of which unfortunately cannot be traced.

The aim of our study was to identify, using morphological and molecular tools (ITS nrDNA sequences) a recent collection of a bright-orange *Sclerotium* found buried in specimens of moss cushions held in E.

Materials and methods

Collections from across Scotland and England held in E were included in this study (see below). Morphological analyses were carried out following Corner (1950). Material from one collection (Watling Wat. 30231) was subjected to molecular analysis, DNA isolation, sequencing and phylogenetic analyses, following the methods described by Ortiz-Rivero *et al.* (2021), which have previously been successfully applied to other members of the Boletales.

DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Venlo, Netherlands), with 60°C overnight incubation. Amplifications of ITS nrDNA were carried out using PuReTaq Ready-To-Go PCR Beads (Cytiva, Marlborough, MA, USA), using the primer pair ITS5–ITS4 (White *et al.*, 1990). Amplimers were purified using the QIAquick Gel Extraction Kit (Qiagen). Purified amplimers were sent to Macrogen (Madrid) for sequencing.

The consensus sequence was aligned with homologous sequences obtained from the GenBank DNA database. *Leucogyrophana mollusca* (Fr.) Pouzar and *Hydnomerulius pinastri* (Fr.) Jarosch & Besl sequences were included as outgroups. The alignment was analysed using a heuristic search option with maximum parsimony and maximum likelihood, using PAUP version 4.0a 147 (Swofford, 2003). Moreover, using the software MrBayes version 3.2 (Ronquist *et al.*, 2012), a Bayesian analysis was carried out. Phylogenetic trees were generated using Tree View (Page, 1996) and edited using Adobe Illustrator CS3 version 11.02 (Adobe Systems).

Results

The sequence from Watling Wat. 30231 (ITS1 region) was aligned with 20 sequences obtained from GenBank, including from *Penttilamyces lichenicola* (Thorn, Malloch & Ginns) Zmitr., Kalinovskaya & Myasnikov, *P. olivascens* (Berk. & M.A.Curtis) Zmitr., Kalinovskaya & Myasnikov, *P. romellii* (Ginns) Zmitr., Kalinovskaya & Myasnikov, and the outgroups. The results of the analysis (Figure 2) show that the sequence from Watling Wat. 30231 from Dawyck Botanic Garden is close to that of *Penttilamyces lichenicola*, and that this relationship is very well supported (maximum-parsimony bootstrap support = 99%, maximum-likelihood bootstrap support = 100%, posterior probability = 1.0). However, 29 base-pair changes were found between the new sequence and sequence GU 187531 (DAOM 194172) of *Penttilamyces lichenicola* from Canada.

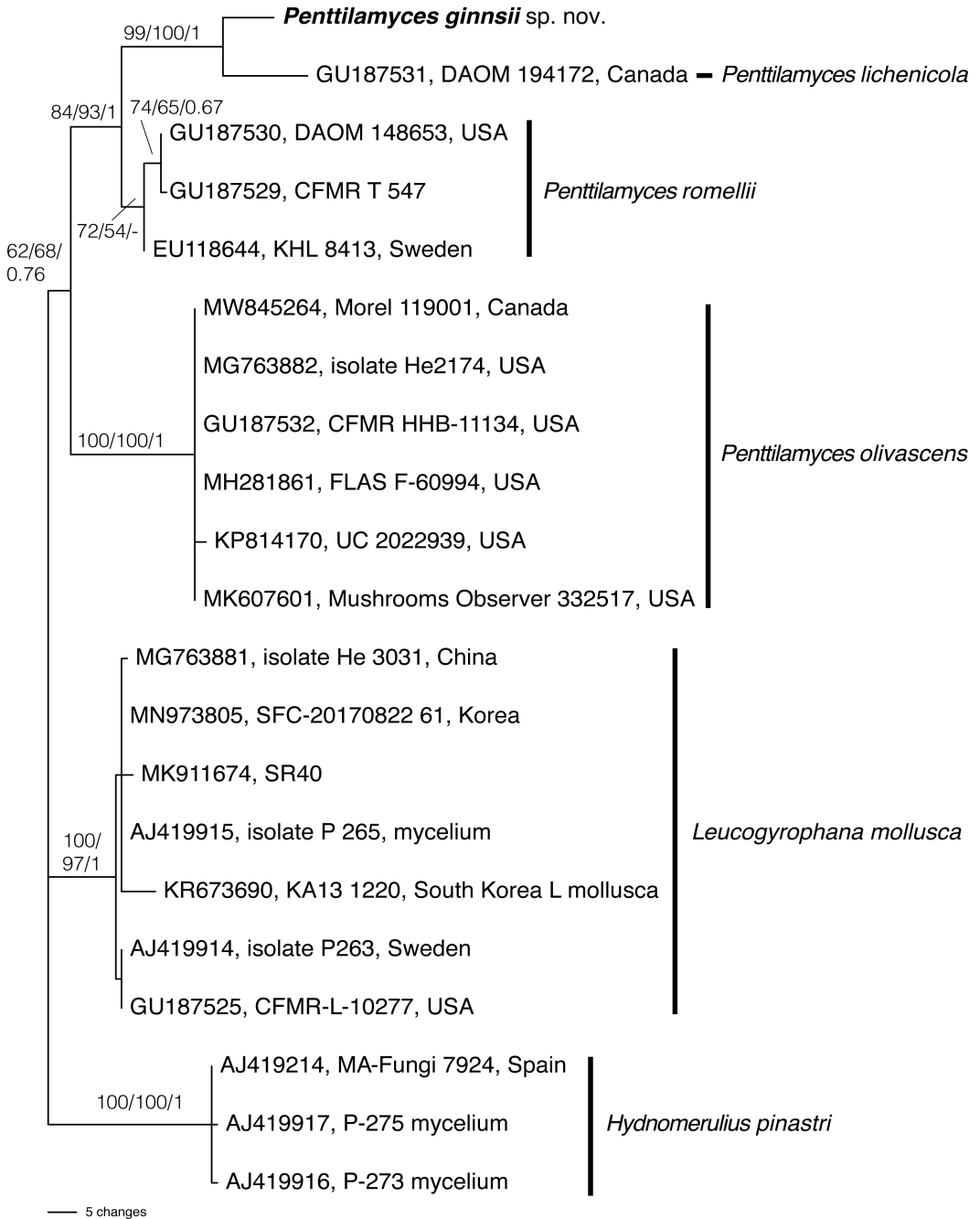


Figure 2. Sequence data for type material of *Penttilamyces ginnsii* compared with those for *P. lichenicola* and species previously placed in *Leucogyrophana*.

Discussion

The molecular information shown in Figure 2 has led us to recognise *Watling* Wat. 30231 as a species new to science, which is here described.

Taxonomic treatment

Penttilamyces ginnsii Watling & M.P.Martin, sp. nov.

This new species differs from all those so far known in *Penttilamyces*, except *P. lichenicola*, by its distinctive bright-orange colour, and from the similarly coloured *P. lichenicola* in its preference for growing with bryophytes and not lichenised fungi. – Type: Scotland, Dawyck Botanic Garden, 24 ix 2006, *Watling* Wat. 30231 (holotype E [barcode E01043381]; MycoBank no. MB840588; GenBank no. [ITS1] MZ648130).

Loosely aggregated or single individual sclerotia, 1–4 mm long, more commonly the latter with some joined, often slightly convoluted, although ranging to entirely smooth in outline, brightly coloured – intense orange, buried among bryopsid mosses, for example *Hylocomium*, *Racomitrium*. The intense colour is confined to the outermost layers, with a uniform, non-distinctive, yellowish white medulla, composed of interlocking, short hyphae giving a mosaic pattern in section; the colour darkens more with age. There are no rhizoids and there appear to be few hyphal connections to the surrounding substrate, although a few may be found among the clusters of sclerotia.

Distribution. Apparently widespread and found from alpine localities with a continental climate.

Habitat and ecology. Possibly always in moss cushions.

Etymology. The specific epithet *ginnsii* acknowledges the monographic work on the meruloid fungi carried out by James Herbert Ginns.

Additional specimens examined (all in E). SCOTLAND. **Argyllshire:** Mull, East Ebudes, Loch na Keal, 7 ix 1968, *Henderson* 9150 (barcode E01043365). **Midlothian:** Edinburgh, “Near Newhaven spring 1822”, *Greville s.n.* (barcode E01043366) [*Greville* (1824a): “Between New Haven and Caroline Park near Edinburgh, on ‘*Hypnum squarrosum*’ Autumn”]. **Peebleshire:** Dawyck, Botanic Garden, Rhododendron Walk, 1 x 2006, *Watling* Wat. 30232 (E E01043589). **Perth and Kinross:** Cross Craigs, Rannoch Moor, 11 x 1980, *P.D. Orton s.n.* (barcode E01234623). **West Lothian:** Muiriston, Livingston, 18 ix 2004, *Watling* Wat. 28576 (barcode E00186878). **Wester Ross:** Applecross, Beinn Bhann, ± 800 m altitude, 11 x 1969, *Henderson* 9450 (barcode E01043364).

ENGLAND. **Cambridgeshire:** Cambridge, 17 iv 1982, *J. Rishbeth s.n.* (barcode E01234625). **Yorkshire:** Hardcastle Craigs, Hebden Bridge, 11 iv 1956, *Watling* Wat. 17c (barcode E01234626) and *Watling* Wat. 667 (barcode E01234627).

Conclusion

Greville's interpretation of *Sclerotium muscorum* in his coloured illustration appears to be a specific bryophilous fungus that, in 1824, was lacking any known teleomorphic stage. Through molecular analysis, it has been ascertained that our fungus is a basidiomycete related to *Penttilamyces lichenicola*, but because of differences in the molecular sequence data between our bryophilous fungus and the lichenicolous taxon described from Canada, we have proposed it as a new species.

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