

A Sequestrate *Galerina* from Scotland

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Summary

A gasteroid brown-spored basidiomycete found in association with *Galerina pseudomycenopsis* in Orkney is shown by molecular methods to be only a morphotype of *G. pseudomycenopsis*. Parallel observations in other fungi are discussed.

Key words: Orkney mycota, agaricoid, *Galerina*, sequestrate, *Weraroa*, DNA sequences, rDNA (ITS1 and ITS2).

Introduction

Much discussion appears in the literature concerning the close relationship between those gasteromycetes which have previously been referred to the unnatural family Secotiaceae and certain families of agarics. These suggestions have been based on anatomical, micro-morphological, developmental and chemical studies. Relatively recently (see Watling, 2000) many hypogeous gasteromycetes have been shown to have parallel close relationships with agarics and the term sequestrate was introduced for both such gasteroid elements, referring to the reduction of an expansion of the fruit-body concealing the spore-producing tissue. More recently molecular techniques have been applied to study this interesting phenomenon and have proved, as theoretically predicted, to be a very powerful tool to demonstrate close, natural genetic links between fungi (Martín, Högborg & Llistosella, 1999; Martín & Rocabruna, 1999) even those with disparate macro-morphology. Reijnders (2000) has summarised these gasteroid/agaric relationships based on classical approaches from the earliest observations to present day studies.

A population of *Galerina pseudomycenopsis* was found in 1990 on South Ronaldsay, Orkney accompanied by a sequestrate form (Fig. 1) and the present paper is the subject of a critical analysis of the two forms to see whether a molecular link could be made between the two.

Methods

During wide ranging field trips undertaken in preparation for a publication on the mycota of Orkney (Watling, Eggeling & Turnbull, 1999), an island group north of Sutherland and Caithness in mainland Scotland, one of us (RW) had the opportunity to visit several of the outlying islands in the archipelago. *Galerina pseudomycenopsis* Pilát & Nannf. was collected in some of these less accessible localities; a familiar agaric having been found at several sites on Mainland and other adjacent islands. It is a widespread fungus of rather acidic, often wet, grassland and, although found throughout the British Isles, it is very probably more northern and montane in its distribution. *G. pseudomycenopsis* has been

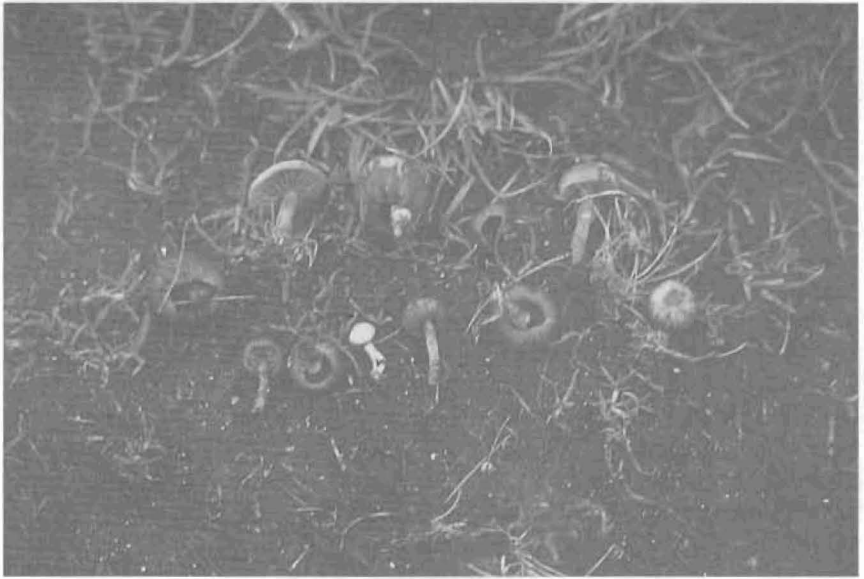


Fig. 1. Basidiomes of gasteroid and agaricoid morphs of *Galerina pseudomycenopsis* in field.

found to be common on the neighbouring archipelago of Shetland (Watling, 1992). It has a circumpolar boreal distribution but is also known from the Palmer Peninsula in the Antarctic (Horak & Miller, 1992).

The survey covered both inland and coastal localities of South Ronaldsay, one of the southernmost islands of the Orkney archipelago 10 km from Duncansby Head on the Mainland. Included in the study area was a cliff-top site at the Brough of Deerness locally called 'The Gloop'; grid. ref. ND 473/856. This is a rather water-soaked coastal site with vegetation consisting of closely sheep grazed turf of *Festuca* and *Plantago maritima*. It was in this plant community that a population of *G. pseudomycenopsis* was found associated with superficially similar basidiomes which differed in the enclosed development and a veil clinging both to the stipe and the pileus. The latter resembled basidiomes of a member of the genus *Weraroa* or *Setchelliogaster tenuipes* (Setchell) Pouzar, the latter which the senior author knows from both California, where it grows with introduced eucalypts, and from Australia its undoubted origin.

These basidiomes found at 'The Gloop' form the subject of this paper. Our combined experiences in classical and molecular techniques have been applied to members of both populations and to a second collection of *G. pseudomycenopsis* from another site on Orkney.

All material is deposited in the herbarium of the Royal Botanic Garden, Edinburgh, Scotland (E).

Material examined: *Galerina pseudomycenopsis*: Orkney, South Ronaldsay, Brough of Deerness, The Gloop, in cliff pasture, 24. ix. 90, Wat. Herb. 22853. Orkney, Ness of Hillswick, in low lying wet grassy area with *Juncus*, 10. ix. 86,

Wat. Herb. 19476. *Weraroa*? sp.: Orkney, South Ronaldsay, Brough of Deerness, The Gloop, in cliff pasture, 24 ix. 1990, Wat. Herb. 22853B.

When unfamiliar material was found during the Orkney and Shetland surveys on return from the field it was immediately described. All collections were dried overnight in a Dorrex fruit-drier operating at about 45°C, packaged and labelled.

A small quantity (10 mg) of the two collections of *G. pseudomycenopsis* as well as the sequestrate form associated with the specimens from 'The Gloop' was subjected to molecular analysis of the internal transcribed spacer regions of rDNA (ITS1 and ITS2), including the 5.8S. According to White *et al.* (1990), the ITS region in fungi can separate taxa at the species or generic level. Total DNA was isolated using E.Z.N.A. Fungal MiniPrep kit (Omega-Biotech, Doraville, USA) as described in Martín & García-Figueroles (1999). Primer pair ITS1F and ITS4 was used to obtain amplifications of both ITS regions, including the 5.8S of the ribosomal RNA gene cluster and small flanking parts of the SSU and LSU genes; primers are described in White *et al.* (1990). Amplifications were carried out using Ready-to-Go (PCR Beads (Amersham-Pharmacia Biotech) as mentioned in Winka, Ahlberg & Eriksson (1998). Amplification products were cleaned using the E.Z.N.A. Clean kit (Omega Biotech) and both strands were sequenced separately using primers ITS1F and ITS4 with an ABI Prism 377 genetic Analyzer and the ABI Prism(BigDye (terminator Cycle Sequencing Ready Reaction kit with AmpliTaq (DNA Polymerase (Perkin Elmer Applied Biosystem). Sequence Navigator (Sequence Comparison software (Perkin Elmer) was used to identify the consensus sequence from the two strands of each ITS region.

Taxonomy

Despite the confusion over the correct name for this fungus and its separation from other small annulate species of *Galerina*, it is now fairly well delimited (Bas, 1960; Gulden, 1980; Horak & Miller, 1992).

Galerina pseudomycenopsis was first described in an account of arctic fungi (Pilát & Nannfeldt, 1954) and is the valid name for a pholiotoid *Galerina* variously called *Galera pumila* (Fr.) Favre f. *oreina* Favre (Favre, 1955), *Galerina moelleri* Bas (Bas, 1960) and *G. pseudopumila* P.D. Orton (1960). The name *Pholiota pumila* has frequently been misapplied to this fungus, e.g. Möller (1945), but this is incorrect as outlined by both Bas (1960) and indicated by Orton in the same year but the latter only by reference to *Agaricus pumilus* Fr., 1838 (Orton, 1960). Bas introduced his epithet to signify that Möller had recognised the true relationships of this agaric, despite using the incorrect name, and Orton introduced his to emphasise the confusion with the true *Galerina pumila*. The latter is now regarded on the basis of Singer's observations (Singer, 1961) to be the same as Kühner's interpretation of *G. mycenopsis* (Fr.) Kühner (1935), a usage which is found in many present day accounts.

Descriptive Information

Basidiome: shortly drum-stick shaped. **Pileus:** 10-15 mm almost globose or broadly ellipsoid, closely clasping the stipe, not expanding, rusty tawny

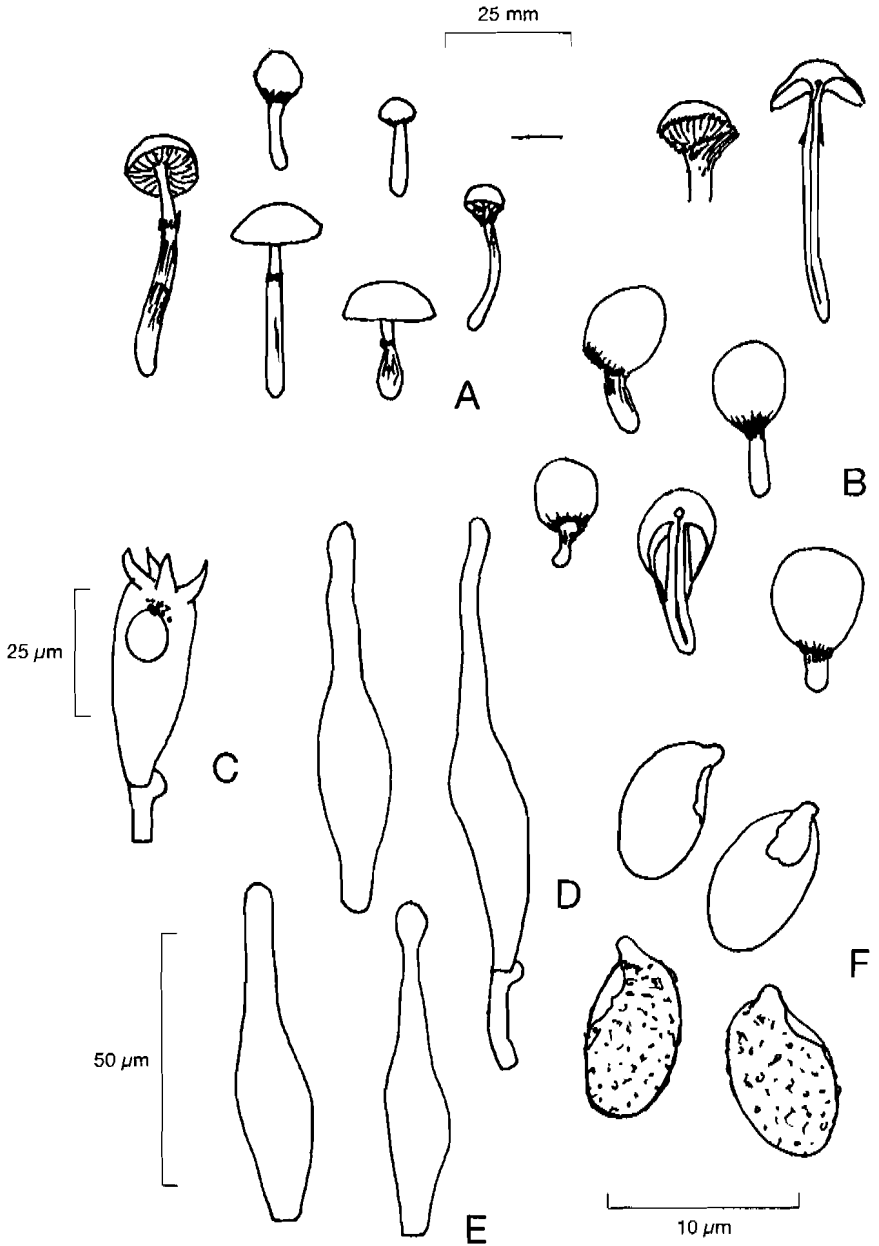


Fig. 2. Agaricoid morph of *Galerina pseudomycesopsis* Wat. No. 22853. B. Gasteroid, *Werarou*-like morph of *G. pseudomycesopsis*. Wat. No. 22853B. C. Basidium with subtending clamp-connection. D. Cheilocystidia. E. Pleurocystidia. F. Basidiospores. C-F from Wat. No. 19476. Magnification indicated by bars; 25 mm for basidiomes; 25 μm basidia; 50 μm cystidia; 10 μm basidiospores.

Table 1. Percent sequence divergences in rDNA (ITS1 and ITS2) from pairwise comparison among isolates of *Galerina pseudomycesopsis* (G.p.)

Pairwise comparison	ITS1	ITS2
G.p. (Wat. Herb. 22853) vs. <i>Weraroa</i> sp. (Wat. Herb. 22853B)	0.8%	1.3%
G.p. (Wat. Herb. 19476) vs. <i>Weraroa</i> sp. (Wat. Herb. 22853B)	0.8%	1.8%
G.p. (Wat. Herb. 22853) vs. G.p. (Wat. Herb. 19476)	1.7%	3.1%

(Henderson, Orton & Watling, 1969) to tawny ochraceous, some slightly darker towards disc, with distinctive ochraceous yellow fringe of fibrils or fluffy flecks joining up with similarly coloured zone on stipe. **Stipe:** 12-15 × 1.75-2.25 mm cylindrical or slightly swollen downwards to white, pubescent-tomentosulate base buried in grass, paling upwards to veil zone, one third hidden within enveloping pileus and there resembling columella, umber to vandyke brown where handled. **Flesh:** thin, concolorous with pileus immediately below disc, paler (ochraceous yellow) elsewhere, darkening downwards from junction with stipe to base; taste and smell indistinct. **Gills:** hidden, fairly closely arranged, distinct although somewhat crumpled, rusty tawny. **Basidia:** 4-spored, clavate, hyaline. **Basidiospores:** ellipsoid-amygdaliform, 10-12 (-15) × 6-8 μm, verruculose from ornamented, closely fitting perispore which separates to give ragged appearance in some views, honey-coloured, darkening in aqueous alkali solutions, with slight evidence of apical callus and smooth hilar plage. **Cheilocystidia:** 30-80 × 7-15 μm, clongate lageniform with narrow neck, slightly swollen venter and apex, hyaline. **Pleurocystidia:** similar to cheilocystidia although often more elongate, scattered with few in some sections, elongate lageniform, 35-90 × 8-16 μm. **Clamp-connections:** present at most septa. **Pileipellis:** with filamentous suprapellis of cylindrical, slightly gelatinised, slightly encrusted, pale rusty tawny hyphae 1-4 μm broad.

Good descriptions of the agaricoid form of *Galerina pseudomycesopsis* can be found in Pilát & Nannfeldt (1954), Bas (1960), Gulden (1980) and more recently in Horak & Miller (1992) in their treatment of arctic-subarctic Alaskan *Galerina* spp. In Gulden (1987) there are SEM electron micrographs.

The sequences of *Galerina pseudomycesopsis* obtained in this study have been lodged in the international data-base (EMBL) with the following accession numbers: AJ300156 (*Galerina pseudomycesopsis*, Wat. Herb. 22853), AJ300157 (*Weraroa* sp., Wat. Herb. 22853B) and AJ300158 (*G. pseudomycesopsis*, Wat. Herb. 19476).

The alignment of the three sequences is shown in Fig. 3 where they are coded with the herbarium numbers. Alignment resulted in 234 sites in the ITS1 region and 222 in the ITS2, without ambiguous areas. There were no differences in the 5.8S rDNA gene and the nucleotide differences among all pairwise comparison for the ITS1 and ITS2 sites were very low and are summarised in Table 1.

There is no doubt that the secotioid sequestrate basidiomes found on South Ronaldsay were of the same genetic parentage as the more normal agaricoid

185
 22853_Wat cattatttqaatlgaclllgggg glgglLgTggtggccclll cgagggatATGTGCACgcccg
 LcaLcLlLlALCPllccacclt GtGCACACtTTCcAcAcTTG
 22853B_Wat cattatttqaatgactttgggg glgglLGTGTGGTGGCCCTTT CGAGGGATGTGHWACGCCCg
 TCATGTTTTATCTTTCCaCCT GtGCACACtTTCGAGACTTG
 19476_Wat cattatttqaatgactttgggt gTggtttatcgcLggccclll cgagggLatLgtgaaCGCCg
 TcAtcTTTATCLLLcCacCT gLgHcAcTTCGAGACTTG

22853_Wat AATAGTATTTTCTGAGGCaA CTCAGTGGGgAGGATTGCTG GTATTATCAGCTCCTCTTGC
 ATTATTCaAGCCTATGTTTT CATATAcCCcAAAAATGTAA
 22853B_Wat AATAGLATTTCCTGAGGCaA CTCAGTGGGAGCAGATTGCTG GTATTATCAGCTCCTCTTGC
 ATTATTCaAGCCTATGTTTT CATATAcCCcAAAAATGTAA
 19476_Wat AATAGLATTTCCTGAGGCaA CTCAGTGGGAgGaTTCG G gLATTATCAGCTCCTCTTGC
 ATTATTCaAGCCTATGTTTT CATATAcCCcAAAAATGTAA

5.8S
 22853_Wat CAGAATGTATCATTGGGcCT TGTGCCATAAACTATATAC AACTTTCAGCAACCGATCTC
 TTGGCTCTCGCATCGATGAA GAACGAGCGAAATGGGATA
 22853B_Wat CAGAATGTATCATTGGGcCT TGTGCCATAAACTATATAC AACTTTCAGCAACCGATCTC
 TTGGCTCTCGCATCGATGAA GAACGAGCGAAATGGGATA
 19476_Wat CaqAATCTATCATTGGGcCT TGTGCCATAAACTATATAC AACTTTCAGCAACCGATCTC
 TTGGCTCTCGCATCGATGAA gAACGAGCGAAATGGGATA

5.8S
 22853_Wat AGTAATCTGAATTGCAGAAT TCAGTGAATCATCGAATCTT TGAACGCACTTTGGCTCTCT
 TCGTATTCCGAGGAGCATGC CTGTTTGGAGTGTCAATTAAT
 22853B_Wat AGTAATCTGAATTGCACAAT TCAGTGAATCATCGAATCTT TGAACGCACTTTGGCTCTCT
 TCGTATTCCGAGGAGCATGC CTGTTTGGAGTGTCAATTAAT
 19476_Wat AGTAATCTGAATTGCAGAAT TCAGTGAATCATCGAATCTT TGAACGCACTTTGGCTCTCT
 TCGTATTCCGAGGAGCATGC CTGTTTGGAGTGTCAATTAAT

22853_Wat TCTCAAGCTTACCAGTTTTc ACAAAATTCCTAATGGCTTTGG AcTTGGGGCTATTTTCTCTG
 CTTCCTAAAgAgGcCTTCCTC CCTTAAATCTATtAgCCCG
 22853B_Wat TCTCAAGCTTACCAGTTTTc ACAAAATTCGTAATGGCTTTGG AcTTGGGGCTATTTTCTCTG
 CTTCCTAAAgAGGCTTCCTC CCTTAAATCTATtAgCCCG
 19476_Wat TCTCAAGCTTACCAGTTTTc ACAAGTTGGTAATGgCTTg AcTTGGGGCTATTTTCTCTG
 CTTCCTAAAgAGGCTTCCTC CCTTAAATCTATtAgCCCG

22853_Wat TACCcGtGGGAGTTCGCTA TtGCTTgATAAATTAATCTac GCGGtGGCCCTTCACTT LAA
 ATGAATTCGLAAdGCTCTA cGjctTytcttAAgTGGqaCA
 22853B_Wat TACCcGtGGGAGTTCGCTA TtGGtGtSAFAAATTAATCTac GCGGtGGCCCTTCACTT LAA
 ATGAATTCGLAAdGCTCTA cGCTCtGCTTAAAGTGGqaCA
 19476_Wat TaccCGtGtggAgTgTCTA TtggLgtgLaalTatctac gccgtggcccttcactttaa
 atgggltgLaclgclLclaa cGjctTytcttAAgTGGqaCA

28S
 22853_Wat AATAATGA/AATTTGAACCLC AAATCA
 22853B_Wat AATAATGA/AATTTGAACCTC AAATCa
 19476_Wat aataatgaaatthgactc aaatca

Fig. 3. rDNA (ITS1 & ITS2) sequence alignment data for the three collections of *Galerina pseudomycesopsis* studied. Capitals Strong sequencing, lower case Wecker sequencing.

basidiomes with which they were associated. The DNA patterns are exactly the same and agree also with material of the agaricoid form from a second locality on Orkney. As the two morphotypes were found together in a locality which was very uniform and which did not differ significantly from other locations where the agaric occurred it can only be assumed that the enclosed basidiomes were aberrant. However, it might be hypothesised that if such aberrations are fixed genetically then sequestrate taxa might arise. Thus Bougher, Tommerup & Malajczuk (1993) have discussed the relationships between *Laccaria*, *Hydnangium* and *Podohydangium* and the senior author has described collections of gasteroid basidiomes in other taxa (e.g. Watling, 1971; 1974). He has also collected many other examples including those in *Lactarius volemus* (Fr.) Fr. and *L. fulvissimus* Romagn. from Scotland and Northern Ireland respectively where a direct relationship can be seen in the field but this is the first time any such relationship has been examined using molecular methods.

Discussion

Bruns *et al.* (1989) have been able to show unequivocally that the hypogeous genus *Rhizopogon* was related to the bolete genus *Suillus*, although this had been hypothesised for some time based on microscopic characters. Many parallel studies have since been made allowing genera with previously unknown affinity to be related to already well-defined bolete and agaric genera, e.g. *Melanogaster* (Bruns *et al.*, 1998). More recently as fresh collections of material of poorly documented secotioid fungi have become available molecular techniques have been used to examine other possible relationships in detail. Thus these techniques have been applied by the junior author to *Setchelliogaster* (Martín & Rocabrana, 1999) to demonstrate even closer links between particular agaric species, e.g. *Descolea rheophylla* (Malençon) Moreno *et al.* In other examples, the overall correspondence of the RFLP patterns (mt-rDNA, ITS region of rDNA) between *Russula messapica* Sarnari and *Macowanites messapicoides* Llistosella & Vidal, discussed in Martín *et al.* (1998), add molecular evidence to the relatedness of *Russula* and *Macowanites*. Calonge & Martín (2000), based on morphological and molecular data, concluded that species of *Gymnomyces* and *Martellia* are gasteroid forms of *Russula*, and those of *Zelleromyces* are gasteroid forms of *Lactarius*. It has also been possible to predict a possible date as to when *Gastrosuillus laricinus* Singer & Both might have separated from its parent form *Suillus grevillei* (Klotzsch) Singer (Baura, Szaro & Bruns, 1992). Now that these links have been made it is hoped that genetic studies will be conducted to attempt to identify the genes involved in producing the sequestrate fruit-body (Watling, 2000).

The present study shows how important it is to marry classical approaches with molecular methods and how the latter are powerful tools in our armoury to unravel relationships within the larger fungi.

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