

A new species of *Ruhlandiella* (Pezizaceae) from Italy

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Abstract This paper describes the newly discovered species *Ruhlandiella peregrina*. Full description and illustrations of macro- and micromorphological features of the new taxon are provided. This species differs from other described species in ascus and ascospore size and in the crested and ridged ornamentation of ascospores. As is the case in two other similar species, the asci of *R. peregrina* do not becoming blue in iodine solutions.

Keywords Australian fungi · Morphology · Taxonomy

Introduction

A collection from Sicily of a small exothecial member of the Pezizales has led us to a review of the genus *Ruhlandiella* Henn. (Hennings 1903). *Ruhlandiella* is a

genus seemingly native to Australia but the type species, *R. berolinensis* Henn., has been transported around the world along with its mycorrhizal hosts (Dissing and Korf 1980). Although it is a known ectomycorrhizal associate of *Eucalyptus* (Warcup and Talbot 1989), Henning's collection came from a potted *Melaleuca* sp. in a Berlin Botanical Garden hothouse, which, like *Eucalyptus*, is an Australian member of the *Myrtaceae*. Along with the original collection, two other Australian mycorrhizal fungi were recorded, viz. *Descomyces albus* (Berk.) Bougher & Castellano and *Hydnangium carneum* Wallr. (Hennings 1903). As summarized by Galán and Moreno (1998), *R. berolinensis* has been collected in Australia but also in *Eucalyptus* plantings around the world, including Tasmania, the Canary Islands, Spain, and California (where it was reported as *Ruhlandiella hesperia* Setch.).

The genus *Muciturbo* Talbot, with three species, is probably best considered a synonym of *Ruhlandiella* as was suggested by Hansen et al. (2001). The described species of *Muciturbo* are ectomycorrhizal and are all associated with Australian plants, *Eucalyptus*, *Acacia* and *Melaleuca* (Warcup 1990; Warcup and Talbot 1989).

In this paper, we present a new species of *Ruhlandiella* that was collected in Italy but probably represents yet another example of an Australian mycorrhizal fungus that has been imported into Europe. In reviewing the literature, it is clear that most of the taxa that might be critical in this study are infrequently collected and so resolution of their identities awaits further collections and investigations. For now, we can confidently say that as described they are not close to our species.

Materials and methods

Field collections were made in the sandy littoral region of Sampieri (Ragusa) in February 2003 and January 2005.

Taxonomical novelties: *Ruhlandiella peregrina* Lantieri & Pfister, sp. nov.

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This area is in the southeastern part of the island of Sicily and it has been strongly influenced by human activity. The natural retrodunal vegetation has been replaced, through reforestation efforts, by *Acacia saligna* Lindley and scattered specimens of *Eucalyptus camaldulensis* Dehnh. Our collections were found at some distance from these trees, in the *Centaureo-Ononidietum Ramississimae* association (Frei 1937), a plant community of the consolidated inner dunes. *Centaurea sphaerocephala* L., *Ononis variegata* L. and *Euphorbia terracina* L. occurred near the collecting area.

Specimens are deposited in the Farlow Herbarium, Harvard University, and Royal Botanic Gardens, Kew. These were studied morphologically and photographed using an Olympus BX50 compound microscope equipped with an Olympus XC50 digital camera. Mounts were made in water, Melzer's reagent, and Cotton blue in lactic acid.

DNA extraction, DNA sequencing, and molecular analyses followed the protocols outlined by Smith and Healy (2009). The sequence used in this study are deposited in GenBank as number JF343549. In order to determine a placement for this collection, a BLAST search of the partial large subunit rDNA was performed.

Results

Morphology

Spore size and ornamentation, ascus size and iodine reactivity, and width and length of paraphyses show this species to be different from previously described species. Table 1 provides comparative data.

Comparison of sequences

In the Blast searches, the 28 s rDNA of the Italian collection matched with 99% similarity (541 out of 542 bp) the sequence of *Ruhlandiella berolinensis* Henn. (GenBank no. AF335175, the neotype of *R. berolinensis* from the Canary Islands). The ITS rDNA sequence of our Italian collection was most similar to species of *Peziza* in the *P. badia* clade (e.g., *Peziza badia*, *P. phyllogena*, *Eremiomyces echinulatus*, and *Terfezia claveryi*) with similarity values of 86–87%. In the most complete published phylogenies of the Pezizaceae, *Ruhlandiella* falls within this clade (Hansen et al. 2001, 2005). GenBank contains no ITS sequences of *R. berolinensis*.

Taxonomy

Ruhlandiella peregrina Lantieri & Pfister, sp. nov.

Mycobank no: MB 561170

Figure 1a–g.

Etymology: *peregrinus* (Latin), meaning travelling about, foreign; referring to the possible origin of this species being Australia.

Ascomata forma exothecii usque ad 3 mm diam., globosa, superficie cerebriformi, brunneovinosa roseo colore suffusa, stipite brevi, interdum parvis mycelii fasciculis cum arenosis reliquis commixtis, praedito. Hymenium fere 500 µm crassum. Asci juvenes parte apicali incrassata, cylindrica vel cylindrico-clavata, 230–300 (350) × 30–32.5 µm, basi attenuata, 8-sporici, haud caerulescentes ope Melzer, inconspicui et verisimiliter dissoluti intus in hymenio. Excipulum incremento magnopere vario, ex globosis vel angularibus cellulis, usque ad 70–75 µm diam., constitutum. Ascospores globosae, 15–19 µm diam. sine ornamento (18–22 µm diam. ornamento addito), uniseriatae, in maturitate brunneae, singulari magna guttula oleosa praeditae, cristis et angulis 2–3 µm altis formantibus reticulum imperfectum exornatae. Paraphyses numerosae, filiformes, saepe septatae, 2 µm latae, excedentes ascos usque ad 50 µm, mersae in materia gelatinosa.

Habitatio subhypogea in solo arenoso, prope *Centauream sphaerocephalam*, *Ononidem variegatam* et *Euphorbiam terracinam*.

Holotypus ut supra, in solo arenoso, prope *Centauream sphaerocephalam*, *Ononidem variegatam* et *Euphorbiam terracinam*, 08 Jan 2005 lectus, legit A. Lantieri, in Herbario FH sub n. 00301074 conservatur.

Ascomata an exothecium up to about 3 mm diam., globose, convoluted and brain-like, brownish with vinaceous tints and rosy highlights, with a short stipe and mycelial tufts to which sandy debris adheres.

Hymenium about 500 µm thick. *Young asci* with a thickened apical cap, cylindrical or cylindrical-clavate, 230–300 (350) × 30–32.5 µm, attenuate at base, 8-spored, not stained blue with Melzer's reagent, indistinct and probably disintegrating within the hymenium. *Excipulum* composed of globose to angular cells, up to 70–75 µm diam. *Ascospores* globose, 15–19 µm diam. excluding ornamentation (18–22 µm diam. including ornamentation), uniseriate, at maturity brown, with a single large oil droplet, ornamented with crests and ridges 2–3 µm high that form an incomplete reticulum. *Paraphyses* numerous, filiform, frequently septate, 2 µm wide, exceeding the asci up to 50 µm, embedded in gel.

Habitat: partly buried in sandy soil, near *Centaurea sphaerocephala*, *Ononis variegata* and *Euphorbia terracina*.

Specimens examined Holotype As above, on sandy soil, near *C. sphaerocephala*, *O. variegata* and *E. terracina*, 08 Jan 2005, A. Lantieri (FH 00301074); Italy, Sicily, Sampieri (Ragusa) region, on sandy soil, near *C. sphaer-*

Table 1 Comparative data among species of *Ruhlandiella* and *Muciturbo*

Taxon	Distribution and host associations	Ascus size	Ascus persistence	Reaction in iodine	Ascospore size	Ascospore color at maturity	Ornamentation	Paraphyses	Anamorph
<i>Ruhlandiella peregrina</i> Lantieri & Pfister	Italy: collected near <i>Centaurea</i> , <i>Ononis</i> , <i>Euphorbia</i>	230–300× 30–32.5 µm	Probably disintegrating	None	15–19 µm w/o ornamentation, 18–22 µm with ornamentation	Brown	Crests and ridges 2–3 µm high	2 µm broad, exceeding the asci up to 50 µm	None reported
<i>Ruhlandiella berolinensis</i> Hennings (1903)	Australia: <i>Eucalyptus</i> . Occurs where <i>Eucalyptus</i> has been introduced	390–440× 32–40 µm	Evanescent at maturity	Amyloid, contents dextrinoid	18–20 µm w/o ornamentation; 24–28 µm with ornamentation	Dark yellowish	Reticulate up to 4 µm high	10–18 µm at tip, walls gelatinous 4–8 µm; exceeding asci by 50–100 µm	None reported
<i>Ruhlandiella parvispora</i> (K.S. Thind & Waraich) G. Hirsch (1983)	India: on soil	200–220× 17–18 µm	Persistent	Amyloid	12.5–15.1 µm w/o ornamentation	Brownish	Reticulate up to 1–2 µm high	5–6 µm at tip, mucilaginous	None reported
<i>Muciturbo reticulatus</i> Talbot (Wareup and Talbot 1989)	Australia: <i>Eucalyptus</i>	290–405 µm	Not reported	Amyloid	26–36 µm w/o ornamentation	Blackish brown	Reticulate flanges 3.5–6 µm high	5.5–8.5 µm at tip, gelatinous, strongly agglutinated	Yes ^a
<i>Muciturbo truncatus</i> Talbot (Wareup and Talbot 1989)	Australia: <i>Eucalyptus</i>	230–300× 38–45 µm	Probably gelatinizing at maturity	None	23–28 µm w/o ornamentation	Blackish brown	Verrucose, truncate warts, up to 3.0–3.5 µm	5.5–6.0 µm. Exceeding the asci by 100–140 µm	None reported
<i>Muciturbo verrucosus</i> Talbot (Wareup and Talbot 1989)	Australia: <i>Eucalyptus</i>	300–350× 60–80 µm	Probably gelatinizing at maturity	None	30–39 µm w/o ornamentation	Blackish brown	Verrucose warts forming meandering ridges	5–10 µm, mucilaginous, exceeding asci 80–100 µm	None reported

^a Described by Wareup and Talbot (1989) as pale pink in powdery masses, conidia on short denticles directly on branching hyphae.

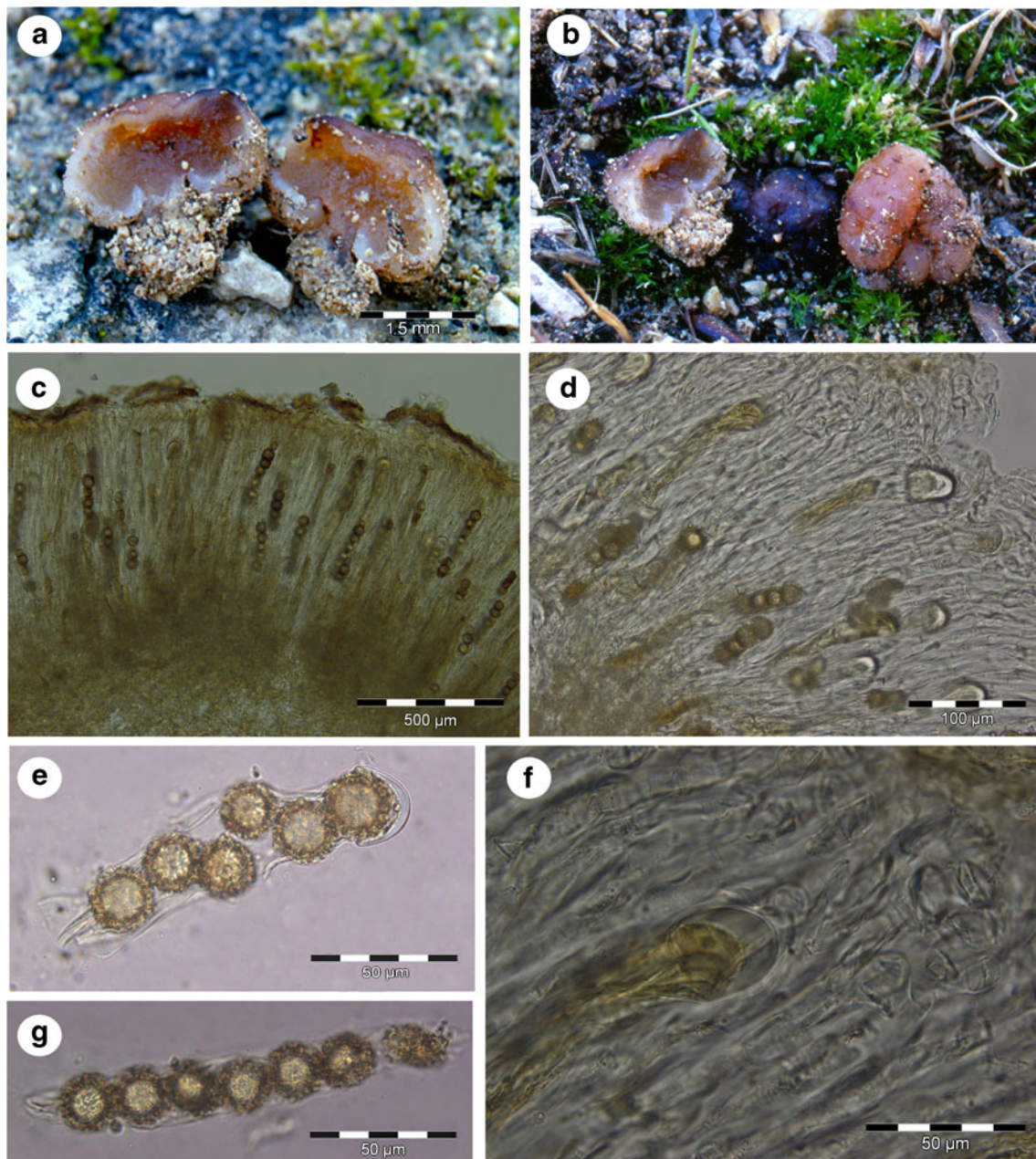


Fig. 1 a–g *Ruhlandiella peregrina*. **a, b** Ascomata in the field, hymenium covering the outer surface; **c** section showing the hymenium with colored ascospores and paraphyses exceeding the

asci; **d** hymenium showing ascospores and refractive ascus tips; **e, f** asci and ascospores; **g** a portion of the hymenium with a thickened ascus tip and paraphyses

ocephala, *O. variegata* and *E. terracina*, 25 Feb 2003, *A. Lantieri* (KM 167991).

Discussion

Molecular and morphological studies support the assignment of this fungus to the genus *Ruhlandiella*. This

collection differs in several ways from *R. berlinensis* and the described species in *Muciturbo*. The apothecia are small compared with those reported by Dissing and Korf (1980) and Galán and Moreno (1998, 1999).

The asci are smaller and do not become blue in Melzers reagent. The reaction is described in *R. berlinensis* as weakly amyloid over the walls. This diffuse bluing can be easily overlooked, but examination of several sections from several ascomata showed no bluing in either fresh or dried

material. The contents of the asci do become somewhat dextrinoid, but there is no sign of the color change on the walls of the asci.

The ascospores are globose and become brown at maturity as is the case in *R. berolinensis*, but they are smaller and the ornamentation is less distinct than in that species. A well-formed and regular reticulum is described and illustrated in *R. berolinensis*, but in our collection the spore markings are more irregular, only rarely forming a well-defined reticulum. The ascospores of *R. peregrina* have lower ornamentations in the form of crests and ridges that anastomose. We have also compared our collection with published descriptions of the described *Mucoturbo* species and find that *R. peregrina* differs in spore size, ornamentation and ascus morphology. Table 1 shows these comparative data.

In Europe, a confusing array of taxa have been put forward for fungi with morphologies similar to that seen in our collections. All of these produce exothecia and are hypogeous or semi-hypogeous. *Sphaerozone ostiolatum* (Tul. & C. Tul.) Setch. is one such fungus. It has persistent asci that are weakly amyloid and ascospores with blunt or truncate warts (Dissing and Korf 1980; Montecchi and Sarasini 2000). The one reliable collection cited by Montecchi and Sarasini (2000) was found with *Pinus*. *Sphaerosoma fuscescens* Klotzsch, as described by Dissing and Korf (1980) and Gamundí (1975) differs from *R. berolinensis* in that its paraphyses do not greatly exceed the asci in length and do not have gelatinous sheaths; the ascospores have a reticulate surface and asci are non-amyloid.

One of the puzzling features regarding this collection is its occurrence in an area dominated by herbaceous plants. *Eucalyptus* species and another Australian plant, *Acacia saligna*, are present but scattered, and they are not close to the collection site of this species.

We find the overall form, the habitat and aspects of the asci and ascospores to be compelling reasons for recognizing this species. We noted peculiar apical thickenings of the asci in our collection, a characteristic not previously reported in species of this genus. We have seen no evidence of active ascospore discharge. The lack of an amyloid reaction of the asci in our collection deserves some additional comment. It has been shown in the Pezizaceae that both amyloid and non-amyloid reactions occur (Hansen et al. 2001). Galán and Moreno (1998) provide a thorough description of fresh material of *R. berolinensis*. They point out that asci do disintegrate and free ascospores within the hymenium. The

breakdown leaves portions of the ascus walls as plates that can be found in the hymenium. These pieces retain their amyloid reactivity. Within several of the clades of the Pezizaceae, the blueing reaction has been lost (Hansen et al. 2001). This seems particularly true in cases where forcible discharge of ascospores has been lost, as in certain of the hypogeous lineages, such as in *Cazia flexascus* Trappe, *Terfezia arenaria* (Moris) Trappe, and species placed in *Pachyphloeus* Tul. & C. Tul.

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Conflict of interest The authors declare that they have no conflict of interest.

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