A NEW RECORD OF *APODOSPORA* FROM AUSTRALIA, A RARELY COLLECTED COPROPHILOUS ASCOMYCETE.

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Abstract

Apodospora peruviana from Australia is described. It was also grown in culture (CBS 118394) and the ribosomal internal transcribed spacer (ITS) region has been sequenced (Genbank EU573703).

Key words: Lasiosphaeriaceae, fungi, systematics.

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Introduction

During continued observation of macropod dung collected from Australia (Bell 2005), a further interesting and seldom seen species of *Apodospora* was found.

Materials and Methods

The Australian sample of *Apodospora peruviana* first developed on incubated wombat dung (*Vombatus ursinus*), collected in Bolaro State Forest, NSW by R. Corringham in 1997 (PDD 83066). This dung sample was kept in a refrigerator in a dried state until it was incubated on damp filter paper in a glass-lidded container in January 2005. Although the age of the collected dung was unknown, we can deduce from this that *A. peruviana* ascospores can remain viable in a dried state for at least eight years. Once incubated, *A. peruviana* took two months of incubation before mature perithecia were observed. Initial microscopic examination of the fungus was made in water mounts and several semi-permanent slides were made using Shear's mounting medium. A second collection of *A. peruviana* was obtained from a sample of unidentified dung in the Deva River area, NSW, collected by P. Cormack in 1997 and incubated by us in March 2006 (PDD 90052). Ascospore sizes were determined by measuring at least 50 ascospores from each collection. Colour references below follow the notation of Kornerup & Wanscher (1989).

A few mature perithecia from the original collection (PDD 83066), were surface sterilized in a 3% solution of hydrogen peroxide for ten minutes after which their contents and fragments of the perithecial walls were spread on to the surface of Difco Corn Meal Agar (CMA) and incubated at 25°C under a daily cycle of 12 hr light/12 hr dark. After seven days, a scrap of pink mycelium growing from a portion of perithecial fragment was plated on to fresh CMA agar where it continued to produce a pink mycelium. After a further 38 days mature perithecia had formed on the agar. Many were submerged in the agar and lay on their sides similar to their habit on the original dung sample. The mycelium was mostly submerged with a few aerial floccose hyphae producing a distinctive pink colour (11A8) in the agar. No anamorph was seen. This culture was inoculated on to further CMA plates augmented with autoclave-sterilized rabbit droppings. Here the fungus grew well, producing many perithecia especially on the rabbit pellets. These axenic cultures were later freeze dried to provide further herbarium samples (PDD 83102, PDD 83103 & PDD 83104). The original culture has been deposited with the Centraalbureau voor

Schimmelcultures, Utrecht, Netherlands (CBS 118394).

In order to sequence the ITS, one of us (Robert Debuchy), grew *Apodospora peruviana* on M2 media (Esser 1974) covered with a cellophane membrane. After three days, a tiny bit of *A. peruviana* mycelium was taken from the cellophane membrane and transferred to a 0.2 mL tube filled with 100 µL of a Taq PCR mixture according to Q-BIOgen instructions (Strasbourg, France) with primers PN3 (5'-ttggtgaaccagcggaggatc-3') and PN10 (5'-tccgcttattgatatgcttaag-3') (Neuvéglise *et al.* 1994). The tube containing the mycelium and the PCR reaction was then frozen at -20°C to disrupt the mycelium. After freezing, the PCR reaction tube was directly submitted to an initial denaturation for 2 min at 94°C, and then to 40 cycles of amplification (1 min at 94°C, 1 min at 60°C, 1 min at 72°C). The reaction ended with a final elongation step of 5 min at 72°C. The success of amplification was checked by depositing 5 µL of the PCR reaction on an agarose gel. The PCR reaction was sent for sequencing with primers PN3 and PN10 to Genome Express (Meylan, France). Sequence files were compared with the electrophoregrams to correct any nucleotide miscalling and the 532 base pairs of the *A. peruviana* ITS were assembled with CAP3 (Huang & Madan 1999). The ITS sequence was deposited at GenBank (accession number EU573703).

Taxonomy

Apodospora peruviana Muroi & Udagawa. (Figures 1A–E & 2A–C).

Perithecia relatively large (approx. 0.5 mm diam), scattered, superficial to slightly submerged, many reclining with a reddish-brown colour (11F6-7) (Fig. 1A). A squash of the perithecial wall shows that a pink colouration (11A7-8) resided in the cells of the interlocking hyphal mat forming a network over the brown pseudoparenchymatous peridial tissue (Fig. 1B). The pink pigment in the hyphae remains after the material was mounted in Shear's mounting medium (at least in the short term). The pink colouration also stains the dung immediately surrounding the perithecia (Fig. 1A). Perithecial necks were black and without vestiture. Paraphyses numerous, hyaline, unbranched, uniform in diameter throughout their length, septate, with rounded apices (Figs 1C & 2A). Asci spore-containing portion cylindrical with long stalk, each containing 8 ascospores (Figs 1C & 2A). Asci approx. 300 µ m long, but difficult to measure accurately due to their elastic nature and to their tapering non-ascospore portions which were not easily released from the hymenial layer where they were obscured by the paraphyses. Ascus ring prominent, approx. 3 µm in diam and remaining as a prominent rigid ring even in discharged asci (Fig. 1E). Ascospores initially uniseriate but quickly becoming irregularly biseriate in water mounts, dark at maturity, single celled, symmetrical, ellipsoidal to ovoid, each surrounded by a gelatinous sheath 5–6 µm wide, which is invaginated over the (usually) apically placed germ pore (Figs 1C & 2B). Ascospore measurements (minus their sheath), 27–38 x 16–21 µm.

Herbarium material: On wombat dung (*Vombatus ursinus*), Bolaro State Forest, west of Bateman's Bay, south west of Nelligen, NSW, lat. 35° 40' 32" S, long. 150° 04' 30" E. Habitat: forest with creek. Vegetation type: mixed *Eucalyptus* with creek community scrub. Collector: R. Corringham, 4 November 1997 (PDD 83066). Axenic freeze dried cultures produced from specimen above: (PDD 83102, PDD 83103 & PDD 83104). On unidentified dung, 17 km W. of Morua, Deva River, NSW, Collector P. Cormack, 25 May 1997 (PDD 90052).

Discussion

Apodospora was described as a genus within the Sordariaceae by Cain & Mirza (1970). Characters for the genus included filiform and abundant paraphyses, a continuous gelatinous sheath around the ascospores and apical germ pores. Cain & Mirza described, illustrated and produced a key to three species: *Apodospora simulans* (type species), *A. viridis* and *A. thescelina*, differing in perithecial colour ("greenish" or "reddish-brown") and ascospore size. They also described *A. simulans* as having a phialidic anamorph state in culture. Their cultures failed to produce the teleomorph even though several unspecified types of agar were used. *Apodospora viridis* also produced a floccose, green, phialidic anamorph on the surface of the dung. The three species of *Apodospora* were variously found on the dung of moose, rabbit, cow or sheep collected in North America, Canada and Mexico. Cain & Mirza (1970) listed 25 collections, the most commonly collected species being *A. simulans*, most of which developed on incubated moose dung. Lundqvist (1972) provided a further key

Table 1. List of main characters separating five species of *Apodospora*.

Species	Peridium colour	Neck	Ascospore size & shape
A. simulans	reddish/brown	glabrous	20–25 x 9–11 µm, oblong/ellipsoid
A. thescelina	dark brown	glabrous	24–32 x 15–19 µm, ovoid/ellipsoid
A. viridis	yellow/green	papillae	48–53 x 29–31 µm (av.), ellipsoid
A. gotlandica	brown	glabrous	30–41 x 15–18 µm, ellipsoidal
A. bulgarica	olive/brown	rigid hairs	22–30 x 13.7–16 µm, ovoid/ellipsoid
A. peruviana	brown/pinkish	glabrous	33–43 x 17–23 µm, oblong/ellipsoid

to the known species including a new one, *A. gotlandica*, collected on horse dung in Sweden. He listed many other collections of *A. simulans* on a variety of dung substrates. He also suggested that *Apodospora*, although looking "very sordarioid", would be better placed in the Lasiosphaeriaceae, since the peridium was vinaceous in colour and there was the occasional appearance of a subapical globule in the asci. He also recorded the invagination in the ascospore sheath at the position of the germ pore. Fakirova (1973) also provided a key to *Apodospora* including a new species, *A. bulgarica*, from cow dung. Muroi *et al.* (1987) described a further new species, *A. peruviana*, from llama dung collected in Peru. They stated that their species is close to *A. simulans*, but the ascospores were larger and the perithecia bare rather than hairy. These four publications represent the only records of this genus to date, which is puzzling, since Cain & Mirza (1970) reported a fairly substantial collection of 25 records in their initial paper, and Lundqvist listed further collections made on a variety of dung substrates. One might thus expect *Apodospora* records to be reasonably common. The main differences attributed to the described species are listed (Table 1).

Although it has been necessary to précis the descriptions of some of the authors in Table 1, due to the non-uniform nature of descriptions, its construction reveals some questions and overlaps. For example, in his general description of *Apodospora*, Lundqvist (1972) mentioned the vinaceous peridial colour common to the genus, stating that Swedish collections of *A. simulans* mostly exhibited a wine-red colour to the peridium, although this colour was sometimes absent. But he described *A. gotlandica* as having a brown peridium and there is no mention of red or pink pigments as being present in this species. There is some overlap/contiguity between the ascospore sizes of *A. gotlandica*, *A. thescelina*, *A. bulgarica* and *A. peruviana* and a certain amount of similarity in the shape of the ascospores as they are illustrated. It seems that what one author considers to be ovoid could very well be described by another as ellipsoid. For example, compare the illustrations of *A. thescelina* by Cain & Mirza (1970, Figs 10–14), with those of *A. gotlandica* by Lundqvist (1972, Fig. 68). The oddly shaped "drop like" ascospores sometimes present in *A. bulgarica* (Fakirova 1973, Fig. 2) could be a reflection of the fact that this collection also exhibited asci containing abnormal numbers of ascospores. We have observed this phenomenon in *Podospora curvuloides*, which also exhibited abnormal numbers of ascospores in the asci. Also, the "rigid brown septate hairs" described for the neck of *A. bulgarica* frustratingly do not match the illustration of same (Fakirova 1973). Instead they resemble the hairy neck characteristic of the young perithecia of *A. simulans* and *A. peruviana*, as described by Cain & Mirza (1970), Lundqvist (1972) and Muroi *et al*. (1987). *A. viridis* stands quite apart from the other five described species insofar as it exhibits green pigmentation and larger ascospores.

Vacillation when matching fungi to published descriptions is familiar to and bedevils all fungal taxonomists, but it is made especially problematical in these days when temporary importation of fumigated, dried and thoroughly dead type material is made prohibitively expensive for mycologists who are independent of large, publically funded organisations. Thus we have to rely on published descriptions alone when comparing our Australian collection of *Apodospora* described above. However, as evidenced from the descriptions of all known species (Table 1), the morphological features of ascospores of *A. thescelina*, *A. gotlandica*, *A. bulgarica*, and to a lesser extent *A. simulans*, show considerable overlap with *A. peruviana*. Until such time as

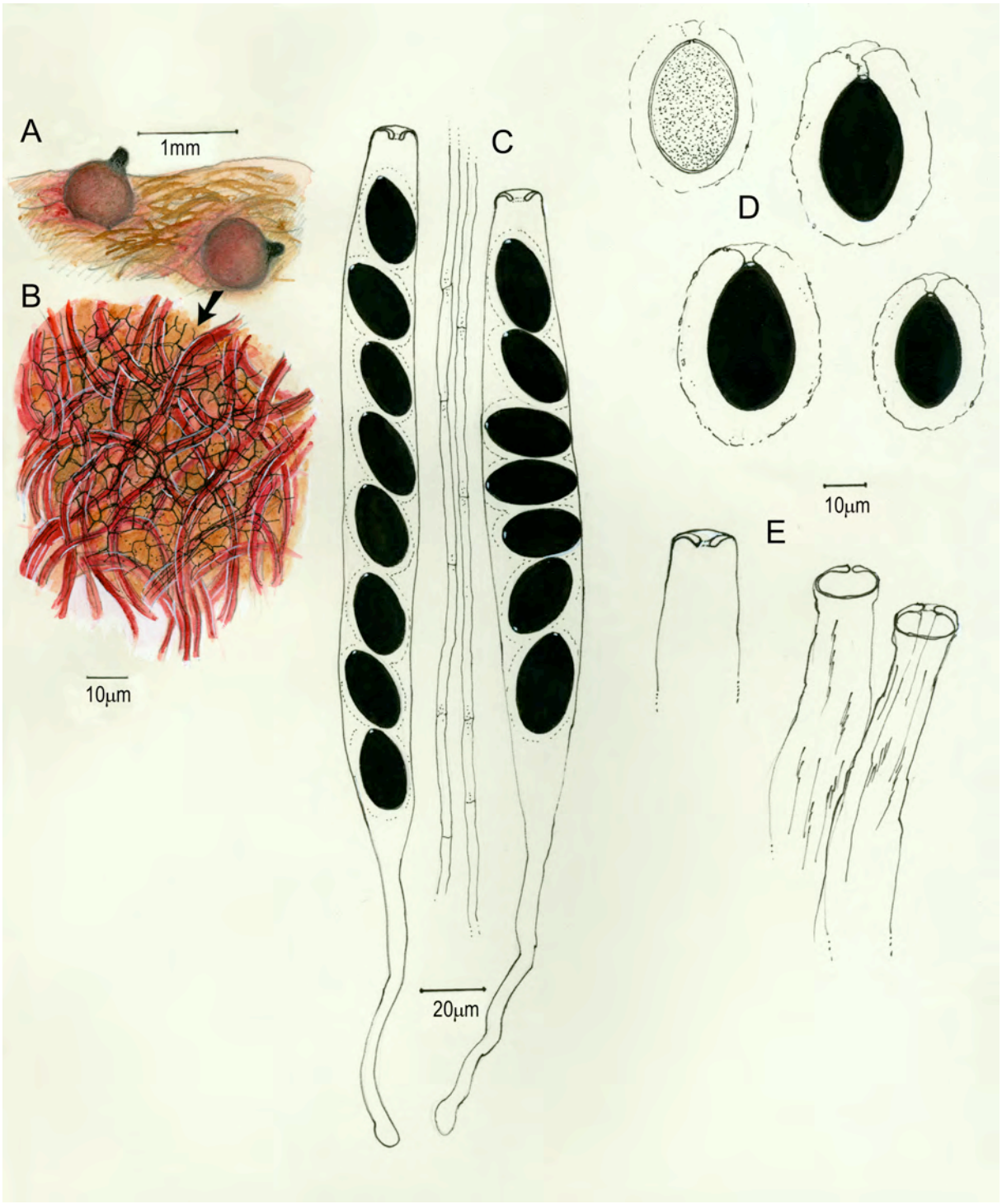

Figure 1. *Apodospora peruviana* A–E. A. Perithecia in situ on dung substrate. B. Detail of peridium showing pink hyphal mat covering perithecial wall. C. Mature asci and paraphyses. D. Immature and mature ascospores. E. Discharged asci showing prominent apical rings.

these other named species are obtained in culture, sequenced and verified against type material, there is no way of ascertaining their validity.

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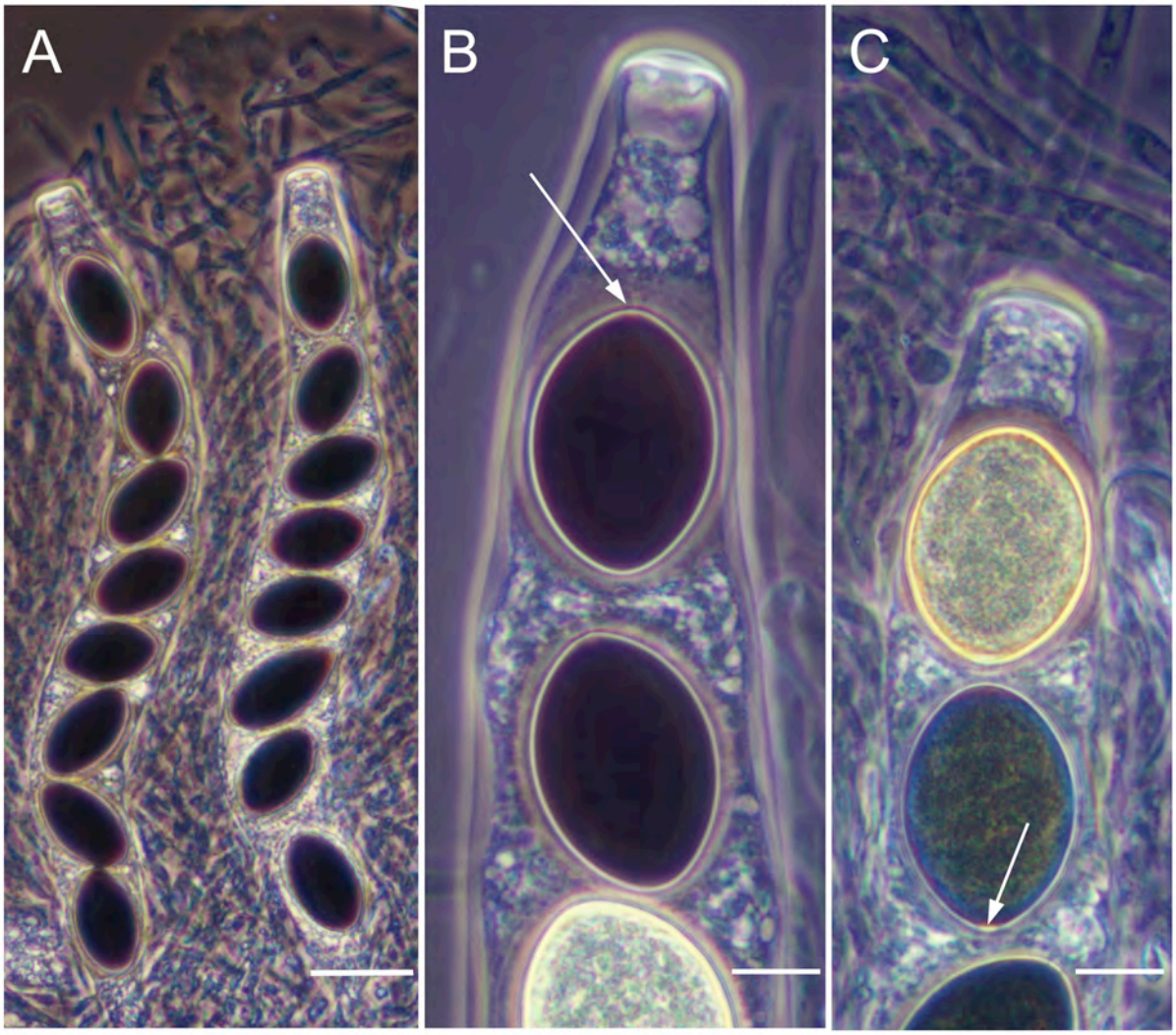

Figure 2. *Apodospora peruviana* CBS 118394. A–C. Photos taken in water mounts of fertile perithecia from a 38 day old axenic Difco CMA culture (incubated at 25°C). A. Asci containing uniseriate ascospores surrounded by paraphyses. B. Ascospores showing the apical germ pores (arrow on one of these) and gelatinous sheaths. C. Illustrating the occasional ascospore with a basal germ pore (arrowed). Scale bars: A. 30 µm, B&C. 10 µm.

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