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Antrelloides atroceracea, a new genus and species in the Pezizaceae (Pezizales) from Australia

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Abstract: A new genus, *Antrelloides*, and a new species, *Antrelloides atroceracea* P.S.Catches. & D.E.A.Catches. (Ascomycota, Pezizales, Pezizaceae) from Australia are described and illustrated. Phylogeny within the Pezizaceae is discussed and an overview of exothecial, cleistothecial and apothecial members of the Pezizaceae given. Morphological and phylogenetic comparisons are made within the Pezizales.

Keywords: Fungi, Pezizaceae, Pezizales, *Antrelloides*, Kangaroo Island, Australia

Introduction

A curious Ascomycete, first found in 2007, appears as small cushion-like black lumps (Figs 1A & 1B) amongst laterite nodules alongside and on sandy tracks of Flinders Chase National Park, Kangaroo Island (Fig. 1E). It has also been collected in grey sand on a track between Denmark and Walpole, Western Australia. The stipe is buried in the soil and, on excavation, the whole fruit body is seen to be turbinate, the obconic base capped by a thin, black, convex disc. In section, the stipe consists of irregular, knobbly columns radiating upwards and outwards from the base. The ascoma resembles a miniature cavern or grotto of continuous columns of stalactites and stalagmites, but gathered at the base and capped by the hymenium (Figs 1C & 1D). When first observed, the black lumps looked like small pieces of discarded patent black shoe leather, hence the fungus was given the informal name 'Shiny Black Shoe Leather'. The hymenial surface of later collections was dull, so the tag name became 'Black Shoe Leather'—an appellation we still use, as it is helpful when describing to other potential collectors what to look for. However, the texture is firm and waxy but brittle, certainly not leathery nor, obviously, has it anything to do with shoes.

Flinders Chase National Park covers an area of approximately 325 km^2 of largely undisturbed remnant vegetation. Its habitats include eucalypt woodland, sandy heath, rugged coastal scrub and dunes. Since we have been surveying in Flinders Chase we have found rare and under-collected fungi such as *Amanita arenaria* (O.K.Mill. & E.Horak) Justo (Catcheside & Catcheside 2005) and the species described in this paper, both of which have also been found in Western Australia.

Materials and methods

Habitat and associated plant communities were noted in the field. Collection locations were recorded by GPS, geodetic datum WGS84/GDA94 (Garmin GPS12) and *in situ* and section photographs were taken with a Nikon E4500 or, for Fig. 1D, a Lumix DMC-GX7 and for Fig. 1E, the habitat shot, an Olympus TG2. Macroscopic characters were described directly from fresh material. Colours are designated using the Royal Botanic Gardens Edinburgh Colour Chart (1969), given as colour descriptor and number, e.g. violaceous black 38, and in general terms. For the more variable colouration in the stipe tissue, colours are given according to Kornerup & Wanscher (1978) (page number, column letter, row number, e.g. 2B4). Fresh material was dried in a food dehydrator at 35°C for 24 h (Hydraflo 1000FD).

Sections of fresh material and dried specimens were hand-cut and mounted in various media. For the amyloid reaction, fresh material was stained with Melzer's reagent and dried material was rehydrated in 5% NH_4OH before staining. Water mounts were used to determine colour of context.

Measurements were made using an Olympus BH-2 microscope at 400× or 1000× with a calibrated ocular micrometer. Spore dimensions are given as: length range × width range (n = 40) and Q ratio (spore length/spore width). Dimensions of asci are given as length range × width range (n = 20). A Nikon 4500 camera was used to photograph microscopic characters. For scanning electron microscopy (SEM) a small piece of hymenial tissue was immersed in 2.5% KOH for 3 mins and rinsed in demineralised water to release

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spores. A drop of the resulting material was mounted on aluminium stubs with double-sided tape, dried and then sputter-coated with platinum at Adelaide Microscopy. Specimens were viewed under 10kV in a JEOL Neoscope JCM 5000 SEM at the State Herbarium of South Australia.

Descriptions of *Antrelloides atroceracea* are based on the type collection, *P.S. Catcheside PSC 2710* (*AD-C* 55811), unless otherwise stated, with outlying measurements for other collections given in brackets. Photographs of fruit bodies and microscopic characters are from the type collection, unless stated otherwise in the figure captions. All South Australian collections have been accessioned into the State Herbarium of South Australia (AD). AD numbers (AD-C #####) are given in the Taxonomy section together with the Collector's number (PSC ####); in other sections only the Collector's number is used.

DNA extraction, amplification and processing were as described in Catcheside *et al.* (2016); primers ITS1 and LR5 were used for amplification and ITS1, ITS4, LR0R, and LR5 for sequencing (White *et al.* 1990). Sequences were manipulated with the Geneious 8.1.9 and 11.0.3 suites of programmes using MUSCLE for alignment and MrBayes 3.2.6 and RAxML 8.2.11 plug-ins for tree building.

Antrelloides atroceracea 28S sequences were aligned with the top 100 blastn hits from GenBank on 7 Feb. 2017, and also 28S sequences from *Boudiera dennisii* Dissing & Sivertsen, *Cazia flexiascus* Trappe (Trappe 1989), *Eremiomyces echinulatus* (Trappe & Marasas) Trappe & Kagan-Zur, *Pachyella adnata* (Berk. & M.A.Curtis) Pfister, *P. babingtonii* (Berk. & Broome) Boud., *P. habrospora* Pfister, *P. punctispora* Pfister, *P. violaceonigra* (Rehm) Pfister, *Ruhlandiella peregrina* Lantieri & Pfister and *Sphaerosoma trispora* McLennan & Cookson. Redundant sequences were removed from the alignment and a phylogram constructed, rooted to *Ascobolus carbonarius* P.Karst. MrBayes used the HKY85 substitution model, 4 heated chains at a temperature of 0.2 for 1,100,000 iterations including a burn-in of 100,000 that was discarded. Following burn-in, trees were sampled every 200 iterations. The average standard deviation of split frequencies reached 0.011. RAxML used the GTR GAMMA nucleotide model with rapid bootstrapping for 1000 iterations and a search for the best scoring maximum likelihood tree.

Taxonomy

Antrelloides **P.S.Catches. & D.E.A.Catches.,** ***gen. nov.***

Type: *Antrelloides atroceracea* P.S.Catches. & D.E.A.Catches.
Mycobank number: MB825520.

Apothecial ascomata up to 40 mm diam. *Hymenium* convex; firm, waxy; black, violaceous-black. *Basal stipe* substantial; obconic; a chambered base of knobby columnar ridges radiating from base; fragile, waxy. *Asci* amyloid; cylindrical; operculate; 8-spored. *Ascospores* ellipsoid; smooth under light microscope. *Paraphyses* longer than asci; septate; tips swollen and tending to aggregate, encrusted with brown amorphous matter; branching occasionally near tips. *Medullary excipulum* of several layers of interwoven hyphae, grading into ectal excipulum. *Ectal excipulum* of large globose to subglobose cells with interwoven hyphae. *Stipe* tissue of *textura globulosa* and chains of subglobose cells.

Etymology. From the Latin *antrellum*, a small grotto or cavern, and the Greek *-oides*, like.

Antrelloides atroceracea **P.S.Catches. & D.E.A.Catches.** ***sp. nov.***

Holotype: South Australia. Flinders Chase National Park, Kangaroo Island: On soil surface in slight depressions on side of path in lateritic sandy soils, 35° 56' 25"S, 136° 43' 58"E, alt. c. 65 m, heath with *Banksia marginata* Cav., *Leptospermum continentale* Joy Thomps., *Melaleuca gibbosa* Labill., *Isopogon ceratophyllus* R.Br., *Petrophile multisecta* F.Muell., *Lepidosperma semiteres* F.Muell. ex Boeck., *Hakea mitchellii* Meisn., 6 July 2007, *P.S. Catcheside PSC 2710 & D.E.A. Catcheside* (AD-C 55811).
Mycobank number: MB825521.

Apothecia solitary, scattered; occasional to frequent; shiny to dull; black, violaceous-black 38; forming circular to irregular cushion-like mounds above soil surface or amongst laterite nodules (Figs 1A & 1B). Whole fruit body broadly obconic, turbinate; diameter (10–) 15–40 mm, height above ground 6–15 mm, total height 15–30 mm. In cross section, the whole fruit body resembles a miniature cavern of continuous columns like stalactites and stalagmites fused at the base and radiating upwards and outwards to the capping hymenium (Figs 1C & 1D). *Disc* convex, irregularly domed; black, violaceous-black 38; shiny to matt; smooth to wrinkled, undulating, occasionally pitted; waxy, brittle; margin smooth to irregularly lobed. *Flesh* 0.6–1 mm thick; exterior black, lower layers pale grey. *Stipe* continuing below soil surface; diameter 17–38 mm, depth 16–24 mm; deeply and irregularly lacunose; forming a series of knobbly columns fused at the base and radiating out from base; height of columns 15–28 mm, diameter of columns 2.5–5 mm; brown-black-grey 5F3, 6F3-5 at top, grading to grey 7E3, 6D3 at base (dark red-brown 6F5-7F5 in WA specimens); texture waxy, fragile. *Asci* cylindrical-clavate (250–) 272–316 (–340) × (8.5–) 11–15 µm, average (264–) 287.8 (–297) × 9.14 (–13) µm (Figs 2B & 2C); 8-spored; amyloid over entire length but more strongly amyloid at apex (Figs 2A & 2D); operculate; tips rounded; base forked, arising from croziers (Fig. 2E). *Ascospores* long ellipsoid; (16.8–) 17.6–22.4 (–24) × 6.4–8.8 µm, average (18.29–) 20.33 (–21.2) × (7.12–) 7.74 (–8.09) µm; Q range 2.4–2.8 (–3.0), Q average (2.35–) 2.61 (–2.76); appearing smooth under light microscope (× 1000) (Fig. 2H), but densely verrucose when viewed with SEM (Fig. 2I); thick-walled; often

Fig. 1. A–E *Antrelloides atroceracea*. **A** Two fruit bodies *in situ*; **B** single fruit body *in situ* (collection FMKI 145); **C** section of fruit body (holotype); **D** section of fruit body (collection PSC 4292); **E** habitat of *Antrelloides atroceracea* in heath. **F** Section of *Ruhlandiella berolinensis* (collection PSC 4322). Scale = 10 mm. Photos: D.E.A. Catcheside.

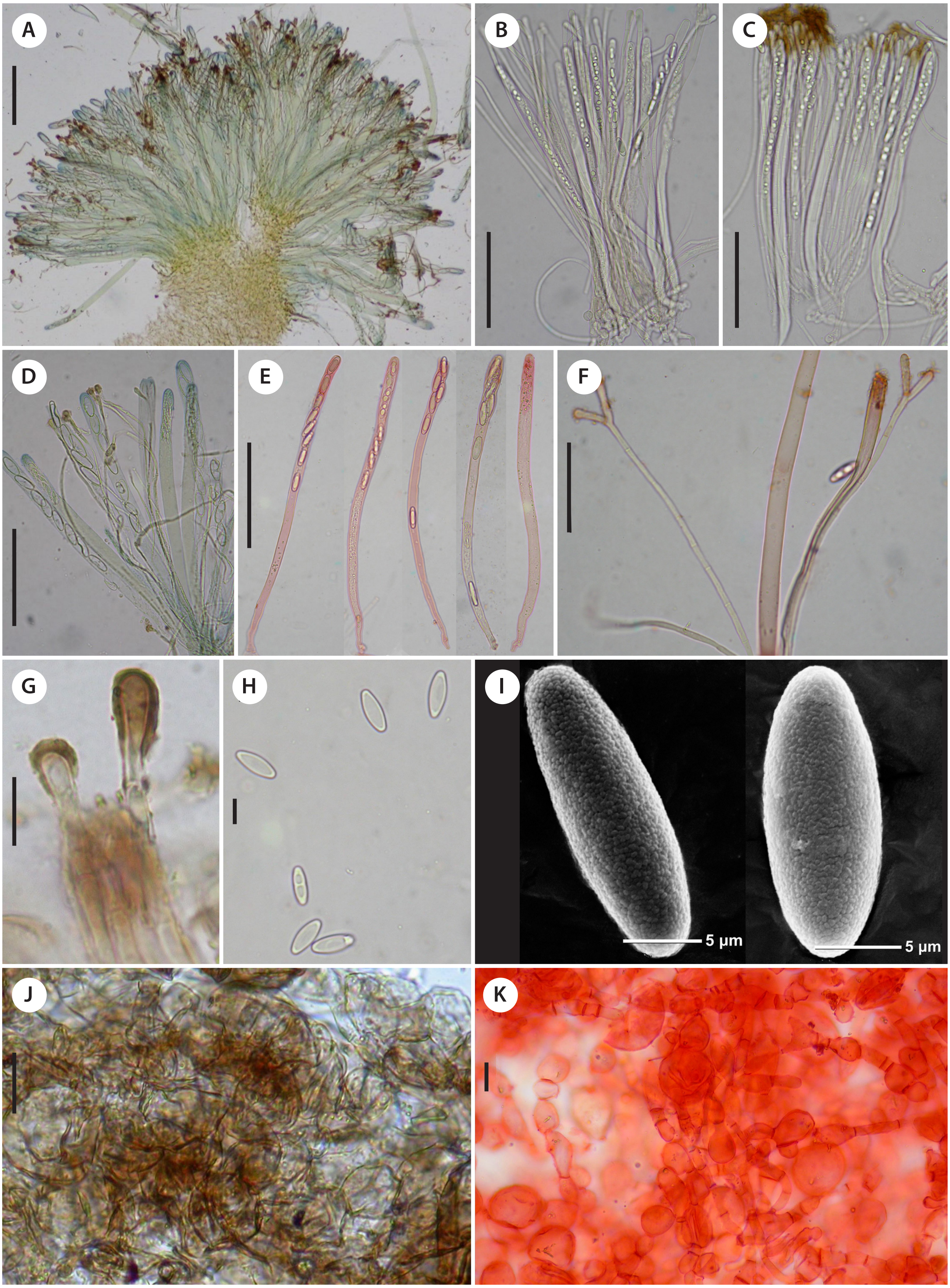

Fig. 2. ***Antrelloides atroceracea*****.** **A** Hymenium showing asci, ascospores, paraphyses and excipular tissue (in Melzer's solution); **B** hymenium showing asci, ascospores, paraphyses (in 5% KOH); **C** hymenium showing asci, ascospores, paraphyses (in water); **D** asci (in Melzer's solution); **E** asci (in Congo Red); **F** bifurcating paraphyses (in Congo Red); **G** tips of paraphyses (in water); **H** ascospores (in 5% KOH); **I** scanning electron micrographs of ascospores; **J** ectal excipulum (in water); **K** stipe tissue (in Congo Red). Scale: A–F 100 µm; G, H, J, K 10 µm; I 5 µm Photos: P.S. Catcheside.

with two largish globules; mostly in upper part or with occasional ascospore in lower half of ascus; uniseriate, irregularly uniseriate, biseriate near top in some asci. *Paraphyses* straight; longer than asci; septate; tips slightly swollen 5–8 µm at tip; tips brown and encrusted with brown amorphous matter (Fig. 2G), brown pigment occasionally extending down into upper part of paraphysis; tips separate or clumped; occasionally bifurcate at tip (Fig. 2F). *Subhymenium* 25–40 µm thick, of densely woven septate hyphae and small cells to 6 µm in length. *Medullary excipulum* a mixture of four types of tissue: i. densely interwoven hyphae of diameter 5–10 µm and not swollen at septa; ii. hyphae composed of cells swollen at septa and appearing 'knuckled'; iii. chains of 'balloon' cells joined by short hyphal cells; iv. regularly and irregularly globose and subglobose cells 15–45 µm diameter. Medullary excipulum grading into the larger cells of the ectal excipulum. *Ectal excipulum* (Fig. 2J) of globose, subglobose, sub-polygonal cells approx 20–65 (–80) µm diameter; cells thin- to thick-walled, contents clear to brown-pigmented, some cells encrusted. *Stipe tissue* mostly of globose, subglobose cells 20–60 (–70) µm diameter but intermixed with chains of small, irregular globose cells and hyphae swollen at septa and appearing 'knuckled' (Fig. 2K).

Habitat. Occurring on sandy and sandy lateritic soils.

Etymology. Latin *ater*, black, *ceracea*, waxy.

Additional specimens examined

WESTERN AUSTRALIA. North of Bow Bridge, Willmott Forest Block on Roe Road, in grey sand on track, 34° 58'S, 116° 57'E, 4 June 2008, *Eucalyptus marginata* Sm. (jarrah), *Andersonia caerulea* R.Br., *Astartea fascicularis* (Labill.) DC., *Melaleuca* sp., *Taxandria parviceps* (Schauer) J.R.Wheeler & N.G.Marchant, *Katrina Syme & Julie Fielder KS2094/08* (AD-C 56009).

SOUTH AUSTRALIA. All collections from: Kangaroo Island, Flinders Chase National Park, Platypus Waterholes Walk in similar soils; on soil surface or in slight depressions on side of path in lateritic sandy, soils, heath with *Banksia marginata* Cav., *Leptospermum continentale* Joy Thomps., *Melaleuca gibbosa* Labill., *Isopogon ceratophyllus* R.Br., *Petrophile multisecta* F.Muell., *Lepidosperma semiteres* F.Muell. ex Boeck., *Hakea mitchellii* Meisn. 35° 56' 25"S, 136° 43' 58"E, alt. c. 60 m, 4 June 2008, *P.S. Catcheside PSC 2899 & D.E.A. Catcheside* (AD-C 59830); 35° 56' 26"S, 136° 43' 58"E, alt. c. 60 m, 27 June 2008, *P.S. Catcheside & Katrina Syme* (FMKI 145; AD-C 55361); 35° 56' 25.5"S, 136° 43' 58"E, alt. c. 60 m, 28 June 2010; *P.S. Catcheside PSC 3422 & D.E.A. Catcheside* (AD-C 57285); 35° 56' 5.6"S, 136° 44' 41.6"E, alt. c. 65 m, 30 June 2011, *P.S. Catcheside PSC 3600 & D.E.A. Catcheside* (AD-C 57335); 35° 56' 5.7"S, 136° 43' 41.8"E, alt. c. 65 m, 23 June 2013, *P.S. Catcheside PSC 3745 & D.E.A. Catcheside* (AD-C 58504); 35° 56' 8"S, 136° 43' 45.7"E, alt. c. 65 m, 23 June 2013, *P.S. Catcheside PSC 3751, D.E.A. Catcheside & H.P. Vonow* (AD-C 58378); 35° 56' 23.5"S, 136° 44' 57.5"E, alt. c. 65 m, 24 June 2015, *P.S. Catcheside PSC 4292, D.E.A. Catcheside & H.P. Vonow* (AD-C 60144); 35° 56' 23.5"S, 136° 44' 57.5"E, alt. c. 65 m, 24 June 2015, *P.S. Catcheside PSC 4386, D.E.A. Catcheside & A. Winston* (AD-C 60145).

Results of molecular analysis

A 1566bp sequence for *Antrelloides atroceracea* (GenBank MH722261), encompassing 18S part, ITS1, 5.8S, ITS2, 28S part, was obtained from PSC 2710 and PSC 3600, which were identical in sequence, and showed no heterozygosity. Bayesian and maximum likelihood phylogenetic analysis places *A. atroceracea* in the Pezizaceae with the closest known relatives being *Peziza natrophila* and *Lepidotia hispida* (Fig. 3). *Antrelloides atroceracea* differs by 4.8% over 835bp in snps, indels and other mismatches in 28S ribosomal sequences from *P. natrophila* (AF335152.1 [isotype] and AF335153.1) and *L. hispida* (as *P. quelepidotia* AY640959.1 and KT869021.1 in GenBank), which are identical over the available 28S sequence. *Lepidotia hispida* (as *P. quelepidotia* KT869020.1 in GenBank) and *A. atroceracea* differ by 5 snps in their 5.8S ribosomal genes and diverge by 31% in ITS1 and 51% in ITS2.

Discussion

Morphological form of *Antrelloides atroceracea*

When we first found 'Black Shoe Leather' we were puzzled about its placement within the Pezizales. *Antrelloides atroceracea* is clearly a member of this order: its ascomata are apothecial and its asci are operculate, paraphyses are present and its ascospores are non-septate. However, its unusual morphological characteristics pose particular challenges when determining its taxonomic affiliations. *Antrelloides atroceracea* has individual morphological characters similar to those of taxa from a number of genera, but the fungus as a whole does not conform to any described genus that we know.

With its above-ground disc and its buried stipe *Antrelloides atroceracea* presents problems as to how to classify its form. Some members of the Pezizales are epigeous, others hypogeous and the ascomata exhibit very varied forms. Weber *et al.* (1997), Moreno *et al.* (2014) and Frey *et al.* (2016) have proposed terms, including apothecium, stereothecium, pulverothecium, cleistothecium and ptychothecium, as well as the less common exothecium, to describe the various fruit body forms. Stereothecia, pulverothecia and cleistothecia are closed structures containing indehiscent asci. The asci may be scattered but they are not organised in a definite hymenium. Stereothecia and pulverothecia are macroscopic and usually hypogeous, while cleistothecia are minute and may be on dung, plants, animals or other fungi. A ptychothecium is hypogeal, has an internal but organised hymenium which may be unfolded or folded and may have one or more openings and its asci are generally indehiscent.

Apothecia at maturity are epigeous with an open exposed hymenium and active ascospore expulsion, are usually discoid or cupulate and may be sessile or stipitate. A few of the Pezizales have exothecia. These comprise a strongly convex external hymenium with paraphyses longer than the asci that often form a tissue, an epithecium, covering the asci.

The ascomata of *Antrelloides atroceracea* are not closed hypogeal structures, so cannot be considered to be stereothecia, pulverothecia or cleistothecia. The sterile base of *Antrelloides atroceracea* is distinct from the hymenium, therefore the fruit body cannot be described as a ptychothecium. Nonetheless, its chambered base does bear some resemblance, albeit without asci, to the convoluted and folded inner structure of hypogean fungi such as *Mycoclelandia* Trappe & G. Beaton Trappe (Beaton & Weste 1982; Trappe & Beaton 1984; Hansen *et al.* 2001), *Peziza whitei* (Gilkey) Trappe (Korf 1973; Trappe 1975; Beaton & Weste 1982; Trappe & Claridge 1975; Hansen *et al.* 2001) and *P. ellipsospora* (Gilkey) Trappe (Hansen *et al.* 2001; Desjardin *et al.* 2015).

The exposed hymenium of *Antrelloides atroceracea* suggests the apothecial form, one of the most common forms amongst the Pezizales. However, its convex above-ground hymenium bears similarities to the exothecium of *Ruhlandiella* (Fig. 1F).

Other taxa: similarities and differences

The definition of an apothecium may be extended to genera such as the stipitate *Helvella* and *Gyromitra* with their often convoluted pilei. Fungi in these genera show some similarities with *Antrelloides atroceracea*. Its convex disc is not convoluted as are the pilei of many species of *Helvella* and *Gyromitra* but its chambered base or stipe is reminiscent of the stipe of some species of those genera, particularly those of *Helvella umbraculiformis* Seaver (family Helvellaceae) (Seaver 1942) and *Gyromitra californica* (W. Phillips) Raitv. (family Discinaceae) (Phillips 1880; Seaver 1942; Kuo 2012; Mykoweb; Beug *et al.* 2014). Neither taxon is known to occur in

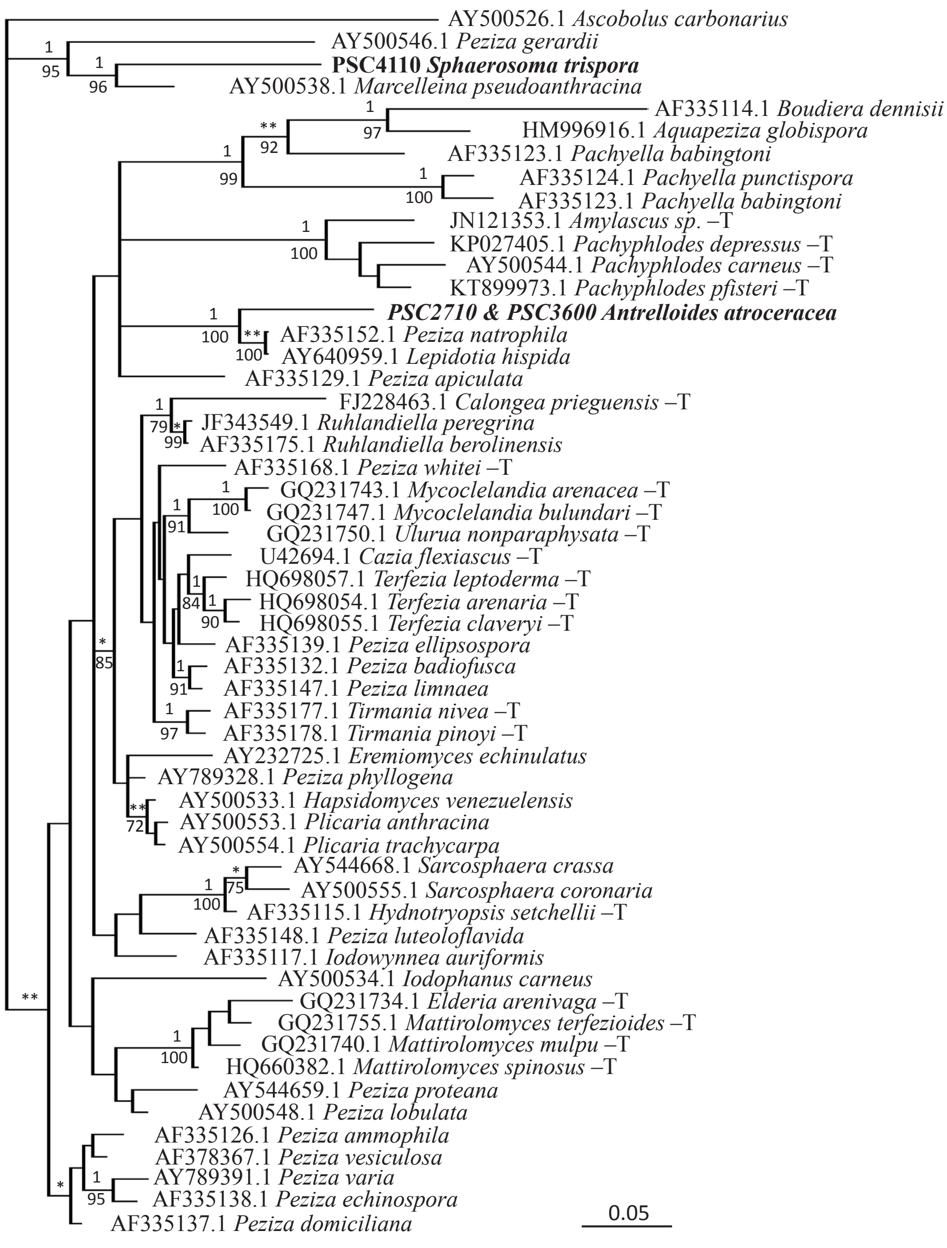

Fig. 3. A Bayesian tree based on sequences of the 28S ribosomal gene showing phylogenetic relationship of *Antrelloides atroceracea* with representative species from genera in the Pezizaceae. Posterior probabilities of ≥ 0.999, ≥ 0.99 and ≥ 0.95 are shown by 1, **, and * respectively above nodes and maximum likelihood values greater than 70% are shown below the nodes. The sequence for PSC 4110 *Sphaerosoma trispora* was derived in this study (GenBank MH722262), other numbers identify GenBank sequences. Truffle like taxa are indicated by –T.

Australia. Both species have convoluted pilei and the surface of the columns or ribs of the above-ground stipe are dry, relatively smooth and whitish. The internal structure of the erect columnar fruit bodies of the Australian species *Underwoodia beatonii* Rifai (family Helvellaceae) (Rifai 1968) is also somewhat similar to the South Australian and Western Australian collections of *Antrelloides atroceracea*. The columns of *U. beatonii* are divided internally by longitudinal ridges separated by alveolar cavities but the ridges are smooth, whitish and not as brittle as those of *A. atroceracea*. A major difference with taxa in the families Helvellaceae and Discinaceae is that they have inamyloid asci (Frey *et al.* 2016), in contrast to the amyloid asci of *A. atroceracea*.

Two families within the Pezizales have amyloid asci, the Pezizaceae (Hansen *et al.* 2001; Hansen *et al*. 2005; Hansen & Pfister 2006; Læssøe & Hansen 2007; Hansen *et al.* 2013) and the Ascobolaceae (Hansen & Pfister 2006; Hansen *et al.* 2013; Frey *et al.* 2016). *Antrelloides atroceracea* may be excluded from the Ascobolaceae since its asci do not protrude at maturity, its opercula are small not large and its spores do not darken on maturity. The asci of *Antrelloides atroceracea* are amyloid along their length but more strongly amyloid at the tips, conforming most closely with type (i) asci (Hansen *et al.* 2001).

The Pezizaceae is a large and very diverse family with an estimated thirty-two genera (Frey *et al.* 2016). It was possible that *Antrelloides atroceracea* was a member of one of these genera. We looked for similarities with genera with apothecia within the Pezizaceae. Such genera include *Pachyella* Boud., *Scabropezia* Dissing & Pfister, *Boudiera* Cooke, *Hapsidomyces* Krug & Jeng, *Iodophanus* Korf, *Iodowynnea* Medel, Guzmán & Chacón, *Sarcosphaera* Auersw., as well as the more common genera *Peziza* Fr. and *Plicaria* Fuckel. We did not discount genera that had not been recorded from Australia since there is a lack of extensive collections of Ascomycetes in this country (Rifai 1968; Hyde 2001).

Pachyella, *Plicaria* and *Scabropezia* (Hansen & Knudsen 2000) have epigeal, sessile fruit bodies, hence differing from *Antrelloides atroceracea* with its substantial subterranean base. While species of *Pachyella* and *Plicaria* are known from Australia, there are no species of *Scabropezia* in the recorded data of the Atlas of Living Australia.

Iodowynnea is a monotypic genus which was described from Africa and tropical America (Medel *et al.* 1996). *Iodowynnea auriformis* (Pat. ex Le Gal) Medel, Guzmán & Chacón does have a hypogeous stipe but this bears caespitose clumps of individual apothecia. The ellipsoid spores are ornamented with warts arranged in longitudinal bands differing from the spores of *A. atroceracea* whose verruculose ornamentation can be detected only with scanning electron micrography.

The coprophilous *Hapsidomyces venezuelensis* J.C.Krug & Jeng and species of *Iodophanus* differ from *A. atroceraea* not only in their ecology but in their size: apothecia seldom reach more than 3 mm in diameter. Moreover the spores of *Hapsidomyces venezuelensis* are globose and reticulate (Krug & Jeng 1984), those of species of *Iodophanus*, though ellipsoid, are covered with a mucilaginous sheath bearing callose-pectate ornamentation (Kimbrough *et al.* 1969). Only *Iodophanus carneus* (Pers. ex Pers.) Korf apud Kimbrough & Korf has been described from Australia (ALA).

The apothecia of *Sarcosphaera* are hypogeous during development, closed at first but split open in a stellate manner when mature to expose a smooth, whitish to pale violaceous hymenium, characters differing from *A. atroceracea*. Asci are amyloid and spores are ellipsoid. The genus has not been recorded in Australia.

Although some truffle-like genera share the amyloidity of asci and smooth, ellipsoid spores, their hypogeal habit and internal hymenium separate them from *Antrelloides atroceracea*.

While the above-ground, broadly convex disc of *Antrelloides atroceracea* may resemble discs of either *Peziza* or *Plicaria*, its distinctive below-ground, chambered base is not like any pseudostipe of any species of those genera. They share the amyloid character of asci but *Plicaria* species have globose spores, thus separating species in that genus from *Antrelloides atroceracea* with its ellipsoid spores, a character they share with species of *Peziza*.

Exothecial taxa

The few genera considered to have exothecial fruit bodies are *Ruhlandiella* P. Henn. (Lantieri *et al.* 2012; Læssøe & Hansen 2007; Frey *et al.* 2016), *Sphaerozone* Zobel (Beaton & Weste 1978; Hansen *et al.* 2001; Læssøe & Hansen 2007) and *Sphaerosoma* Klotzsch. Dissing & Korf (1980) discussed relationships amongst these genera and also *Boudiera* Cooke, separating genera whose spores are actively discharged, *Boudiera* and *Sphaerosoma*, from *Ruhlandiella* and *Sphaerozone* whose asci are indehiscent. All are epigeous.

The genus *Ruhlandiella* is considered native to Australia (Lantieri *et al.* 2012). However, the type species, the mycorrhizal *Ruhlandiella berolinensis* Henn. occurs not only in Australia but in plantings of *Eucalyptus* in Spain (Galán & Moreno 1998), Greece (Agnello & Kaounas 2010), the Canary Islands and California (Dissing & Korf 1980). The tiny fruit bodies from our single collection of *R. berolinensis* were draped over the soil surface (Fig. 1F). Although the hymenium has a similar form to that of *Antrelloides atroceracea*, with paraphyses forming a semi-cover over the asci in both taxa, the sterile lower surface of *R. berolinensis* is miniscule when compared with the large chambered base of *A. atroceracea*. Moreover, the walls of the asci fragment and release the ascospores within the hymenium while the walls of the asci of *A. atroceracea* are not evanescent. Its asci are amyloid, as are those of *R. berolinensis* and *R. reticulata* (P.H.B.Talbot) E.Rubio, Tena, Ormad & A.Suárez, but asci of *R. peregrina*

Lantieri & Pfister and *R. truncata* are inamyloid (Warcup & Talbot 1989; Galán & Moreno 1998; Rubio *et al.* 2010; Lantieri *et al.* 2012).

Sphaerozone has amyloid asci (Dissing & Korf 1980; Læssøe & Hansen 2007); the genus is monotypic, the type species being *S. ostiolatum* (Tul. & C. Tul.) Setch. Zhang & Minter (1989) transferred two species, *Sphaerozone echinulatum* G.W.Beaton and *S. ellipsosporum* Cribb that had previously been included in the genus to the new genus *Gymnohydrotrya*, the new combinations being *Gymnohydrotrya echinulata* (G.W.Beaton) B.C.Zhang & Minter and *G. ellipsospora* (Cribb) B.C.Zhang & Minter. *Sphaerozone ostiolatum* has globose, ornamented spores and its asci are indehiscent (Dissing & Korf 1980; Pegler *et al.* 1993), characters that separate it from *A. atroceracea*, though its subhypogeous and convoluted and infolded fruit body bears some slight resemblance to that taxon. Species of *Gymnohydrotrya* have a subglobose to irregularly lobed ascoma with several internal chambers and canals. The hymenium is external but often extends internally. They differ from *Antrelloides atroceracea* in their totally hypogeous, globose ascomata, inamyloid asci and ornamented ascospores, though the infolded chambers bear some resemblance to the chambered base of *A. atroceracea*.

Dissing & Korf (1980) recognised that *Sphaerosoma* is a particularly problematic genus. *Antrelloides atroceracea* bears little resemblance to the two species that they accepted: *S. fuscescens* Klotzsch and *S. trispora* McLennan & Cookson (McLennan & Cookson 1923). Both have inamyloid asci and globose, highly ornamented spores, while *A. atroceracea* has amyloid asci and ellipsoid spores. Ascomata of the South Australian collections examined were olive-brown to black, irregularly cushion- to saucer-shaped, sessile and attached to the soil surface by a central soil pad (Catcheside 2012). This under-collected genus requires further investigation, though the cryptic nature and probable rarity make this problematic.

Phylogenetic associations of *Antrelloides atroceracea*

Based on the studies of Hansen *et al.* (2001, 2005), Hansen & Pfister (2006), Læssøe & Hansen (2007) and Hansen *et al.* (2013), Frey *et al.* (2016) divide the class Pezizomycetes sensu O.E. Erikss. & Winka, Order Pezizales J. Schröt. into four lineages with lineage A / suborder Pezizinae Rifai (asci often amyloid) divided into two families: Ascobolaceae with asci protruding from the hymenium when mature and Pezizaceae with non-protruding asci. Lineage B covers the *Morchella-Helvella* clade, lineage C the Pyronemataceae s.l. and lineage D the suborder Sarcoscyphinae Rifai. *Sphaerosoma* is amongst genera *incertae sedis*. The phylogenetic data presented here include taxa from all four lineages.

There are some genera which lie in lineages other than the Pezizaceae that have infolded ascomata bearing some resemblance to the chambered base of *Antrelloides atroceracea*. These include *Hydnotrya* Berk. & Broome in lineage B, the *Morchella-Helvella* clade (Trappe 1979; O'Donnell *et al.* 1997; Frey *et al.* 2016). *Hydnocystis* Tul., *Genea*, Vittad. and *Geopora* Harkn. are in Lineage C/Pyrenomycetaceae s.l. (Trappe 1979; Læssøe & Hansen 2007; Moreno *et al.* 2014; Frey *et al.* 2016). However, as well as being hypogean, they are phylogenetically distant from *Antrelloides atroceracea* (Fig. 3).

Phylogenetic analysis of *Antrelloides atroceracea* shows that it is nested within the Pezizaceae (Fig. 3). It is most closely related to *Lepidotia hispida* (Quél.) Boud. (Norman & Egger 1999; Hansen *et al.* 2001, 2005; Hansen & Pfister 2006; van Vooren *et al.* 2015) and *Peziza natrophila* A.Z.M. Khan (Hansen *et al.* 2001; van Vooren *et al.* 2015). Morphologically however, although sharing the amyloidity of asci and globose excipular tissue, they differ in structure and colour. *Lepidotia hispida* is yellow-green, stipitate but its stipe is simple, not chambered (Korf 1973; van Vooren *et al.* 2015). *Peziza natrophila* is olive-brown, later dark brown to black, shallow-cupulate to discoid and sessile (Nowsher & Khan 1976). The habitats of both taxa, *Lepidotia hispida* in peat bogs or swampy areas (Korf 1973; van Vooren *et al.* 2015) and *Peziza natrophila* known mostly from plots treated with sodium and potassium carbonates in pine plantations but also from an old peat bog (Korf 1973; O'Donnell & Beneke 1973; Hansen *et al.* 2001; van Vooren *et al.* 2015) differ substantially from that of *A. atroceracea*, which, although fruit bodies may be subject to inundation, is found in dry sandy to sandy-clay soils. It is possible that *Lepidotia hispida* and *Peziza natrophila* are con-specific (Hansen *et al.* 2001; van Vooren *et al.* 2015).

The 4.8% difference of the 28S ribosomal gene sequence of *Antrelloides atroceracea* to that shared by *Peziza natrophila* and *Lepidotia hispida*, coupled with the very large differences in ITS1 and ITS2 sequence (31% and 51% respectively) from that of *L. hispida*, argue for the erection of the new genus *Antrelloides*.

Taxa in adjacent branches of the phylogenetic tree include species of *Pachyphlodes*, *Amylascus*, *Boudiera*, *Aquapeziza* and *Pachyella*. The truffle-like *Pachyphlodes* and *Amylascus* have globose and echinulate spores, differing from the epigean *Antrelloides atroceracea* with its ellipsoid, faintly verruculose spores. Species of *Boudiera*, *Aquapeziza* and *Pachyella* lie in neighbouring branches. The latter genus has sessile ascomata thus differing from the stiped *A. atroceracea*, *Boudiera* has tiny apothecia which seldom reach more than 3 mm diameter and spores are spiny or reticulate. The monotypic *Aquapeziza globispora* D.M.Hu, L.Cai & K.D.Hyde (Hu *et al.* 2012) has sessile, white apothecia and smooth, globose spores. Its habitat on submerged wood in freshwater streams excludes it from *Antrelloides*. *Peziza apiculata* Cooke is sessile and has ellipsoid spores with needle-like apicules at each end. It grows on rotten wood or moist soil (Moravec 1977).

General discussion

The form of the fruit body and the habitat of *Antrelloides atroceracea* are interesting. Over a limited area fruit bodies are not uncommon. They are almost always on the edges of slightly raised, bare tracks and are subject to inundation, with standing water at least up to the margins of the fruit bodies. One population of immature fruit bodies was found on sticky, sandy clay soil in a slight depression where it was obvious that the water level had reached the margins of the discs. The substantial ribbed base anchors the fruit body in the soil, preventing it from being washed away. Species of *Boudiera* and *Pachyella* grow in swampy ground and have ascomata that are able to withstand flooding. *Boudiera* species are mostly on sand, *Pachyella* species on water-soaked wood or decaying plant debris (Pfister 1973; Hansen *et al.* 2001). Species of both genera are sessile, lacking the chambered base of *A. atroceracea* which anchors that taxon in the ground. The hymenium of species of *Boudiera* occupies the upper surface or covers the whole exposed surface of the apothecium and its asci protrude beyond the paraphyses when mature (Seaver 1942; Eckblad 1968), possibly facilitating spore dispersal in wet conditions. *Pachyella* species have gelatinous tissues, an adaptation to its wet habitat.

Placement of new fungal taxa always presents challenges. When the new fungus has no obvious characters that fit it into any group it becomes more problematic. Phylogenetic analysis is essential, especially in these circumstances. However, molecular data are often not available, especially for cryptic and thus often, in Australia, under-collected taxa such as the Discomycetes. Its genetic characters and the amyloidity of the asci enable *Antrelloides atroceracea* to be placed firmly in the Pezizaceae. Molecular sequencing and phylogenetic analyses enable relationships between species and genera to be understood but demonstrate that, as in this case, there is little relationship between morphological and genetic characters. In addition, Rifai (1968) commented on the lack of extensive collections of Australian Pezizales. From our experience, this has changed little since Rifai's observation.

Additional species examined

Sphaerosoma trispora

SOUTH AUSTRALIA: Mays Cottage to Platypus Walking Track, Flinders Chase National Park, Kangaroo Island, 35° 56'S, 136° 44'E, 27 June 2011, *P.S. Catcheside PSC 3570, D.E.A. Catcheside, T.P. Bridle & H.P. Vonow* (AD-C 56992); 35° 56'S, 136° 43'E, 28 June 2014, *P.S. Catcheside PSC 3583, D.E.A. Catcheside, H.P. Vonow, T.P. Bridle & P. Bridle* (AD-C 56993); Mount Rescue Conservation Park near Tintinara, 35° 55'S, 140° 17'E, 17 Aug. 2014, *P.S. Catcheside PSC4110 & D.E.A. Catcheside* (AD-C 58768) (GenBank MH722262); Meningie, on ground, 29 June 1961, *L.D. Williams 1173* (AD-C 47697); Meningie, on ground, 7 Sep. 1961, *L.D. Williams 1246* (AD-C 47693); Wood's Well, on ground, 27 Aug. 1961, *L.D. Williams 1236* (AD-C 47691).

Ruhlandiella berolinensis

SOUTH AUSTRALIA: Kaiserstuhl Conservation Park, S34° 34', E139° 1', 22 Aug. 2016, *P.S. Catcheside PSC 4322, D. Catcheside & members of Adelaide Fungal Studies Group* (AD-C 59899).

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