Sphaerosoma trispora, an unusual species in the *Pezizaceae* (*Pezizales*) from Australasia

Pamela S. CATCHESIDE
David E.A. CATCHESIDE

Ascomycete.org, 11 (6) : 213–221
Mise en ligne le 24/12/2019
10.25664/ART-0277

Abstract: *Sphaerosoma trispora*, an unusual, three-spored species in the Ascomycota (*Pezizales*, *Pezizaceae*) from Australasia is described and illustrated. Collections of the taxon are discussed, its phylogenetic relationships presented and questions raised about micro-morphological adaptations, methods of spore release and its ecology and biology.
Keywords: Ascomycota, Australia, Fungi, inamyloid reaction.

Introduction

Sphaerosoma trispora McLennan & Cookson is a small to medium-sized black disc fungus known only from Australia and New Zealand (Atlas of Living Australia {ALA}). The species is unusual in having large (to ~70 µm diameter) spores with a distinctive double reticulum and only three spores in most asci. The species was first described by McLennan & Cookson in 1926 from collections made by Ethel McLennan from Ringwood and Chewton, Victoria, Australia in May 1924. Later collections, in 1956 and 1961, were made by L.D. Williams at Meningie and Wood's Well, South Australia, in 1984 by Jack Warcup in Kuitpo Forest, South Australia (Warcup, 1990), by Ross Beever in July 1996 in New Zealand (ALA) and in South Australia by Pam and David Catcheside in June 2011 from Kangaroo Island, and in August 2014 from Mount Rescue.

The collections of McLennan and Warcup seem to have been lost although the University of Melbourne herbarium (MELU) has an envelope containing images and illustrations of *Sphaerosoma trispora* from the original description of the species (McLennan & Cookson, 1926) but no fungal material (Dr Joanne Birch, pers. comm., 30th August, 25th October 2019). Gamundí's description (1976) was based on a collection made by L.D. Williams which is listed in the Kew Fungarium. Rifai (1968) had included it in the "Little known or excluded species" in his treatment of the Australasian *Pezizales* in the Kew Herbarium. We have not seen the Kew collection. However, we have examined three other collections made by L.D. Williams, together with our three collections and that made by Ross Beever.

The known distribution of *Sphaerosoma trispora* lies between 35° and 39° South. South Australian collections were from Kangaroo Island (Catcheside, 2012) — Australia's third largest island that lies approximately 100 km south-west of Adelaide, the state capital —, from Kuitpo Forest approximately 45 km south of Adelaide and from Mount Rescue Conservation Park, Meningie and Wood's Well, all 100–200 km south-east of Adelaide. Collections from Victoria were made at Ringwood, a suburb 20 km from central Melbourne, the state capital, and Chewton approximately 100 km north-west of Melbourne. The New Zealand collection is from the central North Island in the Karapiti thermal area, also known as the Craters of the Moon.

The genus *Sphaerosoma* was established by Klotzsch (1839) with the type *Sphaerosoma fuscescens* Klotzsch which had been collected by him in Grünewald and by others in the Botanical Garden near Berlin, Germany in 1839. Rouppert (1910) expanded Klotzsch's original diagnosis and Setchell (1910) wrote a comprehensive assessment of the studies into the genus up to that time. Dissing & Korf (1980) accepted *Sphaerosoma fuscescens* and *S. trispora* but excluded other taxa that had previously been assigned to the genus. Since then there have been discussions on the genus, the most recent being those of Vizzini (2003), Læssøe & Hansen (2007) and Kraisitudomsook *et al.*. (2019).

The number of species in the genus is unclear. Twelve species of *Sphaerosoma* are listed in Index Fungorum, eleven in Species Fungorum and three in the *Dictionary of Fungi* (Kirk *et al.*., 2008).

Materials and methods

The location of PSC and DEAC collections was determined by GPS, geodetic datum WGS84/GDA94 (Garmin GPS12) and habitat and associated plant communities noted. Photography of habitat (Plate 1A) used an Olympus Stylus TG2, *in situ* and whole collection images (Plate 1B, 1C, 1F) used a Lumix DMC-GX7 with a Leica Macro-Elmarit 45mm lens and whole collection images (Plate 1D, 1E) used a Nikon E4500. Macroscopic characters were described 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. 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 with Melzer's and Lugol's reagents. Water mounts were used to determine context colour.

The description of *Sphaerosoma trispora* given here is based on collections accessioned into the State Herbarium of South Australia (AD): PSC 3570 (AD-C 56992), PSC 3583 (AD-C 56993), PSC 4110 (AD-C 58768), LDW 1246 (AD-C 47693) and LDW 1236 (AD-C 47691). Measurements are based on specimens mounted in water 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). Measurements outside the normal range are given in brackets. Photomicrography used a Nikon E4500 camera. For scanning electron microscopy (SEM) a small piece of hymenial tissue was immersed in 2.5% KOH for 3 min and rinsed in demineralised water to release spores. A drop of the resulting material was mounted on aluminium stubs with double-sided tape, dried, sputter-coated with platinum by Adelaide Microscopy and viewed under 10kV in a JEOL Neoscope JCM 5000 SEM.

Both AD numbers (AD-Cnnnnn) and collector's number (PSC-nnnn) are given in the taxonomy section but only the collector's number is used elsewhere.

Sequence from the large subunit of the nuclear ribosomal cistron of *Sphaerosoma trispora* (Catcheside & Catcheside, 2018) was used in a blastn search of GenBank (19/10/2019) using a window of 28 bp. An RAxML tree of the top 100 hits was used to find the closest taxa

to *S. trispora* and sequences of those, together with a selection of other species from the *Pezizales* were used for phylogenetic analysis. Sequences were manipulated with the Geneious 11.1.5 suite of programmes using the Geneious pairwise alignment tool. Tree building used plugins of RAxML (Stamatakis, 2014) and MrBayes (Huelsenbeck & Ronquist, 2001). MrBayes utilized *Taphrina deformans* (Berk.) Tul. and *Ascobolus carbonarius* P. Karst. as outgroups, the HKY85 substitution model, priors at default values, rate variation gamma with 4 categories, 4 heated chains at a temperature of 0.2 for 1,100,000 iterations and sampling every 200 iterations after a burn in of 100,000. RAxML trees used GTR GAMMA as the nucleotide model for 1000 replicates of rapid bootstrapping for the best scoring maximum likelihood tree.

Taxonomy

Sphaerosoma trispora McLennan & Cookson, *Proceedings of the Royal Society of Victoria*, 38: 73 (1926).

The following description is based on examination of those specimens present in the State Herbarium of South Australia (AD).

Apothecia scattered to gregarious; occasional; sessile with broad basal attachment; discoid, saucer-shaped, pulvinate, becoming irregular and somewhat contorted (Plate 1B & 1C); diameter 4–15(–35) mm, height 3–10 mm; waxy and brittle when fresh, hard when dry. Hymenium covering exposed surface of apothecium. **Disc** shape varied, initially concave, shallow-cupulate, becoming irregularly convex, irregularly undulating to convoluted; black, black-brown, fuscous black 36, olive-brown, olivaceous black 37; smooth, undulating to rough, almost tuberculate, pitted in places; waxy; margin thick, inrolled, irregular, undulating to contorted. **Flesh** 1–3 mm thick; hymenial layer black, lower layer whitish to pale grey; waxy, brittle. **Receptacle** plane to saucer-shaped, shallow-cupulate, contorted discoid; fuscous black 36, olivaceous black 37, fawn 29, mouse grey 35, some with reddish patch; rough, warty, tuberculate, surface appearing finely cracked. Hymenium extending to outer edge of receptacle.

Asci (Plate 2A, Plate 3A, 3B, 3C, 3F) 3-spored, occasionally 4-spored when immature; cylindric, thick-finger shaped; 385–580(–627) × 51–67(–70.5) µm, average 487 × 62.5 µm; inamyloid, not hemiamyloid (no reaction with Lugol's solution); tips rounded, no operculum seen; base rounded, similar to apex or simple-nodulose, rarely tapering; walls irregular, becoming crumpled when ascospores have been discharged; contents initially palely dextrinoid, colour clearing on maturity; immature spores initially spread through the ascus and separate from each other, as they mature the spores group in threes at the centre of ascus, mature spores in groups of three at tip of ascus, uniseriate. **Ascospores** (Plate 2A-F) globose; (45–)51–62(–68) × (42–)50–64(–68) µm, average 55.4 × 54.8 µm; Q range 1–1.07, Q average 1.01; initially hyaline, becoming dark brown, purplish-brown; strongly and regularly reticulate; with double reticulum, meshes of outer reticulum mostly hexagonal, 8.5–14.5 × 8–14 µm across, walls of reticulum wavy, 5–7 µm high, 1.2–2.5 µm thick; inner reticulum of fine, shallow, mostly hexagonal meshes 2.8–3.6 µm across revealed by focussing up and down on the ascospore surface; walls of outer reticulum appearing rough-tuberculate (Plate 2G). **Paraphyses** longer than asci; unbranched; septate; tips clavate to12 µm diameter, containing and encrusted with brown amorphous matter; tips agglutinated and bending over the tips of the asci to form almost a thatch or epithecium (Plate 3A, 3B). Subhymenium 50–100 µm thick; of irregularly arranged, compacted, hyaline hyphae. **Medullary excipulum** (Plate 3F) of irregular subglobose to angular to pear-shaped, hyaline cells, 30–50 µm across (*textura globulosa* to *textura angularis*), intermixed with hyphae; hyphae septate, some not constricted at septa 10-15 µm diameter, others chains of irregular, inflated cells to 25 µm diameter and constricted at hyphae; of *textura porrecta* or irregular *textura prismatica*. **Ectal excipulum** (Plate 3D, 3E) of thick-walled irregular globose, subglobose cells to 25 µm across, interspersed with hyphal elements to 15 µm diameter, with brown pigments.

Collections examined:

Australia. South Australia: Mays Cottage to Platypus Walking Track, Flinders Chase National Park, Kangaroo Island. Heath with *Banksia marginata* Cav., *Banksia ornata* F. Muell. ex Meisn., *Leptospermum continentale* Joy Thomps., *Melaleuca gibbosa* Labill., *Callistemon rugulosus* (Schltdl. ex Link) DC., *Acacia paradoxa* DC, *Eucalyptus cosmophylla* F.Muell.; S 35° 56' 41.1", E 136° 44' 9.8", Alt. c. 65 m; 28 June 2011; P.S. Catcheside PSC 3570, D.E.A. Catcheside, T.P. Bridle & H.P. Vonow (AD-C 56992). S 35° 56' 27.1", E 136° 43' 59.4", Alt. c 65 m; 28 June 2011; 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. Open heath of *Xanthorrhoea australis* R. Br., *Banksia ornata*, *Allocasuarina pusilla* L.A.S. Johnson and mallee with *Eucalyptus fasciculosa* F. Muell. and *E. baxteri* (Benth.) Maiden & Blakely ex J.M. Black, S 35° 55' 36", E 140° 17' 19.7", 55 m, 17 August 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 September 1961, L.D. Williams 1246, AD-C 47693. Wood's Well, on ground, 27 August 1961, L.D. Williams 1236, AD-C 47691.

Collection examined but measurements not recorded:

New Zealand: Bare slightly heated ground with *Eragrostis benthamii* Mattei, Karapiti thermal area (Craters of the Moon), 23 July 1996, Ross Beever PDD 65970. Collection in Landcare Research, New Zealand Fungal and Plant Disease Collection.

Collection not examined by us:

Australia. South Australia: Meningie. On sandy soil amongst moss, 1956, L.D. Williams (ex-herb. WARI 7512) in Kew Fungarium (Accession Number 172439) with type status: Holotype of *Boudiera areolata* var. *macrospora* Dennis 1958. Collection examined and described as *Sphaerosoma trispora* by Gamundí (1976).

Discussion

Characteristics of *Sphaerosoma trispora*

The ascomata of *Sphaerosoma trispora* are unremarkable with no clear characters to distinguish them from other black disc fungi. However, the usually three-spored asci with their very large, deeply reticulate ascospores make the species easy to identify when examined microscopically. Our collections would be unremarkable except that there are few collections of species in this genus available for study (Setchell, 1910; Rouppert, 1910; Seaver, 1914; Gamundí, 1976; Dissing & Korf, 1980; Læssøe & Hansen, 2007; Kraisitudomsook *et al*., 2019) and our collection (P.S. Catcheside PSC4110 & D. E.A. Catcheside (AD-C 58768) (GenBank MH722262.1) provides the only DNA sequence data for this genus currently in GenBank.

The collections examined mostly conform with the descriptions of McLennan & Cookson (1926) and Gamundí (1976), although the upper size limits of both ascospores and asci are considerably larger in the Catcheside & Catcheside and L.D. Williams' collections in AD (Table 1). Our collections were mounted in water but McLennan & Cookson (1926) and Gamundi (1976) do not mention the media in which their specimens were mounted. Mounting media may account for these discrepancies, the large spore size accentuating any differences due to spore shrinkage or expansion in different media. There is disagreement as to whether the asci of *Sphaerosoma trispora* are operculate. McLennan & Cookson (1926) considered them operculate although their illustration shows ascus tips as rounded with no sign of an operculum whilst Gamundí (1976) stated that the asci were inoperculate. We saw no evidence for an operculum (Plate 3A, 3B).

Plate 1 – *Sphaerosoma trispora*

A: Habitat. Platypus Walking Track, Flinders Chase National Park, Kangaroo Island, South Australia. B: Ascocarp *in situ*, bar = 10 mm. C: Ascocarp *in situ*, bar = 10 mm. D: Collection PSC 3570 (AD-C56992), 28.06.2011, scale divisions mm and cm. E: Collection PSC3583 (AD-C 56993) 28.06.2011, scale divisions mm and cm. F: Collection PSC 4110 (AD-C58768), 17.08.2014, scale divisions mm and cm. Images: David Catcheside.

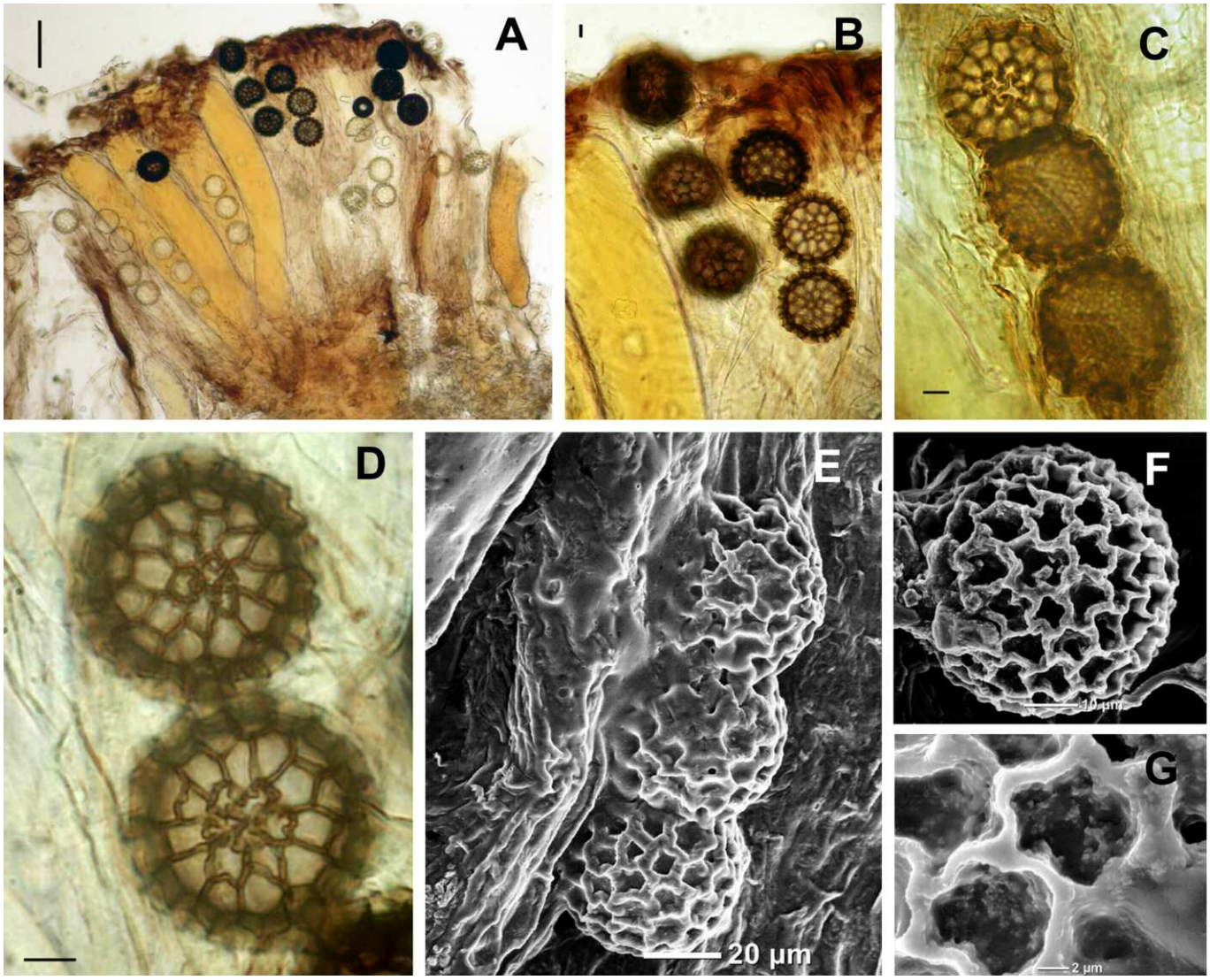

Plate 2 – *Sphaerosoma trispora*. Asci and ascospores

A: Asci with ascospores, in Melzer's reagent, bar = 100 µm. B: Asci with mature ascospores, in Melzer's reagent, bar = 10 µm. C: Ascus with its three mature ascospores, in Melzer's reagent. Top ascospore showing outer reticulum, two lower ascospores showing inner reticula, bar = 10 µm. D: Two mature ascospores showing outer reticula, in Melzer's reagent, bar = 10 µm. E: Scanning electron micrograph of three mature ascospores and ascus wall, bar = 20 µm. F: Scanning electron micrograph of mature ascospore, bar = 10 µm. G: Scanning electron micrograph of part of reticulum showing ornamented inner walls, bar = 2 µm. Images: Pam Catcheside.

Ecology, trophic mode, ascocarp type, spore dispersal

The Kangaroo Island site has sandy, lateritic soils and heathy shrubland; the Mount Rescue, Meningie and Wood's Well sites have sandy soils and open heath vegetation. Soils at Kuitpo Forest are sandy, lateritic loams and vegetation is dry sclerophyll forest. Warcup (1990) recorded *Sphaerosoma trispora* as occurring after fire "in young thickets of *Acacia* and *Pultenaea* on lightly burned sites" in the second year after a wildfire. McLennan & Cookson (1926) did not give details of associated vegetation for the Victorian collections but described specimens as occurring on open, damp, clayey soil. The New Zealand collections were from bare slightly heated ground with *Eragrostis benthamii* Mattei. From the different localities in which *S. trispora* has been found, there seems to be no clear preference for soil type.

It is probable that *Sphaerosoma trispora* is mycorrhizal rather than saprotrophic. Warcup (1990) was unable to grow it in culture and although mycorrhizas formed when pieces of ascocarp were in contact with the radicle of test plant species, he considered the result inconclusive as the ascocarps may have been contaminated by other fungi. However, the large size and decoration of ascospores in *S. trispora* is consistent with a mycorrhizal lifestyle given the correlations between spore characters and trophic mode noted by Calhim *et al.*. (2018) and Halbwachs *et al.*. (2017).

Sphaerosoma has been considered to be exothecial (Dissing & Korf, 1980; Vizzini, 2003). Exothecial taxa have a strongly convex external hymenium with paraphyses that are longer than the asci, often forming a tissue, an epithecium, covering the asci (Weber *et al.*., 1997; Moreno *et al.*., 2014; Jaklitsch *et al.*., 2016). *Sphaerosoma trispora* has long paraphyses with dark agglutinated tips forming a thatch, an epithecium, over the tips of immature asci but the hymenium is not convex and does not extend far over the receptacle (Plate 1B, 1C), unlike the hymenia of clearly exothecial species such as *Ruhlandiella berolinensis* Henn. and *Antrelloides atroceracea* P.S. Catches. & D.E.A. Catches. (Catcheside & Catcheside, 2018). The ascocarps of *Sphaerosoma trispora* are closer to the apothecial form, rather than being exothecial.

How *Sphaerosoma trispora* spores are released and dispersed is problematic. The asci of many apothecial species have opercula, pores or slits. We have not seen any such structures in *S. trispora*, the tips of all asci are rounded (Plate 2A, Plate 3A-C). There is no evi-

dence of fragmentation of ascus walls as occurs in *Ruhlandiella* Henn. (Vizzini, 2003; Catcheside & Catcheside, 2018) and *Stouffera longii* (Gilkey) Kovács & Trappe (Kovács *et al*.., 2011). Microscopic examination of hymenial surfaces of *S. trispora* mounted in water found relatively few discharged spores, with most mature spores remaining within the asci. This compares with other apothecial species such as *Peziza* Fr. and *Plicaria* Fuckel and with the exothecial species *Antrelloides atroceracea* where numerous discharged spores are frequently observed in water mounts. This raises the question not only of how the spores are discharged but how they are dispersed. The potential for spore dispersal by invertebrates is recognised (Malloch & Blackwell, 1992; Lilleskov & Bruns, 2005). Lilleskov & Bruns (2005) found high spore densities of an ectomycorrhizal resupinate fungus, *Tomentella sublilacina* (Ellis & Holw.) Wakef., in the guts, faeces and on the exoskeletons of invertebrates. However, we have not observed any evidence of grazing on the surfaces of the apothecia of *S. trispora*. The spore dispersal mechanism of *S. trispora* is yet to be determined.

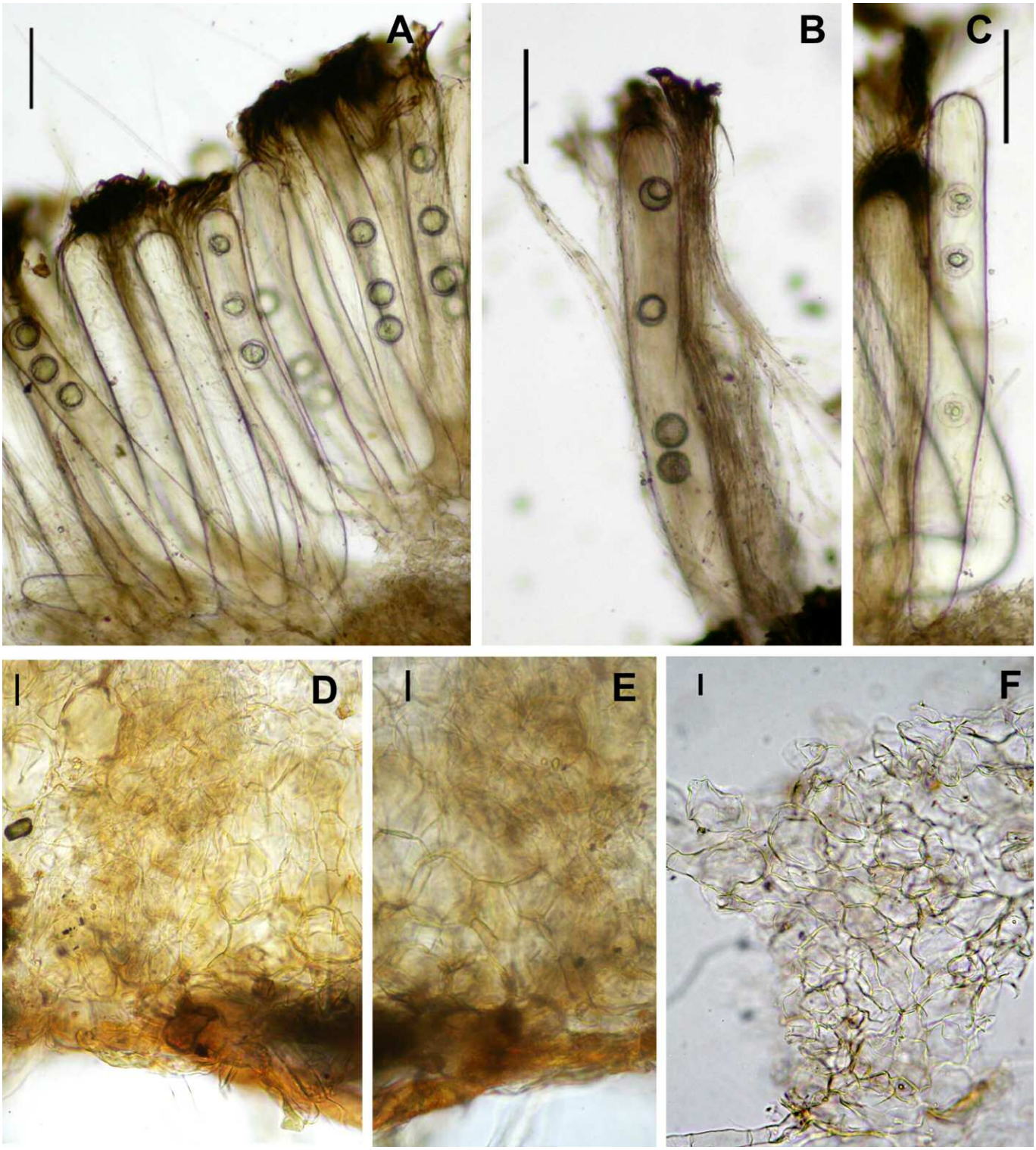

Plate 3 – *Sphaerosoma trispora*. Asci and excipular tissue.
A: Asci, immature ascospores and paraphyses, bar = 100 µm. B: Ascus with four immature ascospores, paraphyses, bar = 100 µm. C: Ascus with three immature ascospores, bar = 100 µm. D: Excipular cells, bar = 10 µm. E: Excipular cells, bar = 10 µm. F: Cells of medullary excipulum, bar = 10 µm. A–C, F in water; D, E, in Melzer's reagent. Images: Pam Catcheside.

Problems with the genus *Sphaerosoma*

Although the species *Sphaerosoma trispora* is relatively easy to identify, the genus *Sphaerosoma* has a long and tortuous history making the taxonomic limits of the genus *Sphaerosoma* difficult to determine. The following is a much abbreviated summary. Klotzsch (1839) established the genus with a Latin diagnosis which translates as: "receptacle subglobose, free; fleshy; sessile; interior solid; exterior bearing asci; base fibrillose; hymenium smooth, peripheral, fused with the receptacle; asci not protruding, close, elongate-clavate, 8-spored; spores lens-shaped, verrucose; paraphyses filiform, tips clavate. Subterranean, however partly emergent at maturity; fleshy; discoloured". Understandably for the times, this diagnosis is silent on essential features of the asci such as amyloidity and whether they are operculate or not. In addition to these characters, Klotzsch described his type, *Sphaerosoma fuscescens*, as having hyaline spores. Rouppert (1910) expanded Klotzsch's original diagnosis of the genus by using the descriptions of Tulasne & Tulasne (1851), Schroeter (1897) and Seaver (1905) to include species with ascomata that were epigeous and hollow and spores that were globose, brown, reticulate or spiny. He also described the asci as operculate.

The type species itself, *Sphaerosoma fuscescens*, presented difficulties. Several authors illustrated and described collections which conformed to some extent with Klotzsch's diagnosis. Setchell (1910), in his comprehensive review of these studies, concluded that "the status of the type of the genus, viz., *Sphaerosoma fuscescens* Klotzsch, is in a most unsatisfactory condition." The same could be said of the whole genus *Sphaerosoma*, in part because of the confusion arising from some of the early descriptions of specimens with Sphaerosoma-like characters and in part because of the lack of well-defined diagnostic characters for the genus.

The name of the genus itself had been problematic, though this difficulty was resolved by Dissing & Korf (1980). Confusion had arisen from the similarity of the names of the fungal genera *Sphaerosoma* and *Sphaerosozma*. Corda (1842) had described a collection with similar characters to *Sphaerosoma fuscescens* as *Sphaerosozma fuscescens* Klotzsch. Zobel (1854) retained the name: *Sphaerosozma fuscescens* (Klotzsch) Corda. Dissing & Korf (1980) synonymised *Sphaerosoma* with *Sphaerozosma*, confirming the name of the type species, *Sphaerosoma fuscescens* Klotzsch. Further confusion is possible because the name *Sphaerosoma* has been applied not only to the fungal genus but also to a genus of beetles in the family *Alexiidae*.

The systematic position of the genus has also been the subject of much discussion, Dissing & Korf (1980) commenting that fresh material is needed before this can be evaluated. The subterranean, partly emergent habit described for the type *Sphaerosoma fuscescens* led to discussion as to whether the genus should be in the *Tuberales* or in the *Pezizales*. Mostly due to the development of molecular analysis, the *Tuberales* have now been put to rest (Læssøe & Hansen, 2007). Thus, *Sphaerosoma* would seem to fit in the Order *Pezizales*. For its position in a family, Læssøe & Hansen (2007) followed Gamundí (1976) and Dissing & Korf (1980) by provisionally placing it in the *Pyronemataceae*. However, phylogeny based on large ribosomal subunit sequences (Fig. 1) suggest *Sphaerosoma trispora* is embedded in the *Pezizaceae*.

A clade containing *Sphaerosoma trispora*

Phylogenetic analysis based on large subunit ribosomal sequences shows the epigeous *Sphaerosoma trispora* nested within the *Pezizaceae* (Fig. 1) in a clade in which it appears basal to the truffles: *Delastria supernova* A. Paz & Lavoise, *Stouffera longii* (Gilkey) Kovács & Trappe, *Temperantia tiffanyae* (Healy) K. Hansen, Healy & Kovács and a species of *Hydnobolites* Tul. & C. Tul.

In addition to the lifestyle difference, the closest species to *S. trispora* in the phylogeny, *Delastria supernova* (Paz & Lavoise, 2013), has substantially smaller ellipsoid to subglobose spores (26 × 22 µm) and smaller subglobose to pyriform asci (130 × 90 µm), thus differing in ascus and ascospore shape from *S. trispora*. However, it is similar in having decorated spores, in this case reticulate-alveolate, and in respect of a short fall (having only 1–3 spores) from the four or eight ascospores expected if all products of meiosis survive.

Others in the terminal branches of the clade also show phenotypic similarities to *Sphaerosoma trispora* with all having relatively highly decorated spores. Like *S. trispora*, *Souffera longii* (synonym *Terfezia longii* Gilkey) has globose spores with an alveolar reticulum (Alsheikh, 1994) but its asci are globose to subglobose and contain eight spores. Kovács *et al.*. (2011) comment on the spores of *S. longii* having 'unusual, double-spore ornamentation' with 'minute hemispheres … enclosed by the reticular walls'. This compares with the double reticulum of *Sphaerosoma trispora* and the rough ornamentation on the walls of the outer reticula (Plate 2G). *Temperantia tiffanyae* (synonym *Mattirolomyces tiffanyae* Healy) differs in having ellipsoid asci, smaller (33.6 µm diameter) though globose spores and no paraphyses. However, like *Sphaerosoma trispora*, spores are ornamented but with a partial reticulum and most asci have an unusual spore complement, mostly 3-spored, occasionally 4-spored and with some asci having as few as one (Healy, 2003; Kovács *et al.*., 2011). Species of *Hydnobolites*, like *S. trispora*, have globose spores which have an alveolate reticulum (Trappe, 1979) but like the other truffle members of the clade differ radically in the ascus shape which here are ellipsoid or subglobose. Of the two accepted species, *Hydnobolites cerebriformis* Tul & C. Tul. and *T. californicus* Fischer, the latter has the larger spores, up to 30 µm (Beug *et al.*., 2014). The asci of both species contain eight spores. Truffles depend on small animals for spore dispersal and have no need of a spore-release mechanism from their asci. *Sphaerosoma trispora* seems to share this lack of spore release. It is interesting that its closest known relatives are truffles.

More basal to *Sphaerosoma trispora* in the clade are *Marcelleina pseudoanthracina* (Donadini) R. Kristiansen & J. Moravec, *Marcelleina persoonii* (P. Crouan & H. Crouan) Brumm. and also, *Marcelleina tuberculispora* K. Hansen & Sandal. Like *S. trispora* the *Marcelleina* species have the apothecial form and inamyloid asci, although the asci in some collections of *M. pseudoanthracina* show a weakly amyloid reaction (Dougoud, 2002). However, the asci of species of *Marcelleina* are eight-spored and spores, though globose, are much smaller, less than 13 µm diameter (Moravec, 1987; Hansen *et al.*., 1998), hyaline, smooth to sculptured rather than highly decorated and the paraphyses are violet-coloured. As well as its three-spored asci, *S. trispora* differs from the *Marcelleina* species in its much larger asci, larger dark brown pigmented, more highly decorated spores and paraphyses lacking violaceous colours. *S. trispora* also has molecular affinities with *Peziza gerardii* Cooke, although it differs morphologically in that *P. gerardii* has amyloid asci and fusoid, ribbed spores (Van Vooren, 2010; Beug *et al.*., 2014).

We incorporated taxa in our phylogenetic analysis that are outside the clade in which *Sphaerosoma trispora* lies because various authors have commented that they show taxonomic similarities with species of *Sphaerosoma*. Rouppert (1910) suggested the reticulate-spored *Ruhlandiella berolinensis* could be synonymous with *Sphaerosoma fuscescens*, but Kraisitudomsook *et al.*. (2019) considered this unlikely based on ascospore colour and ascus and paraphysis characters. Our phylogeny confirms their assessment. Eckblad (1968) suggested that the 'tuberaceous *Sphaerosoma*' was closest to *Boudiera* Cooke because of its spore sculpturing. However, *Boudiera tracheia* (Rehm ex Gamundí) Dissing & T. Schumach. lies on a different branch of the ribosomal large subunit phylogram making this unlikely. In her paper describing *S. trispora*, Gamundí (1976) considered relationship to the genus *Plicaria* Fuckel as well as *Boudiera* and *Sphaerosoma*. Like *S. trispora*, *Plicaria* spores are globose and their paraphyses are reminiscent of those of *S. trispora* in usually being longer than the asci and forming a cover or partial cover over the ascus tips. However. phylogenetically, *Plicaria* is distant from *S. trispora*.

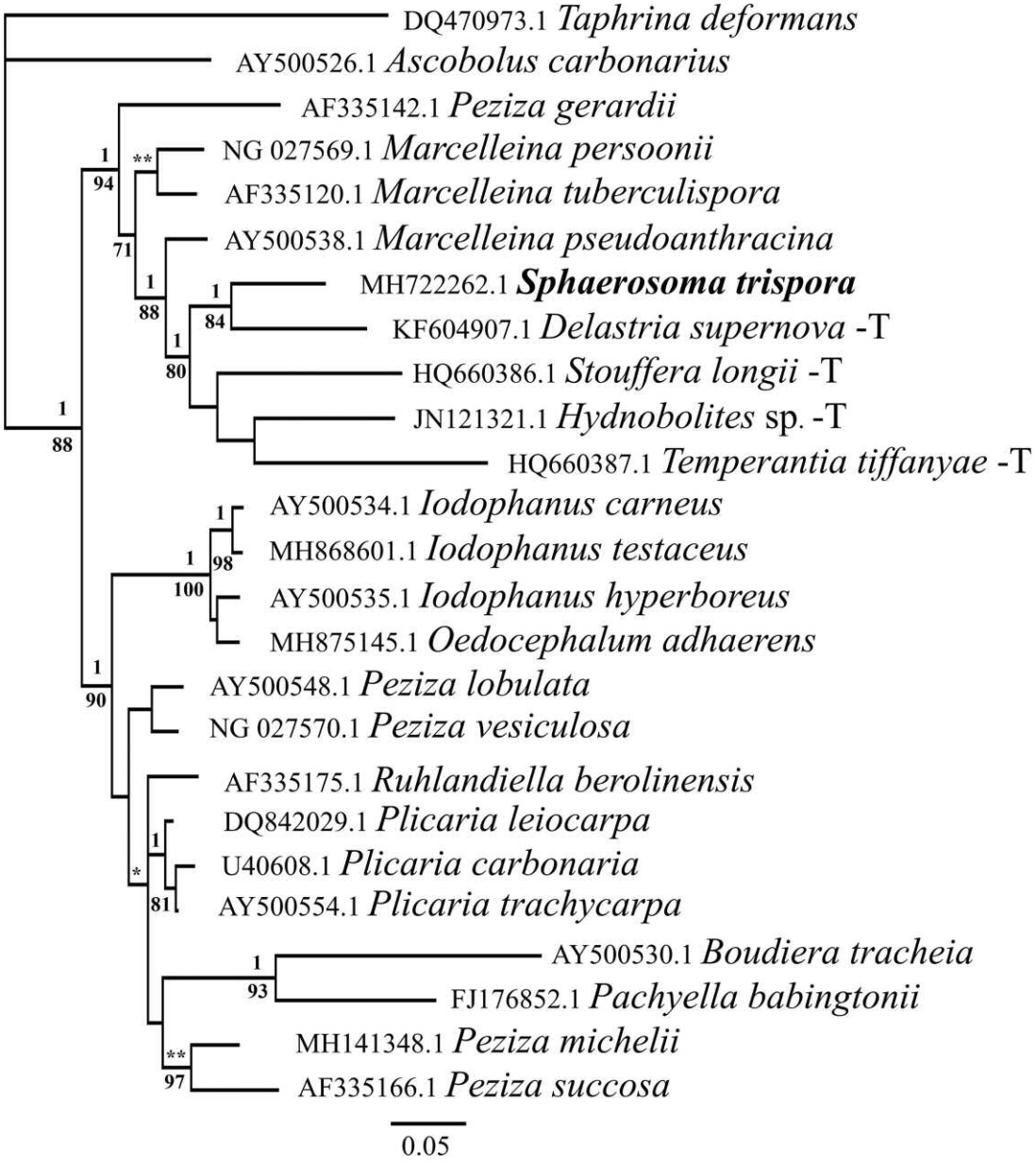

Fig. 1 – A Bayesian phylogram of genera in the *Pezizaceae* based on sequences of the 28S ribosomal gene showing phylogenetic relationship of *Sphaerosoma trispora* to the most closely related species with sequences available in GenBank at 19.10.2019, along with other selected species from genera in the *Pezizaceae*. *Taphrina deformans* (*Taphrinaceae*) and *Ascobolus carbonarius* (*Ascobolaceae*) were chosen as outgroup. 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. Numbers following species names identify the GenBank sequences used. Truffle like taxa are indicated by –T.

Unusual characters of *Sphaerosoma trispora*

Sphaerosoma trispora has some intriguing characters: the apparently inoperculate asci (Plate 3A, 3B), the very large ascospores with their double reticulum (Plate 2C) and particularly the regular shortfall from the more usual four or eight spores normally found in the asci of disc fungi. There are many possible reasons for a shortfall in spore number per ascus including irregular spore wall formation, autonomous spore lethal genes and the presence of meiotic drive elements that kill spores lacking a selfish DNA element such as those known in *Podospora* Ces. (Van der Gaag *et al*., 2000) and *Neurospora* Shear & B.O. Dodge (Turner & Perkins, 1979; Raju & Perkins, 1991, Hammond *et al*., 2012).

In their description McLennan & Cookson (1926) stated "Spores 3, rarely 4". Gamundí (1976) made no comment on any 4-spored asci but described the asci as 3-spored and those of *S. fuscescens* as 8-spored. We have only seen four spores in asci at immature stages of development (Plate 3B) with all mature ascospores in threes per ascus (Plate 2B, 2C, 2E). In one apothecium examined, the asci with four immature spores were particularly frequent. Counts were three spored asci 121, four spored asci 30, a good 3:1 ratio (p=0.285). Determination of why only three spores mature requires genetic experiments which may be impracticable since Warcup (1990) was unable to culture the species.

Conclusions

Our results determine the position of this species in the phylogeny and show that it lies within the *Pezizaceae*. That it nests within a clade where it is basal to truffles emphasises that morphological characters alone are insecure indicators of phylogenetic affinities. Nevertheless, there is a trend within the clade of increasing spore size and decoration. Our collections of *Sphaerosoma trispora* provide specimens of a rare species in a problematic genus. However, fresh collections of other *Sphaerosoma* species are needed to resolve doubts concerning the relationship between species that have been assigned to the genus.

Acknowledgements

We would like to thank a number of people and organisations: Carolyn Ricci for her help with scanning electron microscopy; the State Herbarium of South Australia (AD) for provision of equipment and materials; Lisa O'Donovan at Adelaide Microscopy for advice on preparation of specimens; Adelaide Microscopy for doing sputter-coating of specimens; Frank Kutsche for assistance in obtaining relevant scientific collecting permits for South Australia; Rangers of Conservation and National Parks for their assistance and interest; Lukáš Janošík for help with literature; Tom May and Teresa Lebel, National Herbarium of Victoria, for their encouragement and advice. Roy Halling generously provided literature on the Ascomycota. We are indebted to Helen Vonow, Collections Manager at AD, for her keen ability to spot often cryptic black discomycetes. We are very grateful to the State Herbarium of South Australia and Flinders University for their continual support.

References

Alsheikh A.M. 1994. — *Taxonomy and Mycorrhizal Ecology of the Desert Truffles in the Genus Terfezia*. Thesis for the degree of Doctor of Philosophy in Botany and Plant Pathology at Oregon State University, 239 p.

Atlas of Living Australia. https://bie.ala.org.au/search?q=Sphaerosoma+trispora. Accessed 24 March 2017 and throughout 2017 to 2019.

Beug M.W., Bessette A. & Bessette A.E. 2014. — *Ascomycete Fungi of North America: a mushroom reference guide*. Austin, University of Texas Press, 488 p.

Calhim S., Halme P., Petersen J.H., Læssøe T., Bässler C. & Heilmann-Clausen J. 2018. — Fungal spore diversity reflects substrate-specific deposition challenges. *Scientific Reports*, 8: 5356. doi: 10.1038/s41598-018-23292-8

Catcheside P. 2012. — *Sphaerosoma trispora* - a fascinating little black disc fungus. *Fungimap Newsletter*, 46: 3–5.

Catcheside P.S. & Catcheside D.E.A. 2018. — *Antrelloides atroceracea*, a new genus and species in the *Pezizaceae* (*Pezizales*) from Australia. *Swainsona*, 31: 81–91.

Corda A.C.J. 1842. — *Icones fungorum hucusque cognitorum*. Vol. 5. Prague, J.G. Calve, 92 p. [see page 27].

Dissing H. & Korf R.P. 1980. — Preliminary studies in the genera *Ruhlandiella*, *Sphaerosoma*, and *Sphaerozone* (order *Pezizales*). *Mycotaxon*, 12: 287–306.

Dougoud R. 2002. — Contribution à la connaissance de quelques Discomycètes operculés rares ou méconnus. *Fungi non delineati*, 18: 1–69.

Eckblad F.-E. 1968. — The Genera of Operculate Discomycetes. *Nytt magasin for Botanikk*, 15 (1-2): 1–191.

Gamundí I.J. 1976. — Acerca de los generos *Boudiera* Cooke y *Sphaerosoma* Klotzsch (Fungi, *Pezizales*). *Sydowia*, 28: 339–352.

Halbwachs H., Heilmann-Clausen J. & Bässler C. 2017. — Mean spore size and shape in ectomycorrhizal and saprotrophic assemblages show strong responses under resource constraints. *Fungal Ecology*, 26: 59–64. doi: 10.1016/j.funeco.2016.12.001

Hammond T.H., Rehard D.J., Xiao H. & Shiu P.K.T. 2012. — *Neurospora* Spore killer meiotic drive elements. *Proceedings of the National Academy of Sciences*, 109 (30): 12093–12098. doi: 10.1073/pnas.1203267109

Hansen K., Sandal S.K. & Dissing H. 1998. — New and rare species of *Pezizales* from calcareous woodlands in Denmark. *Nordic Journal of Botany*, 18 (5): 611–626. doi: 10.1111/j.1756-1051.1998.tb01544.x

Healy R.A. 2003. — *Mattirolomyces tiffanyae*, a new truffle from Iowa, with ultrastructural evidence for its classification in the *Pezizaceae*. *Mycologia*, 95 (4): 765–772. doi: 10.1080/15572536.2004.11833078

Index Fungorum. — Accessed throughout 2017 to 2019.

Huelsenbeck J.P. & Ronquist F. 2001. — MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17: 754–755. doi: 10.1093/bioinformatics/17.8.754

Jaklitsch W., Baral H.-O., Lücking R. & Lumbsch H.T. 2016. — Ascomycota. *In*: Frey W. (ed). *Syllabus of plant families* — Adolf Engler's Syllabus der Pflanzenfamilien. Stuttgart, Borntraeger, 322 p.

Kirk P.M., Cannon P.F., Minter D.W. & Stalpers J.A. 2008. — *Ainsworth & Bisby's Dictionary of the Fungi*. 10th edition. Wallingford, CABI, 771 p.

Klotzsch J.F. 1839. — *Sphaerosoma fuscescens* Kl., Bräunender Kugelkörper. *In*: Dietrich A.G. (ed.). *Flora regni Borussici: Flora des Königreichs Preussen oder Abbildung und Beschreibung der in Preussen wildwachsenden Pflanzen*. Berlin, Ludwig Ochmigke, Vol. 7: No. 467.

Korf R.P. 1972. — Synoptic key to the Genera of the *Pezizales*. *Mycologia*, 65 (5): 937–994. doi: 10.1080/00275514.1972.12019349

Kovács G.M., Trappe J.M., Alsheikh A.M., Hansen K., Healy R.A. & Vági P. 2011. — *Terfezia* disappears from the American truffle mycota as two new genera and *Mattirolomyces* species emerge. *Mycologia*, 103 (4): 831–840. doi: 10.3852/10-273

Kraisitudomsook N., Healy R.A., Mujic A.B., Pfister D.H., Nouhra E.R. & Smith M.E. 2019. — Systematic study of truffles in the genus *Ruhlandiella*, with the description of two new species from Patagonia. *Mycologia*, 111 (3): 477–492. doi: 10.1080/00275514.2019.1574490

Læssøe T. & Hansen K. 2007. — Truffle trouble: what happened to the *Tuberales*? *Mycological Research*, 111 (9): 1075–1099. doi: 10.1016/j.mycres.2007.08.004

Lilleskov E.A. & Bruns T.D. 2005. — Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. *Mycologia*, 97 (4): 762–769. doi: 10.1080/15572536.2006.11832767

Malloch D. & Blackwell M. 1992. — Dispersal of fungal diaspores. *In*: Carroll G.C. & Wicklow D.T. (eds.) *The fungal community: its organization and role in the ecosystem*. New York, Marcel Dekker: 147–171.

McLennan E. & Cookson I. 1926. — Additions to the Australian Ascomycetes, No II. *Proceedings of the Royal Society of Victoria*, 38: 69–76.

Moravec J. 1987. — A taxonomic revision of the genus *Marcelleina*. *Mycotaxon*, 30: 473–499.

Moreno G., Alvarado P. & Manjón J.L. 2014. — Hypogeous Desert Fungi. *In*: Kagan-Zur V., Roth-Bejerano N., Sitrit Y. & Morte A. (eds.) *Desert Truffles. Phylogeny, Physiology, Distribution and Domestication*. Heidelberg, Springer: 3–20.

Paz A. & Lavoise C. 2013. — Propuesta de dos nuevas especies de hongos hipogeos y una primera cita para la península Ibérica. *Boletín Micológico de FAMCAL*, 8: 71–86.

Raju N.B. & Perkins D.D. 1991. — Expression of Meiotic Drive Elements Spore killer-2 and Spore killer-3 in Asci of *Neurospora tetrasperma*. *Genetics*, 129: 25–37.

Rifai M.A. 1968. — The Australasian *Pezizales* in the Herbarium of the Royal Botanic Gardens, Kew. *Verhandelingen der Koninklijke Nederlandse Akademie van Wetenschappen, afd. natuurkunde, sect. 2*, 57 (3): 1–295.

Royal Botanic Garden, Edinburgh. 1969. — *Flora of British Fungi: Colour Identification Chart*. Edinburgh, Her Majesty's Stationery Office.

Rouppert C. 1910. — Révision du genre *Sphaerosoma*. *Bulletin International de l'Académie des sciences de Cracovie, Classe des Sciences Mathématique et Naturelles*, 2e sem. 1909: 75–94.

Schroeter J. 1897. — *Helvellineae*. *In*: Engler A. & Prantl K. (eds.). *Die natürlichen Pflanzenfamilien*, Teil 1, Abteilung 1: 172.

Seaver F.J. 1905. — A new species of *Sphaerosoma*. *Journal of Mycology*, II: 2–5, I pl.

Seaver F.J. 1914. — Observations on *Sphaerosoma* and Allied Genera. *Mycologia*, 6: 103–108.

Setchell W.A. 1910. — The genus *Sphaerosoma*. *University of California Publications, Botany*, 4 (5): 107–120, plate 15.

Species Fungorum. — Accessed throughout 2017 to 2019.

Stamatakis A. 2014. — RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30 (9): 1312–1313. doi: 10.1093/bioinformatics/btu033

Trappe J.M. 1979. — The orders, families, and genera of hypogeous ascomycotina (truffles and their relatives). *Mycotaxon*, 9: 297–340.

Tulasne L.R. & Tulasne C. 1851. — *Fungi Hypogaei: Histoire et monographie des Champignons hypogés*. Paris, Klincksieck, 222 p.

Turner B.C. & Perkins D.D. 1979. — Spore killer, a chromosomal factor in *Neurospora* that kills meiotic products not containing it. *Genetics*, 93: 507–606.

Van der Gaag M., Debets A.J.M., Oosterhof J., Slakhorst M., Thijssen J.A.J.M. & Hoekstra R.F. 2000. — Spore-Killing Meiotic Drive Factors in a Natural Population of the Fungus *Podospora anserina*. *Genetics*, 156 (2): 593–605.

Van Vooren N. 2010 [2009]. — Description de *Peziza gerardii* (Ascomycota, Pezizales) et présentation d'une récolte à petites spores. *Mycologia Montenegrina*, XII: 33–40.

Vizzini A. 2003. — Il genere *Tuber*: la sua posizione nelle *Pezizales* (origine dei taxa ipogei nelle *Pezizales*). *Bolletino del Gruppo Micologico G. Bresadola, n.s.*, 46 : 97–153.

Warcup J.H. 1990. — Occurrence of ectomycorrhizal and saprophytic discomycetes after a wild fire in a eucalypt forest. *Mycological Research*, 94 (8): 1065–1069. doi: 10.1016/S0953-7562(09)81334-8

Weber N.S., Trappe J.M. & Denison W.C. 1997. — Studies on Western American *Pezizales*. Collecting and describing ascomata — macroscopic features. *Mycotaxon*, 61: 153–176.

Zobel J.B. 1854. — *Augusti Cae. Jos. Corda Icones fungorum hucusque cognitorum*. Vol. 6. Prague, Ehrlich, 91 p., XX pl.


1


2

1: P.S. Catcheside – State Herbarium of South Australia, G.P.O. Box 1047, Adelaide, South Australia 5001 – Pam.Catcheside@sa.gov.au

2: D.E.A. Catcheside – College of Science and Engineering, Flinders University, P.O. Box 2100, Adelaide, South Australia 5001 – David.Catcheside@flinders.edu.au