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Boletus edulis (Boletaceae), a new record for Australia

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Abstract

Fungi belonging to *Boletus* section *Boletus*, known in Italy as porcini, in France as ceps, are prized for their good flavour and command a high price. They are ectomycorrhizal, forming symbiotic associations with a wide range of host plants, and although endemic to the Northern Hemisphere have been introduced into South Africa and New Zealand. We report here that *Boletus edulis* Bull. has become established broadly across the higher parts of the Adelaide Hills in South Australia in mycorrhizal association with at least three species of exotic trees. ITS sequence and morphological data were utilised to confirm the identity of the fungus and a full description of the Adelaide Hills collections is provided.

Keywords: naturalised and introduced fungi, edible fungi, *Boletus*, porcini, ceps, *Quercus*, *Pinus*, *Castanea*, Australia.

Introduction

Boletus section *Boletus* comprising *Boletus edulis* and several allied species are of significant economic importance due to their excellent flavour both when fresh and dry and to their high nutritional value (Çaglarlrmak et al. 2002; Ribeiro et al. 2008; Sitta & Floriani 2008). Annual worldwide consumption of porcini has been estimated to be between 20,000 and 100,000 tons (Hall et al. 1998) with a world market value exceeding $250 million. Prices range from $20−$80/kg in the northern hemisphere autumn but have reached as high as $231/kg in a poor fruiting season in New York in 1997 (Hall et al. 2003).

Boletus sect. *Boletus* is characterised by the spongy hymenial surface of tubes and pores which is initially whitish due to the pores being covered with a white hyphal mass. The tubes and pores become yellow to olive-yellow and lose the hyphal web on maturity. The flesh does not discolour on cutting or bruising. The stipe is usually bulbous and has a raised reticulum, at least on the upper part. The pileus is hemispherical when young and brown to chestnut in colour (Watling 1970; Singer 1986; Breitenbach & Kranzlin 1991; Cortecuisse 1999; Beugelsdijk et al. 2008).

Species in *Boletus* sect. *Boletus* are ectomycorrhizal, growing in association with a wide range of tree species in the families Fagaceae, Betulaceae, Malvaceae, Cistaceae, Salicaceae and Pinaceae (Hall et al. 1998; Agueda et al. 2006; Beugelsdijk et al. 2008; Dentinger et al. 2010). *Boletus* sect. *Boletus* is endemic in the northern hemisphere and does not occur naturally in the southern hemisphere. However, *B. edulis* has been introduced into South Africa (Marais and Kotze 1977; Hawley 2008) and New Zealand (Wang et al. 1995; Hall et al. 1998; Stringer et al. 2001). *B. edulis* has not previously been reported from Australia (Watling & Li 1999; Roy Halling, pers. com., 2010) although an endemic true porcini with the provisional generic name "*Inferiboletus*", estimated to have diverged from *Boletus* 34 Mya, has recently been reported for Australia (Dentinger et al. 2010).

We first observed and collected material (*PSC 2651*) consistent with the macroscopic and microscopic characters of *Boletus* sect. *Boletus* in South Australia in May 2007 associated with *Quercus robur* L., although it is likely to have been present and known to European immigrants somewhat earlier. More material was found fruiting in May 2009, April 2010 and March 2011 (Fig. 1) at the same and a nearby site and another collection (*PSC 3004*) was made. Further reports in April and December 2010 of porcini-like boletes associated respectively with *Castanea sativa* Mill. and *Pinus radiata* D. Don, led to more collections (*PSC 3273* and *PSC 3458*). Following early autumn rain in 2011, a report appeared in the Adelaide press on 2 Apr. 2011 of porcini being collected in the Adelaide Hills and sold at up to $120/kg in the Adelaide Market (Wilkinson 2011). A short survey on 12 Apr. 2011 led to finding a further six locations in the Adelaide Hills where the fungus was associated with *Q. robur* or *P. radiata*. A full evaluation of the collections was made due to the importance of porcini as a high value commercial crop and their potential to form mycorrhizal associations with a wide range of tree species.

Fig 1. *Boletus edulis*. Adelaide Hills, March 2011, under *Quercus robur*. See back cover of journal for colour reproduction of this photograph.

Methods

Morphology

Habitat and associated plant communities were noted in the field. Collection locations were recorded by GPS (Garmin GPS12) and in situ photographs taken using a Nikon 4500 camera. Macroscopic characters were described directly from fresh material. Colours are described in general terms and more precisely according to the Royal Botanic Gardens Edinburgh Colour Chart (1969) (given as colour descriptor and number e.g. rust 13) and 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 and dried material were mounted in 5% aqueous KOH, then stained with ammoniacal Congo Red. Measurements were made at ×400 or ×1000 with an ocular micrometer. Illustrations of microscopic characters were made using an Olympus drawing tube system. Measurements are the normal range observed with outliers, if any, in brackets. Spore dimensions are given as length range × width range (n = 30). The length: width ratio (Q) of individual spores is presented as the range and mean of Q values. Measurements do not include the apiculus. Basidia and cystidia dimensions are recorded as length range × width range (n = 20). All illustrations are based on collections *PSC 2651*, *PSC 3004*, *PSC 3273* and *PSC 3458*. All collections have been accessioned into the State Herbarium of South Australia (AD).

DNA Extraction, amplification and processing

DNA was extracted from 5–10 mg of dried specimens by freezing with liquid nitrogen and grinding in a pestle and mortar with 500 µl of pH 8.0 isolation buffer (50 mM Tris-HCl, 170 mM EDTA, 1% N-lauroylsarcosine). The frozen paste was allowed to thaw, transferred to a 1.5 ml Eppendorf tube and incubated at 65°C for 5 min. Following addition of 300 µl 7.5 M ammonium acetate, the tubes were mixed by inversion, incubated on ice for 10 min. and then centrifuged at 13,000 g for 5 min. The supernatant (700 µl) was transferred to a fresh tube, mixed with 500 µl of isopropanol and held on ice for 10 min. Following centrifugation at 13,000 g for 3 min., the supernatant was discarded and the tubes drained by inversion on paper towel. The pellet was dissolved in 250 µl Tris EDTA buffer (10 mM Tris 1mM EDTA

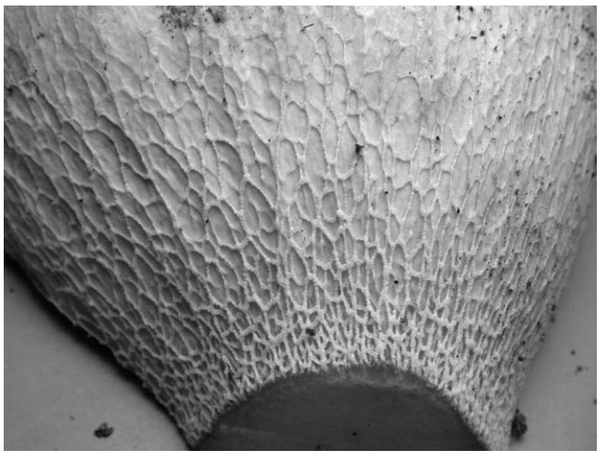

Fig 2. *Boletus edulis*, Adelaide Hills, *PSC 3273*, reticulum on stipe, under *Castanea sativa*.

Fig 3. *Boletus edulis*, Adelaide Hills, *PSC 2651*, showing extended reticulum on stipe, under *Quercus robur*.

pH 8.0), if necessary by incubation at 50°C for 5 min. and brief vortex mixing. PCR amplifications (20 µl) employed Phusion polymerase (New England Biolabs) in HF buffer, ITS1 and ITS4 primers (White et al. 1990) and 1 µl of a 1/20 to 1/100 dilution of the DNA extract. Amplification employed 5 min. at 98°C followed by 40 cycles (98°C 30 s, 57°C 15 s, 72°C 15 s) then 5 min. at 72°C prior to storage at 4°C. PCR products were purified using a PCK-1 kit (AdBiotec) and sequenced (AGRF) on both strands using the PCR primers. DNA sequence was assembled from unidirectional reads and sequences compared using Sequencher 4.9 (Gene Codes Corp., Ann Arbor, Michigan, USA) and blastn searches for similar sequences in GenBank conducted using NCBI software.

Results

Morphology

Specimens examined: *PSC 2651*, 19.v.2007; *PSC 3004*, 31.v.2009; *PSC* 3273, 15.iv.2010; *PSC 3458*, 27.xii.2010.

Description. *Pileus* (70–) 100–250 (–275) mm diameter, (22–) 40–50 mm high, more or less hemispherical when young, later becoming plano-convex, irregularly plane to slightly concave; brown, light brown, 6D4–7, 7D8, 7E6–8, 7E5–6 (Kornerup & Wanscher), fulvous 12, rust 13, rusty-tawny 14, brick 15 (Edinburgh Colour Chart); initially covered with white-grey bloom; dry becoming lubricous in centre; smooth to almost phlebioid in patches; margin rather irregular, projecting beyond tubes, wavy, finely revolute or involute, smooth, even, margin edge whitish with whitish bloom. *Flesh of cap* thick, solid, becoming rather spongy; white, cream, 4A2; not discolouring though turning slightly brown or reddish immediately under cap cuticle but not above stipe. *Tubes* emarginate to adnexed, with sulcus around stipe; separating easily from flesh; deep, 8–25 mm in centre; parallel; white, cream, becoming pale yellow 3A3–4, dull yellow, greenish-yellow 2B4, 2C4, 3B3–5, darkening to greyish-yellow 3C3 on cutting, not blueing. *Pores* initially white, cream, with whitish plug of tangled hyphae, later losing plug and becoming concolourous with tubes; not changing colour on bruising; small, 1–3/mm; rounded to angular, some irregular, slightly elongated; dissepiments thin to medium. *Stipe* (80–) 135–195 mm long, 30–68 (–98) mm diameter under cap, (32–) 70–98 mm in centre, 17–56 mm diameter 1 cm above base; ventricose or with bulbous base, occasionally cylindric; whitish, dull white-grey, pale brown, paler than cap, 5C4, 6C3–4, 7D4, creamy-brown under cap, creamy clay-pink, closest to 30, 5B4, paler, whitish-brown at base; surface covered with whitish reticulum (Fig. 2) to half way down stipe, rarely over most of stipe (Fig 3), reticulations raised, white, white-beige, slightly greyer than 2B; reticulations small at top, approx. 1 × 0.5 mm, larger below to 4.5 × 2 mm and more when most of the stipe is reticulated, lacunae shallow; surface rather waxy. *Flesh of stipe* thick, solid, white, not changing colour on bruising. *Spore print* olive brown, yellowish-brown, hazel 27, 5E5–7. *Spores* (Figs. 4A, 5A) fusiform-ellipsoid, cylindrical, more or less smooth, rather thick-walled, pale yellow-brown in KOH; with oil globules; (12–) 13.6–18.4 × (4.0–) 4.8–5.6 µm, mean 16.8 × 5.3 µm; Q: 2.4–3.8, mean Q: 3.17. *Basidia* (Figs. 4B, 4C, 5B) clavate, (24–) 34–53 × (7–) 8–10 µm, mean 35.8 × 9.6 µm; with (2–) 4 sterigmata; without clamp connections. *Cheilocystidia* (Figs. 4D, 5C) fusiform, lageniform, ventricose-digitate, 40–72 × 5.5–10 µm, hyaline in KOH. *Pleurocystidia* similar to cheilocystidia. *Pileipellis* a trichoderm of irregular and regular interwoven hyphae, hyphae 3–10 µm, many hyphae ascending, erect, exserted; slightly gelatinised; terminal elements 4–10 µm; septa without clamp connections. *Hymenial elements in reticulum of stipe* (Figs. 4E, 5D): *caulocystidia* abundant; fusiform, cylindric, subclavate, 42–75 (–85) × (6.5–) 8–13 µm, mean 62.2 × 8.9 µm (n=10); *basidia* clavate, 2–4 spored but mostly 3-spored, 36–44 × 11–14 µm. *Hymenophoral trama* of parallel hyphae.

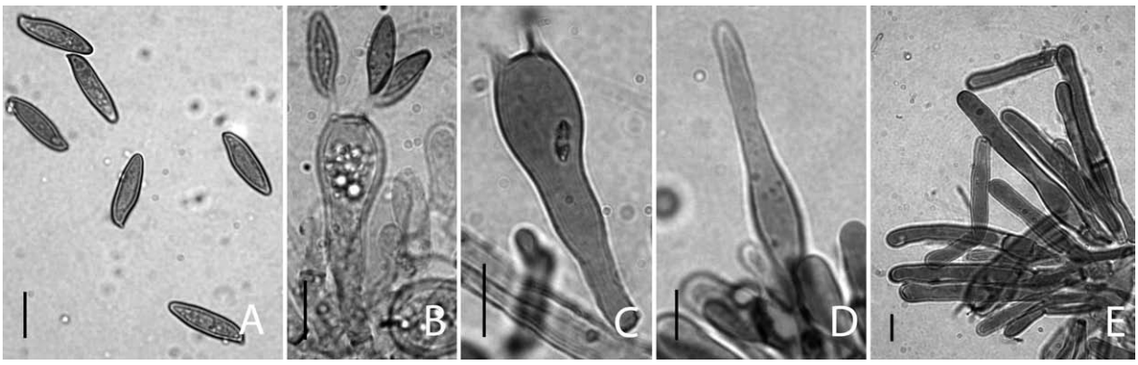

Fig 4. *Boletus edulis* micrographs of *PSC 2651*. **A** spores; **B, C** basidia; **D** cystidium; **E** caulocystidia. Scale bar: 10 µm.

Habitat. PSC 2651 in soil and deep leaf litter under *Quercus robur* L. with *Amanita muscaria* (L.) Lam., *Lepista nuda* (Bull.) Cooke and *Russula* sp., *Araucaria bidwillii* Hook. was nearby; *PSC 3004* in litter under *Quercus robur* L.; *PSC 3273* under 25 year old *Castanea sativa* Mill.; *PSC 3458* in soil and pine needle litter under *Pinus radiata* D.Don. with *Suillus granulatus* (L.) Roussel*, Rhizopogon rubescens* (Tul.) Tul., *Lactarius deliciosus* (L.) Gray. *Populus alba* L. and *Eucalyptus microcarpa* (Maiden) Maiden were also nearby.

Molecular data

PSC 2651, *3273* and *3458* each had the same ITS sequence (Genbank accession JQ277466). Blast searches of the NCBI database at 1 November 2011 identified 39 entries having a sequence identical to JQ277466: DQ990838 (Peintner et al. 2007), EU417846, EU417847, EU417849, EU417851, EU417852, EU417855, EU 417856, EU417857, EU417858, EU417859, EU417861, EU417862, EU417863, EU417864, EU417868, EU417869, EU417874 (Beugelsdijk et al. 2008), GU373493 (von Cräutlein et al. 2011), GU198977, GU198981, GU198982, GU198991 (Korhonen et al. 2009), JF728991, JF728992, JF728994, JF728995, JF728999, JF729002, AY680981 (De la Varga et al. 2011), AY680983, AY680984, AY680985, AY680991, AY680992, AY680993, AY680994 (Leonardi et al. 2005), DQ002921 and DQ131622 (Águeda et al. 2006).

Discussion

The porcini mushrooms, *Boletus* sect. *Boletus*, comprise a number of described species (Singer 1986; Wang et al. 1995; Beugelsdijk et al. 2008; Dentinger et al. 2010) whose relationships have been clarified considerably by molecular data (Leonardi et al. 2005; Beugelsdijk et al. 2008; Dentinger et al. 2010). *B. edulis* is now recognised as a taxon having variable morphology, a wide range of mycorrhizal partners and an extensive geographical range in the Northern Hemisphere. The molecular data suggest *B. edulis* includes the previously recognised species: *B. betulicola, B. chippewaensis*, *B. persoonii*, *B. quercicola* and *B. venturi*.

Morphologically, the Adelaide collections conform well to descriptions of *Boletus edulis* sensu stricto (Watling 1970; Singer 1986; Breitenbach & Kranzlin 1991; Cortecuisse 1999; Beugelsdijk et al. 2008) with respect to their microscopic and macroscopic characters. They differ from descriptions of the three other lineages recognised by Beugelsdijk et al. (2008) in *Boletus* sect. *Boletus*: *B. aereus*, *B. reticulatus* and *B. pinophilus.* None of the Adelaide collections have the dry, velvety darker brown cap and darker brown stem with a rust-brown reticulum of *B. aereus*; all had whitish or, at most, a pale brown stipe and the white to off-white reticulum usually reached no further than the stipe's centre, unlike the reticula of both *B. aereus* and *B. reticulatus* which cover the whole stem. Collection *PSC 3458* was found under *Pinus radiata* but no specimens had the thickly gelatinous cap pellicle or wide terminal elements of *B. pinophilus*; those in all our collections had terminal elements to 10 µm while those of *B. pinophilus* may reach 27 µm.

ITS sequences identical to those of the South Australian collections PSC 2651, PSC 3273 and PSC 3458 include AY680991, AY680992, AY680993, AY680994, AY680984, EU 417846, EU17847, EU17849, EU417851, EU417852, EU417855, EU417856, EU 417857, EU417858, EU417859, EU417861, EU417862, EU417863, EU417864 and EU417874 which are in the clade recognised by Beugelsdijk et al. (2008) as *B. edulis* and also DQ002921 which is within the clade recognised by Dentinger et al. (2010) as *B. edulis* sensu stricto. This sequence is dissimilar to the ITS sequence of any of the additional taxa within the *Boletus* sect. *Boletus* clade recognised by Dentinger et al. (2010). There is thus no doubt that *B. edulis* has arrived in Australia.

Matching ITS sequences to the Adelaide Hills *B. edulis* biotypes have been found in collections made in Austria, Belgium, Finland, Italy, Netherlands, Spain and Sweden, variously associated with *Betula*, *Castanea*, *Cistus*, *Fagus*, *Picea*, *Pinus* and *Quercus*. In consequence, the currently available molecular data provide insufficient information to identify the source of the introduction into South Australia.

All currently known populations of *B. edulis* in Australia are within 8 km of one another and within 7 km of Mount Lofty at altitudes ranging from 330 m to 470 m above sea level. The extent of this range and

association with three species of mature trees suggests that the introduction is not recent.

B. edulis is an obligate ectomycorrhizal fungus. Fruiting bodies have not been produced in culture, although efforts to cultivate the fungus in association with known plant hosts have had some success (Hall et al. 1998). These authors suggest that plantation forests of *Pinus* would be ideal habitats for *B. edulis*. In later work, Hall et al. (2005) observed *Amanita excelsa* ectomycorrhizas (ECMs) together with rhizomorphs of *B. edulis* penetrating the mantle around roots of their host plant and suggest that such co-symbionts may be mutually beneficial for all partners.

The discovery of *Boletus edulis* in South Australia raises the possibility of harvesting and managing this potentially valuable resource. A problem that might arise from deliberate cultivation of an introduced ectomycorrhizal fungus with a wide host range, such as *B. edulis*, is the possibility of its switching host to native trees. Although Malajczuk et al. (1982) found that ECMs of *B. edulis* did not form on any of the eleven species of eucalypt they tested for ECM formation, the possibility of *B. edulis* forming such symbioses with other Australian native species should not be discounted. Dunk et al. (2011) report on the invasion of *Amanita muscaria* into native *Nothofagus* forests in Tasmania and express the concern that such invasion may have detrimental effects on the ecosystem. Dunk et al. (2011) and Orlovich & Cairney (2004) comment on the lack of knowledge about the effect of the spread of exotic ECMs and emphasise the need for research into the long term consequences of the introduction or encouragement of potentially invasive mycorrhizal species.

Although recognition of the presence of the choice edible *Boletus edulis* in South Australia provides opportunities for exploitation of a new resource, there are corresponding risks which should be assessed before implementation of any actions to expand its distribution.

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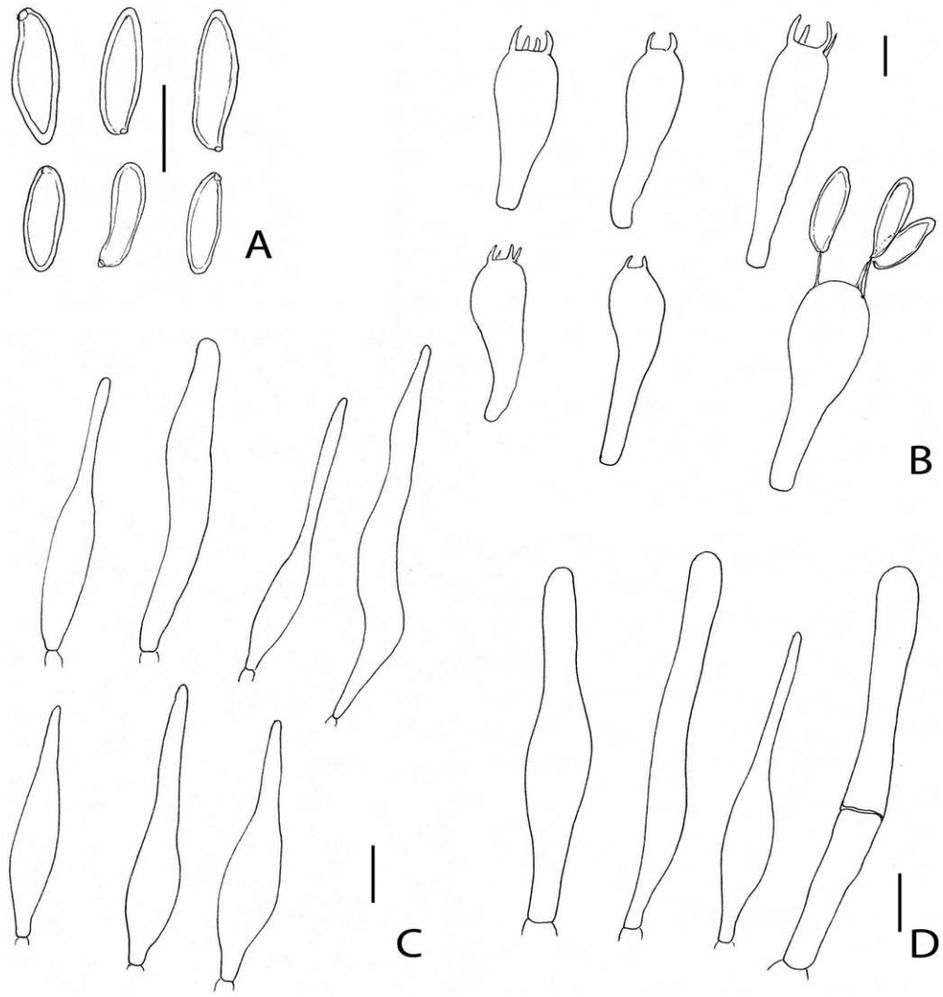

Fig 5. *Boletus edulis*, drawings of *PSC 2651*. **A** spores; **B** basidia; **C** cystidia; **D** caulocystidia. Scale bar: 10 µm.

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