Gymnogaster boletoides J.W. Cribb (Boletaceae, Boletales), a striking Australian secotioid bolete

Matteo Gelardi¹, Nigel Fechner^{2*}, Roy E. Halling³, Federica Costanzo¹

Summary

Gelardi, M., Fechner, N., Halling, R.E. & Costanzo, F. (2017). *Gymnogaster boletoides* J.W. Cribb (Boletaceae, Boletales), a striking Australian secotioid bolete. *Austrobaileya* 10(1): 121–129. The austral secotioid species *Gymnogaster boletoides* J.W. Cribb has been reported from various localities in southern Queensland, New South Wales, Victoria and Western Australia. A detailed, modern description of the species including macro- and micromorphological characters is provided, accompanied by colour images taken *in situ;* photomicrographs; and line drawings of the main anatomical features. Comparative assessments of morphologically closely allied species are also presented.

Key Words: Boletales, Boletaceae, *Gymnogaster boletoides*, Australia fungi, Queensland fungi, secotioid fungi, taxonomy

¹M. Gelardi & F. Constanza, Via Angelo Custode 4A, I-00061 Anguillara Sabazia, RM, Italy.

²N. Fechner, Queensland Herbarium, Department of Science, Information Technology & Innovation, Mt. Coot-tha Road, Toowong, Brisbane, Queensland 4066, Australia.

³R.E. Halling, Institute of Systematic Botany, The New York Botanical Garden, Bronx, NY 10458-5126, USA.

*Corresponding author: Email: Nigel.Fechner@dsiti.qld.gov.au

Introduction

The taxonomic placement of the Australian secotioid bolete *Gymnogaster boletoides* J.W.Cribb has long been uncertain and the species has been placed in either the Secotiaceae Tul. & C. Tul. (Cribb 1956) or the Agaricaceae Chevall. (Kirk *et al.* 2008). However, molecular analysis carried out by Halling *et al.* (2012) clearly demonstrated that the species belongs to the family Boletaceae Chevall. and clusters in a separate, independent lineage with high phylogenetic confidence (Tedersoo & Smith 2013; Wu *et al.* 2014, 2016b; Zhao *et al.* 2014, 2015; Smith *et al.* 2015).

Intensive mycological field research carried out by Roy Halling (REH) and Nigel Fechner in eastern and south-eastern Australia over the years 2005–2015 resulted in several collections of *Gymnogaster boletoides* (Figs. 1–6). Two additional samples were

found by REH and Matteo Gelardi during the forays of the combined annual meeting of the Australasian Mycological Society, and the Queensland Fungi Festival held in Brisbane in late April 2014. Specimens of *G. boletoides* examined for this paper constitute a representative sample of the collections held in the Queensland Herbarium, one of which was gathered on Mt Glorious, the type locality where Joan Cribb first collected the species in 1956.

Materials and methods

Specimens examined were collected at different localities in Queensland, Australia, dried on a commercial portable food dehydrator and deposited in BRI, NY (Thiers 2015) and the personal herbarium of Matteo Gelardi. Herbarium collections are cited for those from which morphological features were examined. Author citations follow the *Index Fungorum, Authors of Fungal Names* (www. indexfungorum.org/authorsoffungalnames. htm).

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Fig. 1. *Gymnogaster boletoides.* Basidiomes *in situ* (*Halling et al. MG605*, BRI). Scale bar = 10 mm. Photo: M. Gelardi.

Macroscopic descriptions, habitat notations and associated plant communities were based upon detailed field notes of fresh basidiomes. Colours were recorded under daylight and described in general terms only. Micromorphological features were observed from dried material; sections were either rehydrated in water, 5% potassium hydroxide (KOH) or in ammoniacal Congo Red. Observation of structures and measurements of anatomical features were performed by mounting preparations in ammoniacal Congo Red. Colours and amount of pigmentation were described after examination in water and 5% KOH. Measurements were made at $1000 \times$ with a calibrated ocular micrometer (Nikon Eclipse E200 optical light microscope). Spores were measured from the hymenophore of mature basidiomes. Dimensions are given as (minimum-)a-b(-maximum), where the range a-b contains a minimum of 90% of the measured values, Q = length/width ratio with minimum and maximum values in parentheses, Qm = average quotient (length/ width ratio) \pm standard deviation, while average spore volume was estimated as a rotation ellipsoid V = 4/3*(length/2)*((width/2)*width) $\pi/2 \pm$ standard deviation]. The notation [n/m/p] indicates that measurements were made on "n" randomly selected spores from "m" basidiomes of "p" collections.



Fig. 2. *Gymnogaster boletoides.* Habit. (*Halling 9455*, BRI). Scale bar = 10 mm. Photo: R.E. Halling.

Metachromatic, cyanophilic and iodine reactions were tested by staining the spores in Brilliant Cresyl blue, Cotton blue and Melzer's reagent respectively. Line-drawings of microstructures were made free hand from rehydrated material and based on photomicrographs.

Commonly used abbreviations in the specimen citation include NP for National Park.

Taxonomy

Gymnogaster boletoides J.W. Cribb, *Pap. Dept. Bot. Univ. Queensland* 3(13): 110 (1956). **Type:** Queensland. MORETON DISTRICT: Mt. Glorious, 19 February 1955, *J.W. Cribb s.n.* (holo: BRI [BRIP10509]). *Mycobank number*: MB 298018.

Illustrations: Cribb (1956: 111, figs 1–6).

Basidiomes secotioid, epigeous, 1–3.5 cm high, 0.7–5 cm broad, with hymenophore (fertile portion) surrounding percurrent stipe columella, ovoid, subglobose to elongate in outline or irregularly shaped, sometimes barely pileate, stipitate, evelate. *Pileus* a small apical, appressed disc, depressed, 4–8 mm broad, sometimes absent; surface of the disc matt, dry, very finely tomentose, dark brown to reddish-brown or orangish-brown, not cracked. *Hymenophore* completely



Fig. 3. *Gymnogaster boletoides.* Spores in KOH, showing thin wall (germ pore) at apex (*Halling 9800 & Fechner*, BRI). Scale bar = $10 \,\mu$ m. Photo: R.E. Halling.



Fig. 4. *Gymnogaster boletoides.* Dextrinoid spores (*Halling 9800 & Fechner*, BRI). Scale bar = 10 μm. Photo: R.E. Halling.

and permanently exposed, *Morchella*-like, moderately fleshy, firm but progressively softer with age, slightly subdecurrent, undulate-loculate, consisting of labyrinthine to irregularly arranged, folded chambers tending to radiate out from the columella; surface and inner portions whitish with some brownish red stains at first then grayishyellow, later bright lemon yellow to oliveyellow with scattered rusty brown spots and finally dull yellowish-brown, immediately staining dark blue on handling and finally fading to sordid brown. *Stipe* 0.5–1.5 × 0.1– 0.8 cm, reduced, central, straight, cylindrical but tapered towards the base, protruding within the hymenophore as a columella, not or only faintly rooting; surface short sulcate at apex, dry, smooth to subpruinose, bright lemon yellow to orange–yellow at apex, rhubarb red elsewhere and progressively darker downwards, staining blue when pressed. *Columella* present, usually columnar and percurrent (not percurrent but dendroid with scattered, thin branches extending into the fertile hymenophore in collection *Gelardi et al. MG607*), context evenly whitish to



Fig. 5. *Gymnogaster boletoides.* Cyanophilic spores (*Halling 9800 & Fechner*, BRI). Scale bar = 10 μm. Photo: R.E. Halling.

yellowish, sometimes whitish only in the peripheral zones and rhubarb red inwards, staining blue on exposure. *Stipe context* solid but at times becoming hollow, whitish to rhubarb red or purple–red and gradually darker downwards, staining blue throughout when bruised.

Odour faint to sometimes pungent, agreeable. Taste mild. Spore print not obtained. Basidiospores [66/3/2] (10.0–)11.3–13.1(– 14.0) × (6.7–)7.1–8.1(–8.6) μ m, Q = (1.22–) 1.36-1.80(-1.84), $Om = 1.60 \pm 0.11$, Vm = 370 \pm 68 μ m³ (n = 66 from the hymenophore of mature specimens) (several anomalous spores originating from 1-spored basidia have been observed in collection Gelardi et al. MG607, with size up to $26.0 \times 13.2 \ \mu m!$), bilaterally symmetric in all views, amygdaliform to citriform or rarely subglobose, apex rounded to sometimes pointed and with a thin germ pore-like region, smooth, with a pronounced, prominent apiculus and without suprahilar depression, thick-walled $(0.4-0.6 \mu m)$, honey vellow to ochraceous in water and 5% KOH, with one or, more rarely, two or three large oil droplets when mature, inamyloid or a very

small minority dextrinoid, acyanophilic to less frequently cyanophilic and with a faint metachromatic reaction. Basidia 23-44(-46) \times 9–14 µm (n = 23), cylindrical-clavate to clavate, vesciculose to subglobose in the inner hymenophoral tissues, moderately thickwalled (0.5–1.0 µm), predominantly 4-spored but also 1- or 2-spored, usually bearing short to moderately long sterigmata (2–7 μ m), hyaline to pale yellowish and not containing straw-yellow oil guttules in water and 5% KOH, bright yellow to ochraceous (inamyloid) in Melzer's, without basal clamps; basidioles cylindrical-clavate, clavate to subglobose in the inner hymenophoral tissues, about the same size as basidia. Cheilocystidia (26-)27- $65(-70) \times (5-)6-17(-21) \ \mu m \ (n=23), \ very$ common, short to moderately long, straight to flexuous, versiform, irregularly cylindrical, cylindrical-fusiform, ventricose-fusiform, mucronate to less frequently bottle-shaped or sublageniform, with neck ranges from narrow and short to very long, with rounded to subacute tip, smooth, moderately thickwalled (0.5-1.0 µm), hyaline to pale straw yellow in water and 5% KOH, bright yellow to ochraceous (inamyloid) in Melzer's, without



Fig. 6. *Gymnogaster boletoides.* A. basidiospores. B. basidia. C. cheilo- and pleurocystidia. D. hymenophoral trama (direction of hyphae from the bottom to the top, stippling in the drawing representing a gelatinous matter). E. pileipellis. All from *Halling et al. MG605* (BRI). Scale bars: A–C: 10 μm, D–E: 20 μm. Del. M. Gelardi.

epiparietal encrustations. Pleurocystidia $(36-)38-65(-68) \times (5-)6-20(-27) \ \mu m \ (n=20),$ uncommon, shape, size, colour and chemical reactions as in cheilocystidia, usually showing a narrow and short neck. Pseudocystidia not recorded. Pileipellis (when pileus present), trichoderm consisting of subparallel а (towards the margin of the disc) to moderately interwoven (at the center of the disc), erect, elongated, filamentous and sinuous, rarely branched hyphae not embedded in gelatinous matter; terminal elements (10-)12-30(-36) \times 4–9(–11) µm (n = 16), relatively short, irregularly cylindrical, bullet-shaped or acorn-shaped to less frequently cystidioid or clavate, rarely diverticulate, apex roundedobtuse to sometimes pointed, moderately

thick-walled (up to 0.9 µm), honey yellow to ochraceous in water and 5% KOH or with a slowly soluble red pigment in hydroxide mounting media, inamyloid in Melzer's, smooth; subterminal elements similar in shape, size and colour to terminal elements. Hvmenophoral trama 30 - 50(-60)um broad, consisting of very slightly divergent or almost parallel or subparallel, loosely arranged, gelatinized hyphae with nearly no differentiation between mediostratum and lateral strata [hyphae in transverse section remaining separate and (1-)2-6 µm apart, $2-10 \ \mu m$ broad, with a tendency to spread from the inner hymenophoral tissues towards the peripheral areas], straw yellow in water and hyaline to very pale yellowish in 5%

KOH, inamyloid in Melzer's. Stipitipellis a texture of slender, subparallel to loosely intermingled and longitudinally oriented, smooth walled, appressed hyphae, 2-10 µm wide, straw yellow to honey yellow in water and in 5% KOH; stipe apex covered by a welldeveloped caulohymenial layer consisting of sterile caulobasidioles, very sparse, 1-, 2- and 4-spored, fertile caulobasidia (these latter not detected at all in collection MG607) and abundant short to projecting caulocystidia similar in shape and colour to hymenial cystidia but distinctly smaller, irregularly cylindrical, cylindrical-fusiform, ventricosefusiform to sublageniform or bullet-shaped, $(14-)18-42(-46) \times 4-12 \ \mu m \ (n = 20), having$ a wall up to 1 µm thick. Columella composed of densely arranged, subparallel to loosely interwoven, filamentous to slightly inflated. smooth, inamyloid hyphae, 3-19 µm broad. Stipe trama composed of densely arranged, strongly interwoven, smooth, inamyloid, filamentous hyphae intermixed with inflated or vesciculose to nearly subglobose cells, 3–20(–26) µm broad. Oleiferous hyphae present. Clamp connections absent in all tissues. Figs. 1–6.

Additional specimens examined: Australia: Queensland. DARLING DOWNS DISTRICT: Road from Dalby to Bunya Mountains NP, Feb 2013, Halling 9800 & Fechner (BRI, NY); Cunningham's Gap, Main Range NP, Mar 2011, Halling 9455 & Fechner (BRI, NY). MORETON DISTRICT: Rainforest Circuit, Maiala NP, Mt Glorious, Apr 2014, Gelardi MG607 et al. (BRI); Thylogale Track, along Mt Nebo Road between Boombana and Jolly's Lookout, Mt Nebo, Apr 2014, Halling et al. MG605 (BRI); Main Range NP, road from Boonah to Killarney, Mar 2012, Halling 9664 (BRI, NY).

Distribution and habitat: Gymnogaster *boletoides* is currently known primarily from eastern Australia (Queensland, New South Wales, Victoria), with a single collection recorded from Western Australia (Map 1). Fruiting bodies are gregarious or scattered amongst litter in wet sclerophyll forests dominated by Myrtaceae (Eucalyptus L'Hér., Lophostemon Schott. and Corymbia K.D.Hill & L.A.S.Johnson). Details of non-vouchered observations can be found on Mushroomobserver.org (Observations #66652, #163760), and on the Atlas of Living Australia (ALA); http://www.ala.org.au/.

Typification: The holotype of Gymnogaster boletoides consists of two incomplete halves of a fruiting body < 10 mm diameter. This material has been stored in various liquid media for the past 60 years and is now a discoloured, fragile, deficiently prototypical collection which would be best served by augmentation with a fresher, well annotated and photographed dried collection as, at a minimum, a representative specimen which is more interpretive of the protologue description of both the genus and species. We therefore propose the collection Halling 9455 & Fechner (BRI) as a representative collection for the species. Cunningham's Gap is less than 90 km from the original holotype location, Mt. Glorious. The representative specimen has the added benefit of having been sequenced for DNA analyses, with six associated sequences lodged in GenBank: nrLSU: JX889673; tef1: JX889683; 28S: KT990572; tef1: KT990768; RPB1: KT990928: RPB2: KT990406.

boletoides Affinities: *Gymnogaster* undoubtedly approaches Neoboletus thibetanus (Shu R. Wang & Yu Li) Zhu L. Yang, B. Feng & G. Wu from the morphological viewpoint. However, as already pointed out by Wu et al. (2016a), and based on the protologue by Wang et al. (2014) and our own observations, the latter species can be readily delimited by the apical peridial remnants being essentially absent (if a peridiopellis is present, then it shows a hymeniderm structure with inflated terminal elements, 8-15 µm broad, or a cutis with repent filamentous hyphae); presence of a short, shallow reticulum at the stipe apex; much larger basidiospores, $16-19(-20) \times$ (9-)9.5-11(-11.5) µm; shorter and differently shaped cystidia; narrower columella hyphae and stipe trama hyphae (3-7 µm broad), and its occurrence in south-western China in subalpine conifer forests dominated by Abies Mill. and Betula L. Furthermore, recent molecular phylogenetic analyses indicate that N. thibetanus clusters in a distantly related clade focused on the complex of the boletoid, pileate-stipitate species N. luridiformis (Rostk.) Gelardi, Simonini & Vizzini (Wu et al. 2016a).

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Gymnopaxillus nudus Claridge, Trappe & Castellano resembles G. boletoides but diverges from the latter species in having a (sub-) hypogeous habit; absence of stipe, or only rarely with a very rudimentary stipe; unchanging tissues; presence of white basal rhizomorphs; pileus surface (whenever present) consisting of interwoven, collapsed hyphae; hymenium devoid of cystidia, and differently shaped, cyanophilic, boletoid basidiospores which are longer and narrower, being $(10-)11-16(-17.5) \times (4.5-)5.5-6.5(-7.5)$ um (Claridge et al. 2001). Moreover, the genus Gymnopaxillus E. Horak is phylogenetically allied to Austropaxillus Bresinsky & Jarosch in the family Serpulaceae Jarosch & Bresinsky (Claridge et al. 2001; Jarosch 2001; Binder & Hibbett 2006; Watling 2008; Skrede et al. 2011).

Notes: Gymnogaster boletoides is easily circumscribed on account of the following set of unique and distinctive macro- and micromorphological characters: (1) small to tiny basidiomes (1–3.5 cm high \times 0.7–5 cm broad); (2) completely exposed morchellioid, whitish to lemon yellow and eventually olive-brown hymenophore with irregularly shaped chambers; (3) pileus surface reduced to a small brownish-red disc at top; (4) reduced stipe continuing as a columella inside the hymenophore; (5) tissues definitely cyanescent overall on exposure or handling; (6) symmetrical, amygdaliform to citriform, smooth basidiospores; (7) pileipellis а trichoderm consisting of subparallel to moderately interwoven cylindrical hyphae; (8) hymenophoral trama consisting of subparallel and loosely arranged, strongly gelatinized hyphae; (9) fertile caulohymenium; (10) occurrence in wet sclerophyll forests in association with Myrtaceae.

The single, disjunct occurrence of this taxon in Western Australia, as illustrated on the ALA website (**Map 1**), could not be ignored. Whilst the authors did not get the opportunity to examine this specimen, the description accompanying the specimen record was sufficiently detailed enough to convince the authors that the identification was correct. In terms of the list of prescriptive characters

outlined in the preceding paragraph, the following descriptors matched very closely, including spore dimensions: "Pileus crimson red, smooth hymenium greenish yellow, with faint and inconsistent dull greenishblue bruising and some dull reddish bruising when cut; forming a broad mass of convoluted plates with empty locules in between. Stipe columella: solid, extending to apex of fruit body; upper part dull greenish-blue entirely when cut, lower part dull yellowish with red bruising when cut spores dull yellowishbrown in KOH and in Melzers, ellipsoid, some with slightly attenuated apex, thick and smooth-walled, $10.5-13.0(-13.3) \times (6.3-)6.9-$ 8.4 µm"

From a morphological perspective *Gymnogaster boletoides* may be considered an intermediate transition of an aboveground bolete to a truffle-like form in which a vestigial pileus and a reduced stipe combined with an epigeal growth are still retained. The similarities to boletes are quite evident when considering the bluing reaction and the mycorrhizal habit. According to present knowledge, *G. boletoides* seems to have an ecologically restricted distribution range and appears to be endemic to Australia.

Etymology: the specific epithet is derived from the Latin *boletus* (mushroom) and the suffix -ides (resembling), referring to the similarity of its characters to species in the genus *Boletus s.l.*

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Map 1. Distribution of *Gymnogaster boletoides* ○. ★ represents the collection (PERTH 07628005) from Dorrigo National Park, NSW which lacks a georeference and is therefore absent from the ALA map for this taxon. Source: Atlas of Living Australia occurrence; download at http://www.ala.org.au. Accessed 2 December, 2016.