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# Evolutionary relationships of *Heimioporus* and *Boletellus* (Boletales), with an emphasis on Australian taxa including new species and new combinations in *Aureoboletus, Hemileccinum* and *Xerocomus*

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**Abstract.** Boletellus and Heimioporus, two genera of Boletaceae with ornamented basidiospores, are shown to be distinct genera on the basis of phylogenetic analyses of nuclear ribosomal large-subunit and translation elongation-factor  $1\alpha$  DNA sequences. Comparison of spore ornamentation type – longitudinally ribbed in Boletellus v. punctate, alveolate-reticulate in Heimioporus – are further evidence for distinction. Analyses of multiple accessions from the Americas, Asia and Australia support the monophyly of Heimioporus and a 'core Boletellus' clade, containing the type species, B. ananas (M.A.Curt.) Murrill, and approximately seven additional species. Tests of alternative phylogenetic topologies could not reject monophyly of a more inclusive group containing the core Boletellus clade and six other species. Heimioporus australis Fechner & Halling, H. cooloolae Fechner & Halling, Boletellus deceptivus Halling & Fechner, B. reminiscens Halling & Fechner and B. sinapipes Fechner, K.Syme, R.Rob. & Halling are described as new species. Phylogenetic analyses also support the following new combinations: Aureoboletus projectellus (Murrill) Halling, A. mirabilis (Murrill) Halling, Hemileccinum subglabripes (Peck) Halling and the new name, Xerocomus tenax Nuhn & Halling.

Additional keywords: biogeography, boletes, Boletineae, phylogeny, ribosomal DNA, translation elongation-factor 1a.

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# Introduction

*Boletellus*, as originally conceived by Murrill (1909), included one species from the south-eastern USA, *B. ananas* (M.A.Curt.) Murrill, which was based on *Boletus ananas* (Curtis 1848) from South Carolina. Singer's (1986) concept of the genus included 33 species in seven sections, with either smooth or variously ornamented basidiospores. Other features, including moisture content, scaliness of the pileus, or both, or the degree of stipe ornamentation, were used to define sections. Recent phylogenetic studies by Nuhn *et al.* (2013) and Wu *et al.* (2014) suggested that such characters may not delimit clades in Boletales. However, this apparent failure of morphological characters to reflect underlying phylogeny may instead be a consequence of loose application of generic concepts; once concept drift is corrected (Halling and Ortiz-Santana 2009), the morphological characters of described species can have a bearing on phylogenetic inference. *Heimioporus* was proposed by Horak (2004) to replace *Heimiella* Boedijn non-Lohmann (1913). The original species included by Boedijn (1951) was *Boletus retisporus*, described by Patouillard and Baker (1918) from specimens gathered in Singapore. In the recent nomenclatural overview by Horak (2004), 16 species were placed in two subgenera distinguished by the type of spore ornamentation (alveolate v. pitted). However, one smooth-spored taxon was admitted (*Boletus mirabilis* (Murrill) Murrill).

With the appearance of Corner's (1972) book on Malaysian boletes, *Heimioporus* E.Horak (as *Heimiella* Boedijn) received recognition as a distinct genus with six species, based primarily on spore morphology (the lack of an adaxial patch distinguished it from *Strobilomyces* Berk.) and tube trama type (truly boletoid). In contrast, Singer (1945, 1986) classified the group as *Boletellus* sect. *Retispori* Singer. Interestingly, Corner (1972) relegated

*Boletellus* to a subgenus of *Boletus* L. *s.l.* by virtue of extreme variation in spore shape and ornamentation, basidiome stature and tube trama type. Watling and Gregory (1986) initiated a study on boletes of the Cooloola Sandmass in south-eastern Queensland, the first installment of which covered those taxa with ornamented spores (*Austroboletus* (Corner) Wolfe, *Heimiella, Boletellus*). This latter publication has provided a starting point from which we have assembled more material with detailed observations.

We studied multiple accessions morphologically assignable to *Boletellus* and *Heimioporus* from the Americas, Australia, Japan, South-east Asia and Zambia, to investigate the limits of *Boletellus v. Heimioporus*, with a particular emphasis on taxa occurring in Australasia. The preliminary analyses of Osmundson (2009), Nuhn *et al.* (2013) and Wu *et al.* (2014) indicated that additional material and loci would be appropriate in our dataset.

# Methods and materials

# Morphological datasets

Macromorphological data were obtained from fresh specimens. General colour terms are approximations, and the colour codes (e.g. 7D8) are page, column and grid designations from Kornerup and Wanscher (1983). Microscopic structures were observed with an Olympus BHS compound microscope equipped with Nomarski differential interference contrast (DIC) optics, and measured from dried material revived in 3% KOH. The letter abbreviation Q refers to the mean length : width ratio measured from *n* basidiospores, and *x* refers to the mean length  $\times$  mean width. Scanning electron micrographs of the spores were captured digitally from a Hitachi S-2700 scanning electron microscope operating at 20 kV. Hymenophoral fragments were removed from dried basidiomata, mounted directly on aluminum stubs with carbon adhesive tabs, and coated with 10 nm of gold by using a Hummer II sputter coater. Herbarium codes (Thiers 2014) are cited for all collections from which morphological features were examined.

# Sampling and molecular datasets

Taxon sampling focused on species that have been placed in or proposed to be closely related to *Boletellus*, or that have longitudinally ornamented spores. The sample of taxa was informed by prior molecular studies (Binder and Hibbett 2006; Halling *et al.* 2012*a*, 2012*b*; Nuhn *et al.* 2013), as well as preliminary maximum likelihood (ML) analyses (not shown) that included as many genera of Boletaceae as possible. Genera or species that are not pertinent to *Boletellus* were removed, e.g. all members of the Chromapes group as reported by Halling *et al.* (2012*b*). Newly generated sequences were submitted to GenBank (Table 1); sequence alignments and phylogenies were submitted to TreeBase (#16346, http://purl.org/phylo/treebase/phylows/ study/TB2:S16346, accessed September 2014).

# DNA extraction, polymerase chain reaction (PCR) amplification, sequencing and alignment

Genomic DNA was extracted using the phenol–chloroform method described by Lee and Taylor (1990), with modifications (Halling *et al.* 2012*a*, 2012*b*). Purified gDNA was suspended in 50 or 100  $\mu$ L HPLC H<sub>2</sub>O. PCR amplification of nuclear large-subunit

rRNA (*nuc-lsu*) and translation elongation-factor  $1-\alpha$  (*tef1*) genes was performed with respective primer pairs LR0R/LR5 and TEF1-983F/TEF1-2218R. PCR protocols were as previously established (Halling *et al.* 2012*a*, 2012*b*; Nuhn *et al.* 2013). PCR products were purified using a series of ethanol-centrifuge washes and sequenced by using the BigDye 3.1 terminator sequencing kit (Applied Biosystems, Foster City, California, USA). In addition to the primers used in PCR reactions, two internal primers were used for both genes: LR3R and LR3 for *nuc-lsu* and TEF1-1577F and TEF1-1567R for *tef1*. Raw sequence files were edited and assembled into contigs using Geneious R7 v.7.1.2 (Biomatters, Auckland, New Zealand; https://www. geneious.com, accessed March 2014).

Curated sequences were aligned using MAFFT v7.017, as implemented in Geneious R7 (Katoh *et al.* 2002). The G-INS-i settings were used for *nuc-lsu* and the E-INS-i settings were used for *tef1*. Thirty-nine publicly available DNA-directed RNA polymerase Subunit 1 (*rpb1*) sequences were included in the analyses and were aligned using the same methods as for *tef1* (Nuhn *et al.* 2013).

# Phylogenetic analyses

Individual gene alignments were analysed using the RAxML Blackbox server (http://embnet.vital-it.ch/raxml-bb/, accessed May 2014; Stamatakis *et al.* 2008). The resulting trees were manually compared for conflicts with bootstrap support (BS) values greater than 70% (trees not shown). No conflict was observed. Therefore, a supermatrix, including 130 *nuc-lsu*, 111 *tef1* and 37 *rpb1* sequences, was assembled and analysed.

Maximum likelihood and Bayesian analyses of the supermatrix used the CIPRES Science Gateway (Miller *et al.* 2010). For all phylogenetic analyses, the supermatrix was partitioned by locus, and members of the Paxillaceae were used as the outgroup. The tree topology and BS values were estimated using ML, as implemented by RAxML HPC2 on XSEDE (Stamatakis 2014), with the following parameters: GTRGAMMA model of molecular evolution, 1000 rapid bootstrap replicates, and a simultaneous best-scoring ML tree search.

Bayesian posterior probability (PP) values were estimated with MrBayes v3.2.2 (Huelsenbeck and Bollback 2001; Ronquist *et al.* 2012). The supermatrix was analysed with two runs of four Markov chain Monte Carlo (MCMC) chains using the GTR model, gamma distribution rates, and 30 million generations. Sampling occurred every 100 generations and a burn-in fraction of 0.3 was used. Convergence was checked using Tracer (Rambaut *et al.* 2014) and AWTY (Wilgenbusch *et al.* 2004).

# Alternative-topology testing

The optimal topology recovered in ML analyses suggests that *Heimioporus* is nested within a weakly supported paraphyletic grade that contains most of the species referred to *Boletellus* (Fig. 1). An alternative topology that forces monophyly of *Boletellus* (excluding only *Boletellus russellii* (Frost) E.-J.Gilbert and *B. shichianus* (Teng & L.Ling) Teng), with *Heimioporus* as the sister group, was evaluated. Topology testing was performed using the following three methods: Bayesian analysis (Ronquist *et al.* 2012), the Shimodaira–Hasegawa (SH) test (Stamatakis 2014)

	Table 1. Vou	icher information and GenBank accession numb	bers (new submissio	ns in bold) for the specim	ens studied		
Species	Isolate ID	Location	Date	Collector identifier	nuc-lsu	tefl	rpbI
Afroboletus luteolus	00-436	Zambia		D. Arora	KF030238	KF030397	KF030392
Aureoboletus gentilis	Pugl	Maindreieck, Germany	3 Oct. 1995	J. Schreiner	DQ534635	KF030399	I
Aureoboletus innixus	MB 03-104	Lincoln, Massachusetts, USA	3 Aug. 2014	M. Binder	KF030239	KF030400	I
Aureoboletus mirabilis	REH8717	Humboldt County, California	15 Nov. 2005	R.E. Halling	KF030299	I	Ι
Aureoboletus mirabilis	REH9765	Mendocino, California, USA	10 Dec. 2012	R.E. Halling	KP327661	KP327709	I
Aureoboletus moravicus	Xle1	Maindreieck, Germany	19 Sep. 1998	J. Schreiner	DQ534641	KF030403	Ι
Aureoboletus projectellus	NYBG13392	North Collins, New York, USA	20 Sep. 1987	E. Both	KP327622	KP327675	I
Aureoboletus projectellus	NYBG13393	North Collins, New York, USA	23 Aug. 1986	E. Both	KP327623	KP327676	Ι
Aureoboletus projectellus	AFTOL-713;	Cape Cod, Massachusetts, USA	14 Sep. 2003	M. Binder	AY684158	AY879116	AY662660
	MB03-118						
Aureoboletus roxanae	DS626-07	Chestnut Ridge Park, New York, USA	25 Oct. 2006	E. Both	KF030311	KF030402	KF030381
Aureoboletus thibetanus	AFTOL-450	Kunming, Yunnan, China	I	ZL. Yang	AY700189	DQ029199	DQ435800
Boletellus ananas	ARB1223	Crooked River, Georgia, USA	19 Oct. 2012	A.R. Bessette	KP327618	KP327671	I
Boletellus ananas	REH7763	Alajuela Province, Costa Rica	27 June 1998	R.E. Halling	KP327626	KP327679	I
Boletellus ananas	REH7924	Cartago Province, Costa Rica	24 June 2000	R.E. Halling	KP327627	KP327680	I
Boletellus ananas	REH8548	Belize District, Belize	14 Oct. 2003	R.E. Halling	KP327628	KP327681	I
Boletellus ananas	REH8613	Puntarenas Province, Costa Rica	11 June 2004	R.E. Halling	KP327629	KP327682	I
Boletellus ananas?	REH8788	Chiang Mai Province, Thailand	4 June 2006	R.E. Halling	KP327636	KP327689	Ι
Boletellus sp.	00-335	Zambia	2000	D. Arora	KF030330	I	Ι
Boletellus ananiceps	REH9484	Fraser Island, Queensland, Australia	17 May 2011	R.E. Halling	KP327655	KP327705	Ι
Boletellus ananiceps	REH9690	Cooloola, Queensland, Australia	12 Mar. 2012	R.E. Halling	KP327660	KP327708	Ι
Boletellus ananiceps	Syme	Denmark, Western Australia, Australia	25 May 2011	K. Syme	KP327667	1	I
Boletellus ananiceps	3794	Victoria, Australia	7 May 2010	N.H. Sinnot	KP327666	KP327712	I
<b>Boletellus</b> badiovinosus	REH8923	Davies Creek, Queensland, Australia	14 Mar. 2007	R.E. Halling	KP327640	KP327693	I
Boletellus betula	DD9852	Orange County, North Carolina, USA	28 Feb. 2013	D. Drehmel	AY612797	1	I
Boletellus betula	134/96	Massachusetts, USA	31 Aug. 1996	N. Arnold	AF050642	I	I
Boletellus chrysenteroides	3838	Towy Park, North Collins, New York, USA	10 Oct. 1995	E. Both	KF030312	KF030432	KF030383
Boletellus chrysenteroides	54/97	Massachusetts, USA	21 Aug. 1997	N. Arnold, H. Besl	DQ534634	I	Ι
Boletellus chrysenteroides	REH9015	Pennsylvania, USA	10 Aug. 2008	R.E. Halling	KP327645	I	I
Boletellus deceptivus	REH8937	Mount Baldy, Atherton State Forest,	21 Mar. 2007	R.E. Halling	KP327641	I	Ι
		Queensland, Australia					
Boletellus deceptivus	REH9017	Fraser Island, Queensland, Australia	7 Feb. 2009	R.E. Halling	KP327646	KP327697	Ι
Boletellus deceptivus	REH9634	Mount Baldy, Atherton State Forest,	21 Feb. 2012	R.E. Halling	KP327619	KP327672	Ι
		Queensland, Australia					
Boletellus deceptivus	REH9653	Springbrook National Park, Oueensland. Australia	2 Mar. 2012	R.E. Halling	KP327658		I
Roletellus decentivus	RFH9786	Oueen Mary Falls Oneensland Australia	14 Feb 2013	R F Halling	KP327663		I
Boletellus dissiliens	REH8943	Mount Baldy. Atherton State Forest.	21 Mar. 2007	R.E. Halling	KP327642	KP327694	I
		Queensland, Australia		0			
<b>Boletellus dissiliens</b>	REH9435	Fraser Island, Queensland, Australia	18 Feb. 2011	R.E. Halling	JX889674	JX889684	Ι
<b>Boletellus</b> dissiliens	REH9466	Fraser Island, Queensland, Australia	6 Mar. 2011	R.E. Halling	KP327654		Ι
<b>Boletellus dissiliens</b>	REH9624	Davies Creek, Queensland, Australia	15 Feb. 2012	R.E. Halling	KP327657	KP327706	Ι
<b>Boletellus dissiliens</b>	REH9688	Cooloola, Queensland, Australia	11 Mar. 2012	R.E. Halling	KP327659	KP327707	Ι
<b>Boletellus dissiliens</b>	REH9768	Vicinity Canungra, Queensland, Australia	12 Feb. 2013	R.E. Halling	KP327662		Ι
Boletellus elatus	TMI22101	Tottori, Japan	6 Sep. 1996	E. Nagasawa	KP327620	KP327673	I

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Species	Isolate ID	Location	Date	Collector identifier	nuc-lsu	tef1	rpbI
Boletellus elatus	06-204NR	Tottori, Japan	14 Sep. 2006	E. Nagasawa	KP327621	KP327674	Ι
Boletellus emodensis	REH8734	Davies Creek, Oueensland, Australia	4 Feb. 2006	R.E. Halling	KP327631	KP327684	Ι
Boletellus emodensis	REH8742	Davies Creek, Oueensland, Australia	9 Feb. 2006	R.E. Halling	KP327633	KP327686	I
Roletellus emodensis	REH8808	Chiano Mai Province Thailand	11 June 2006	R F. Hallino	KP327637	KP327690	I
Roletellus emodensis	REH8904	Davies Creek Oneensland Australia	11 Mar 2007	R F Hallino	KP327638	KP327691	I
Rolatallus obsenvacionais	DEH8741	Deluma Oneancland Australia	6 Eab 2006	D F Halling	1237627	289762J	
Rolatellus obscurenceireus	REH8787	Daluma Queensland Australia	0100.2000 25 Eab 2006	R F Halling	KP377634	KP337687	I
	DETRONE	Daries Cuells Automatica Automatica	11 Mar 2007	D E II.IIi		100/7C IXI	
Boletellus obscurecoccineus	CU68H3N	Davies Creek, Queensland, Australia	11 Mar. 2007	K.E. Halling	NF32/039	NF32/092	I
Boletellus obscurecoccineus	REH9034	Fraser Island, Queensland, Australia	8 Feb. 2009	R.E. Halling	KP327648	KP327699	I
Boletellus obscurecoccineus	REH9038	Fraser Island, Queensland, Australia	9 Feb. 2009	R.E. Halling	KP327649	KP327700	I
<b>Boletellus</b> obscurecoccineus	REH9061	Fraser Island, Queensland, Australia	12 Feb. 2009	R.E. Halling	KP327650	KP327701	Ι
Boletellus reminiscens	REH9284	Fraser Island, Queensland, Australia	27 Mar. 2010	R.E. Halling	KP327651	KP327702	I
Boletellus russellii	DPL6698	Texas	22 June 2005	D. Lewis	KF030325	I	I
Boletellus russellii	MEN11-031	Mount Wachusetts, Massachusetts, USA	3 July 2005	M.E. Nuhn	KP327625	KP327678	I
Boletellus russellü	73887		•		AF050651	I	I
Boletellus shichianus	AFTOL-532	Yunnan. China	25 June 2005	ZL. Wang	AY647211	DO408145	I
Boletellus sinapipes	REH9018	Fraser Island, Oueensland, Australia	7 Feb. 2009	R.E. Halling	KP327647	KP327698	I
Boletellus sinapipes	REH9408	Fraser Island, Oueensland, Australia	15 Feb. 2011	R.E. Halling	KP327653	KP327704	Ι
Boletellus sinapipes	REH9592	Denmark. Western Australia. Australia	17 July 2011	R.E. Halling	KP327656		I
Boletellus sinapipes	25511	Denmark. Western Australia. Australia	25 May 2011	K. Svme	KP327668		I
Boletellus sinoeri	VB4530	Veracruz, Mexico	5 Sen 2012	V M Bandala	KP327669	KP327713	I
Roletellus singeri	VB4460	Veracruiz Mexico	27 Oct 2009	V M Bandala	KP327670		I
Dolotic abuintibuiltie	1500	Come Con Died Elevide 110 A	1 1 2001 2000	F Doth	VED20200	VE020401	V EU 2 0 2 0 0
bolems abruptionus	4000	Cape San Blas, Florida, USA	CUU2 .14A 1	E. Boun	NFU3U3U2	NFU30401	NFU2U288
Boletus amygdalinus	112605ba	Mendocino, California, USA	26 Nov. 2005	B. Neill	JQ326996	JQ327024	KF030360
Boletus aurantioruber	MEN11-0MB	Cape Cod, Massachusetts, USA		M. Binder	KF030342	I	I
Boletus bicolor var. bicolor	MB 07-001	Chestnut Ridge Park, New York, USA	28 July 1995	E. Both	KF030370	KF030405	I
Boletus bicolor var. borealis	2858	Erie County, New York, USA		E. Both	JQ326998	JQ327021	I
Boletus calopus	Bc1	Bavaria, Germany	7 Sep. 1994	N. Arnold	AF456833	JQ327019	I
Boletus carminipes	MB 06-061	Erie County, New York, USA	4 Aug. 2006	M. Binder, E. Both	JQ327001	JQ327022	KF030363
Boletus dupainii	JAM 0607	Butner, New York, USA	8 Mar. 2000	E. Both	KF030251	KF030413	KF030361
Boletus edulis	Be1	Bavaria, Germany	14 Sep. 1994	M. Binder	AF050643	JQ327018	I
Boletus firmus	MB 06-060	Chestnut Ridge Park, New York, USA	3 Aug. 2006	M. Binder	KF030278	KF030408	KF030368
Boletus inedulis	MB 06-044	Erie County, New York, USA	3 Aug. 2006	M. Binder, E. Both	JQ327013	JQ327020	KF030362
<b>Boletus luridiformis</b>	AT2001087	Berkshire, England, UK		A.F.S. Taylor	JQ326995	JQ327023	I
Boletus morrisii	8206	Concord, Massachusetts, USA	2 Aug. 2006	B. Neill	KF030433	Ι	Ι
Boletus peckii	3959	Erie County, New York, USA	4 Aug. 1995	A.R. Clark, E. Both	JQ326999	JQ327026	I
Boletus pseudosensibilis	DS615-07	Chestnut Ridge Park, New York, USA	7 July 1995	E. Both	KF030257	KF030407	I
Boletus pulchriceps	DS 4514	Chiricahua Mountains., Arizona, USA	1 Aug. 91		KF030261	KF030409	KF030376
Boletus pulverulentus	9096	West Newton, Massachusetts, USA	9 June 2006	Bill Neill	KF030313	KF030418	KF030364
Boletus regineus	REH8721	Redwood National Park, Humboldt County,	17 Nov. 2005	R.E. Halling	KF030339	KF030426	KF030377
		California, USA					
Boletus rhodosanguineus	4252	Chestnut Ridge Park, New York, USA	12 July 1998	E. Both	KF030252	KF030412	I
Boletus roseopurpureus	MB 06-059	Chestnut Ridge Park, New York, USA	30 July 2006	A. Taylor, M. Binder	KF030262	KF030410	KF030372
Boletus rufomaculatus	4414	Chestnut Ridge Park, New York, USA	6 Aug. 1997	E. Both	KF030248	KF030406	KF030369
Boletus subalpinus	27882			J. Trappe	KF030430	KF030427	KF030379
Boletus variipes var. fagicola	4249	Cheboygan County, Michigan, USA	10 Aug. 1968	A. H. Smith	JQ327014	JQ327017	KF030378

 Table 1.
 (continued)

Borofutus dhakanus	HKAS73785	Bangladesh	7 June 2011	Md. Iqbal Hosen	JQ928615	JQ928577	JQ928585
Buchwaldoboletus lignicola	Pull	Maindreieck, Germany	9 Sep. 1995	J. Schreiner	JQ326997	JQ327040	I
Butyriboletus appendiculatus	Bapl	Bavaria, Germany	10 Aug. 1995	J. Schreiner	AF456837	JQ327025	KF030359
Butyriboletus autumniregius	11265	Mendocino County, California, USA	26 Nov. 2005	Bill Neill	KF030267	KF030411	I
Chalciporus piperatus	MB 04-001	Rutland, Massachusetts, USA	28 Sep. 2004	M. Binder	DQ534648	GU187690	GU187453
Chalciporus pseudorubinellus	4302	Deer Meadows, Tulet, New York, USA	14 Sep. 1998	E. Both	KF030284	KF030441	I
Chalciporus rubinus	DS4640-3	Germany		J. Schreiner	KF030283	KF030440	I
Fistulinella prunicolor	REH9502	Fraser Island, Queensland, Australia	18 May 2011	R.E. Halling	J889648	JX889690	I
Fistulinella viscida	238			I	AF456826	I	I
Gyrodon lividus	GII	Bavaria, Germany	11 Aug. 1995	H. Besl, W. Helfer	AF098378	GU187701	GU187461
Heimioporus australis	REH9288	Fraser Island, Queensland, Australia	28 Mar. 2010	R.E. Halling	KP327652	KP327703	Ι
Heimioporus cooloolae	REH9817	Cooloola, Queensland, Australia	22 Feb. 2013	R.E. Halling	KP327664	KP327710	I
Heimioporus cooloolae	REH9852	Cooloola, Queensland, Australia	28 Feb. 2013	R.E. Halling	KP327665	KP327711	I
Heimioporus fruticicola	REH8958	Davies Creek National Park,	22 Mar. 2007	R.E. Halling	KP327643	KP327695	Ι
2		Queensland, Australia		)			
Heimioporus fruticicola	REH8962	Kuranda, Queensland, Australia	23 Mar. 2007	R.E. Halling	KP327644	KP327696	I
Heimioporus ivoryi	REH8620	Puntarenas Province, Costa Rica	12 June 2004	R.E. Halling	KP327630	KP327683	I
Heimioporus mandarinus	FRIM4636	Sungai Kejar, Malaysia	19 June 2007	)	KP327624	KP327677	I
Heimioporus mandarinus	REH8785	Chian Mai Province, Thailand	3 June 2006	R.E. Halling	KP327635	KP327688	Ι
Heimioporus retisporus	REH8074	Java, Indonesia	16 Jan. 2001	R.E. Halling	KP327617	Ι	Ι
Heimioporus retisporus	MS6			)	AF050650	Ι	Ι
Hemileccinum impolitum	Bim1	Bavaria. Germany	21 Sen. 1995	J. Schreiner	AF139715	JO327034	KF030375
Hemileccinum subolabrines	72206	Jefferson. New Hampshire, USA	22 July 2006	B. Neill	KF030303	KF030404	KF030374
Leccinellum corsicum	Buf 4507				KF030347	KF030435	KF030389
Leccinellum crocinodium	930809/1	France	9 Aug. 1993	G. Lannov	AF139694	KF030434	I
Loccinum alballum	MR 06-040	Frie County New Vorb 11SA	30 Inly 2006	M Binder	10327007	10327038	
Leccmum arbenum	I al	Austria	14 Son 1005	M. Dinder	A E1 20705	00170201	I
Leccinum scaorum	LSI	Ausuria	14 Sep. 1995	M. Binder	AF139/00	95U/25U	I
Paragyrodon sphaerosporus	MB 06-066	lowa City, lowa, USA	22 Sep. 2006	M. Binder	GU187593	GU187737	I
Paxillus filamentosus	Pfl	Bavaria, Germany	21 Aug. 1995	L. Krieglsteiner	AF167680	GU187736	I
Paxillus obscurisporus	Pol	Bavaria, Germany	21 Aug. 1998	Ch. Hahn	AY177256	KF030442	Ι
Paxillus vernalis	Pv2	Canada	31 Aug. 1997	T. Lohmeyer	AY645059	DQ457629	I
Phylloporus pelletieri	Pp1	Bavaria, Germany	9 Sep. 1995	M. Kronfeldner	AF456818	JQ327036	$\rm KF030390$
Pseudoboletus parasiticus	Xpa1	Bavaria, Germany	9 July 1995	A. Bresinksy	AF050646	KF030443	KF030394
Retiboletus griseus	202/97	Massachusetts, USA	30 Aug. 1997	N. Arnold, W. Helfer,	AF456834	KF030414	KF030373
				W. Steglich			
Spongiforma thailandica	DED 7873	Thailand	7 July 2005	D.E. Desjardin	EU685108	KF030436	KF030387
Strobilomyces floccopus	Sfl	Bavaria, Germany	12 Aug. 1995	J. Enzmann	DQ534626	JQ327037	AY858963
Strobilomyces sp.	REH8514	Cayo District, Belize	3 Oct. 2003	R.E. Halling	EU685109	KF030398	I
Tylopilus badiceps	78206	Holliston, Massachusetts, USA	28 July 2006		KF030335	KF030429	I
Tylopilus felleus	AT2001011	Stadsskogen, Uppsala, Sweden	17 Sep. 2001	A.F.S. Taylor	JQ326993	JQ327015	$\rm KF030380$
Tylopilus ferrugineus	MB 06-053	Erie County, New York, USA	3 Aug. 2006	E. Both	JO326994	JO327016	Ι
Xerocomellus chrysenteron	Xch1	Bavaria, Germany	8 Aug. 1995	M. Binder	AF050647	KF030415	KF030365
Xerocomellus cisalpinus	AT2005034	Uppsala, Uppland, Sweden	30 July 2005	A.F.S. Tavlor	KF030354	KF030417	KF030367
Xerocomellus zelleri	REH8724	Redwood National Park. Humboldt	18 Nov. 2005	R.E. Halling	KF030271	KF030416	KF030366
		County, California, USA		0			
Xerocomus perplexus	MB 00-005	Rutland, Massachusetts, USA	5 Aug. 2000	M. Binder	JQ003702	KF030438	I
Xerocomus subtomentosus	Xs1	Bavaria, Germany	10 Aug. 1995	J. Enzmann,	AF139716	JQ327035	KF030391
Voucoomine tonav	DEH6871	Decon Night USA	אןייז 2005	A. Bresinsky D E Halling	VE030370	VE020427	
Aerocomus tenax	KEH00/1	Bronx, New YOIK, USA	CERT VINL C2	K.E. riaming	Nrusus V	Nr U2U42 /	I



and the 'approximately unbiased' (AU) test (Shimodaira and Hasegawa 2001).

For the Bayesian analysis, two models were tested, one that forces the monophyly of *Boletellus* species in the indicated clade (see Fig. 1, inset), and another that forces the absence of this *Boletellus* clade. Unlike the analyses using the SH and AU tests, a sister-group relationship between *Boletellus* and *Heimioporus* was not enforced. Two MCMC analyses were performed, one for each model, using the same settings (e.g. number of generations, sampling) as described previously. Additionally, the arithmetic and harmonic mean for each MCMC analysis was calculated.

For the SH and AU tests, a constraint tree was manually generated from the ML topology by swapping branches to force the sister relationship between *Boletellus* and *Heimioporus*, and collapsing all other ingroup nodes to a polytomy. For the SH test, 100 trees were generated using RAxML under the settings described previously, except that each inference was independent and constrained to have the sister-clade topology, and bootstrapping and ML optimisation were not performed (Stamatakis 2014). The 100 trees were then compared to the unconstrained ML tree using the default SH test settings (Stamatakis 2014).

The AU test requires per site log-likelihood values, which were calculated using RAxML, under the GTRGAMMA model for all 101 trees (Shimodaira and Hasegawa 2001; Stamatakis 2014). Consel version 0.2 (http://en.shimolab.com/consel, accessed May 2014) was used to perform the AU test for all 101 trees, with default settings, and the resulting *P*-value for each tree was recorded.

# Results

# Phylogenetic analyses

The concatenated alignment contains ~49% gaps or undetermined characters. The RAxML analysis reported 2354 unique alignment patterns in the concatenated alignment. A sample of 380 682 trees was used to estimate Bayesian PP. The Paxillaceae and Boletaceae were strongly supported (Fig. 1). As in prior analyses (Binder and Hibbett 2006; Halling *et al.* 2012*a*, 2012*b*; Nuhn *et al.* 2013), clades that largely correspond to genera are often supported, but backbone nodes between these clades and the early most diverging clades are generally weakly supported. Clades of non-target species that are strongly supported include *Boletus sensu stricto*, *Tylopilus sensu stricto*, and the clade containing *Xerocomus sensu stricto* and *Phylloporus pelletieri* (Lév.) Quél. (Fig. 1).

Seventeen strongly supported clades containing only *Boletellus* species (including nested clades) were recovered, as well as a clade with 1.0 PP that contains *Aureoboletus mirabilis* 

(Murrill) Halling and *A. projectellus* (Murrill) Halling (which were formerly placed in *Boletellus sensu* Singer 1945), *Boletellus russellii, B. shichianus, A. gentilis* (Quél.) Pouzar (the type of *Aureoboletus*) and others (Fig. 1). The most inclusive strongly supported group of *Boletellus* species (including the type species, *B. ananas*) is labelled as the 'core *Boletellus*' clade (Fig. 1). The core *Boletellus* clade is nested within a paraphyletic assemblage including three lineages that contain (1) *Boletellus chrysenteroides* (Snell) Snell and *B. badiovinosus* E.Horak, (2) *B. reminiscens* and (3) *B. sinapipes*. Monophyly of the core *Boletellus* clade plus *B. chrysenteroides*, *B. badiovinosus*, *B. reminiscens* and *B. sinapipes* is weakly supported.

A clade containing six species of *Heimioporus* (including the type species, *H. retisporus* (Pat. & C.F.Baker) E.Horak) is strongly supported (BS = 99, PP = 1.0), with *Boletus morrisii* Peck as its sister group (PP = 0.95). The *Heimioporus–B. morrisii* clade is weakly supported as the sister group of the group that contains the core *Boletellus* clade (Fig. 1).

Two strongly supported lineages containing two isolates each of *Boletellus betula* (Schwein.) E.-J.Gilbert and *Boletellus elatus* Nagasawa are weakly supported as sister taxa, and the *B. betula–B. elatus* group is weakly supported as the sister group of the clade containing *Heimioporus* and the core *Boletellus* clade (Fig. 1).

# Alternative-topology testing

The Bayesian MCMC analysis that forced monophyly of Boletellus species (excluding B. russellii and B. shichianus) returned a harmonic mean average of both runs of -45 935.69, whereas the analysis that forced non-monophyly of Boletellus species had a harmonic mean average of -45 932.64 (a difference of -0.03172 log-likelihood units). In the SH test, the unconstrained optimised ML tree had a score of -49 784.032084, whereas all the constrained trees had higher likelihoods than did the unconstrained topology, with an average score of -49 743.86979, and none of the constrained trees could be rejected. P-values for all constrained trees were greater then 0.05. None of the constrained topologies could be rejected using the AU test (P = 0.361 - 0.756). Collectively, these results suggest that monophyly of the core Boletellus clade plus B. chrysenteroides, B. badiovinosus, B. betula, B. elatus, B. reminiscens and B. sinapipes cannot be rejected.

# Biogeography

*Boletellus* and related taxa have complex biogeographic relationships. The core *Boletellus* clade contains taxa from North and Central America (USA, Mexico, Belize and Costa

**Fig. 1.** Phylogenetic relationships between species of the genera *Boletellus* and *Heimioporus* within the Boletaceae. The topology was inferred from the nucLSU, tef 1- $\alpha$ , and rpb2 using maximum likelihood (ML) methods with likelihood optimization of the final topology using RAxML. Bootstrap values  $\geq$ 70% are reported, as are posterior probabilities (PP), generated using Mr Bayes, if PP $\geq$ 0.95 but <1. Bold branches represent PP values  $\geq$ 0.95. Closed circles indicate a clade that has an alternative topology that cannot be rejected based on an AU test, as implemented in Consel, and the SH test as implemented in RAxML. The topology recovered in the ML analysis results in a polyphyletic grouping of *Boletellus* species with *Heimioporus* species. The alternative topology (inset) places *Boletellus* and *Heimioporus* as sister clades; and clades that have different topologies are indicated. Names in bold represent the taxa of interest. Locations of Australian exemplars are abbreviated: QLD (Queensland), VIC (Victoria), WA (Western Australia). The polyphyletic nature of *Caloboletus* is indicated with a broken line uniting *Boletus calopus*, *B. inedulis*, *B. firmus*, and *B. peckii*, but not *B. rhodosanguineus*, *B. dupainii*, *B. amygdalinus*, and *B. luridiformis*.

Rica), Africa (Zambia), Thailand and Australia. Similarly, the *Heimioporus* clade contains taxa from Australia, Costa Rica, Java, Malaysia and Thailand. *B. betula*, collected in the USA, is the sister group to *B. elatus*, which was collected in Japan. A single Thailand collection of *B. emodensis* (Berk.) Singer is nested within a clade of Australian specimens. A regional geographical pattern of a north–south split was recovered for *B. obscurecoccineus* (Höhn.) Singer specimens collected in Queensland, Australia.

# Taxonomy

**Boletellus ananas** (M.A.Curt.) Murrill, *Mycologia* 1:10 (1909) (Figs 2A, 3A, 4A)

- $\equiv$  Boletus ananas M.A. Curt., Amer. J. Sci. Arts 6: 351 (1848).
- =? Boletus coccineus Fr., Epic. Myc. 423 (1838), nom. illeg., non Boletus coccineus Bull., Hist. Champ. France 364 (1791).
- $\equiv$  Strobilomyces coccineus Sacc., Syll. Fungorum 6: 50 (1888).
- ≡ Boletellus coccineus (Sacc.) Singer in Singer, García & Gómez, Beih. Nova Hedwigia 105: 6 (1992).
- = Boletus isabellinus Peck, Bull. Torrey Bot. Club 24: 146 (1897), nom. illeg., non Schweintiz, Schr. naturf. Ges. Leipzig 1:96 (1822).

Mycobank number: MB 100683.

This iconic bolete, originally described from the south-eastern USA (South Carolina, Curtis 1848), is the type species of the genus and has been described, illustrated and characterised in more detail in several publications on North American boletes (Coker and Beers 1943; Singer 1945; Thiers 1963; Smith and Thiers 1971; Bessette et al. 2000). The species has also been collected, described and illustrated from Mexico, Central America and Colombia (Singer 1970; Singer et al. 1983, 1992; Halling and Mueller 2005; Ortiz-Santana et al. 2007). It was originally diagnosed as parasitic on pine trees (Murrill 1909), but this has since been discounted (Singer 1945; Thiers 1963). Without justification, Corner (1972) went so far as to suggest that B. ananas, B. emodensis and B. dissiliens (Corner) Pegler & T.W. K. Young appeared not to be mycorrhizal. On the basis of our (REH, NF) observations of the latter two species in Queensland (including B. deceptivus and B. ananiceps (Berk.) Singer), these taxa can appear (but not always) attached to bark at the base of living trees or coming from logs on the ground. Thiers (1963) noted that this type of habit is '... the result of the activity of mycelium which has migrated from the soil to the outer bark...' (p. 37).

Corner (1972) summarised the reports of *B. ananas* known to him, and Horak (2011) later added information for the USA, South-east Asia and Australasia (Australia, New Zealand and New Caledonia). Heim and Perreau-Bertrand (1963) discussed possible synonymy of the *ananas*-group in their treatment of *Boletellus* from Madagascar and New Caledonia. Watling and Gregory (1986) compared *B. ananas* in some detail to concepts of Singer (1955), Corner (1972), Pegler and Young (1981) and Singer *et al.* (1983). Their commentary is largely based on Australian specimens lacking good field notes, but they essentially conclude that *B. ananas* is an American species. With the possible exception of *Halling 8788* from Thailand, we would agree that the reports of *B. ananas* from Australasia and South-east Asia actually describe any combination of *B. emodensis*, *B. deceptivus* or *B. dissiliens*, and possibly *B. ananiceps* if the spores were not examined for presence or absence of cross-striae. Similarly, Zeng and Yang (2011) noted that the occurrence of *B. ananas* in China has not yet been confirmed.

The best, detailed descriptions of B. ananas are those of Singer (1945, but excluding Strobilomyces pallescens Cooke & Massee as a synonym), Thiers (1963) and Ortiz-Santana et al. (2007, as B. coccineus (Sacc.) Singer). It is important to note here that these accounts feature the idiosyncrasies of the morphology of the pileus, stipe and context and the specific location of oxidation reactions. Although there have been descriptions published since, critical details on changes in the temporal integrity of pigments, squamules and oxidation reactions are often not recorded. This is likely to be due to the age of specimens or isolated environmental conditions when collected. In particular, the true colour of the context (both pileus and stipe before oxidation), and the specific colour and location of the oxidations are distinctive. As noted by the authors above, the colour of the pileus context is some shade of yellow at first and is quickly cyanescent. The oxidation reaction quickly masks the true colour and makes it seem white. The stipe context is white (rarely yellowish-cream), reddening or cyanescent (sometimes slowly). These localised context colours and oxidation reactions are similar to those observed for B. ananiceps, B. deceptivus and B. dissiliens (see below). So far, in this group, only B. emodensis has yellow flesh throughout and is cyanescent throughout.

### Material examined (among 47 specimens in NY)

USA. Georgia. Camden County, Crooked River State Park, 19 Oct. 2012, *A.R. Bessette 1223* (NY). North Carolina. Carteret County, Kohlmeyer property, Broad Creek, 6 July 1971, *J.J. Kohlmeyer 3390* (NY). South Carolina. Society Hill, Aug. 1847, *M.A. Curtis* (FH); Santee Canal, *Ravenel (M.A. Curtis 1649)* (isotype: FH). BELIZE. Belize District. Western Highway: Foster Property, near Belize Zoo, 14 Oct. 2003, *Halling 8548* (BRH, NY); 16 Oct. 2003, *Halling 8560* (BRH, NY). COSTA RICA. Alajuela Province Grecia, Bosque del Niño, 27 June 1998, *Halling 7763* (NY, USJ); Cartago Province Palo Verde, 4.5 km E of km 31 of Interamerican Highway near town of Palo Verde, *Halling 7924* (NY, USJ); Guanacaste Province Area Conservación Guanacaste, Parque Nacional Rincon de la Vieja, sector Pailas, 18 June 2003, *Halling 8391* (NY, USJ). Puntarenas Province Zona Protectora Las Tablas, Sitio Tinieblas, *Halling 8613* (NY, USJ). THAILAND. Chiang Mai Province km 22 on Highway 1095, *Halling 8788* (MFLU, NY).

# **Boletellus ananiceps** (Berk.) Singer, *Sydowia* 9: 423 (1955) (Figs 2B, 3B, 4E)

- ≡ Boletus ananaeceps Berk., J. Linn. Soc., Bot. 13: 161 (1873).
- ≡ Strobilomyces ananaeceps (Berk.) Sacc., Syll. Fung. 6: 50 (1888).

### Mycobank number: MB 810820.

Singer (1955) was the first to recognise this taxon as a *Boletellus* and concluded that it was similar to *B. ananas* but was distinguished by the lack of cross-striae on the ribbed spore ornamentation. Berkeley's (1872) terse protologue described a specimen from Victoria, Australia, and included a macroscopic description with nine words that could describe any mushroom



Fig. 2. A. Boletellus ananas (Halling 8548). B. B. ananiceps (Halling 9893). C. B. deceptivus (Halling 9785). D. B. dissiliens (Halling 9022). E. B. emodensis (Halling 8761). F. B. reminiscens (Halling 9063). Scale bar: 1 cm.

with squamose warts on the pileus. Since then, without a definitive macroscopic concept, the ribbed spores lacking cross-striae were the defining feature of the species (but see Commentary). Watling and Gregory (1986) provided the best macroscopic description up to that time based on a specimen from New South Wales. They suggested that *B. ananiceps* was less closely related to *B. ananas* than the latter was to *B. emodensis*, precisely because the latter two feature cross-striae on the spore ornamentation.

*Boletellus ananiceps* was treated by Bougher and Syme (1998) and illustrated via a watercolour of material from Western Australia. Halling and Fechner (2011*b*) brought the

macroscopic concept forward with colour photographs that had been lacking previously. The key features are the pink to pale red colour that is confined to a portion of the pileus that is beneath an overlying, fine superficial layer of hyphae. Later, those superficial hyphae have coalesced and gained an ochre colour, and the pale red to pink pigmentation has remained below or between the developing squamae; the pigmentation may disappear with time and exposure. Finally, the squamae can become thick and coarse (but not always), flattened on and near the disc, to somewhat so towards the margin, but are not, and never were, pigmented. In *B. ananiceps* (and the others in this group), the true colour of the pileus context is yellow, but is



Fig. 3. Scanning electron micrographs – Basidiospores. A. *Boletellus ananas* (Halling 8548). B. *B. ananiceps* (Halling 9276).
C. *B. deceptivus* (holotype, Halling 9786). D. *B. dissiliens* (Halling 9805). E. *B. emodensis* (Lectotype). F. *B. reminiscens* (Halling 9063). Arrows point to lacunae. Scale bar: 5 µm.

quickly obscured by the bluing so as to appear white. In the stipe, the context is white, but there is a slow change to a brownishorange or a pinkish-brown (a rufescence), especially towards the base, although this reaction can sometimes be localised. The fresher the basidiome, the more convincing and obvious is this reaction. The species has been noted in other publications on Australian boletes in a list compiled by May and Wood (1997). Zeng and Yang (2011) noted that reports of *B. ananiceps* from China represent specimens of *B. emodensis*.

### Commentary

We would note here that the exemplars from Victoria and Western Australia are on a branch separate from those from Queensland, indicating some biogeographical structure. Further, on the basis of the description and habit illustration in Bougher and Syme (1998), the pink pigmentation is less obvious than but disposition of the pileus squamules is approximately the same as for the specimens found in Queensland. On an extremely



**Fig. 4.** Differential interference contrast (DIC) light micrographs – Basidiospores of *Boletellus*. A. *B. ananas* (Halling 8515). B. *B. deceptivus* (Halling 9786). C. *B. dissiliens* (Halling 9816). D. *B. emodensis* (Halling 8734). E. *B. ananiceps* (Halling 9484). Scale bar: 5 μm.

rare occasion, a spore of *B. ananiceps* can exhibit a few crossstriae, but these are never so obvious as those seen in *B. ananas*, *B. emodensis*, *B. deceptivus* and *B. dissiliens*. Furthermore, the ribs of the latter four species are thicker than in *B. ananiceps*.

# Material examined

AUSTRALIA. Victoria. Wangaratta, *612* (holotype, isotype: K); Gippsland, 1880, *Mrs. Campbell* (3 specimens: K); Gippsland Plain, Mornington Peninsula, Buckley Nature Reserve (Myers and Balnarring roads), 38°20'32"S, 145°5'49"E, 7 May 2010, *N.H. Sinnot 3794* (MEL2341322, NY). New South Wales. Paramatta, HMS Challenger Expedition (2 specimens: K; = *B. emodensis*). Queensland. Wide Bay

District. Great Sandy National Park, Fraser Island, walking track S of Central Station, 25°29'S, 153°03'27"E, 155 m, 3 June 2009, *Halling 9139* (BRI, NY); Fraser Island, road from Lake McKenzie to Central Station, 25°27'S, 153°02'17"E, 50–65 m, 6 June 2009, *Halling 9178* (BRI, NY); 25°24'08"S, 153°01'54"E, 47 m, 28 Mar. 2010, *Halling 9287* (BRI, NY); Fraser Island, Birrabeen Road, between Lake Boomanjin and Dillingham Road, 25°34'08"S, 153°03'43"E, 131 m, 9 June 2009 *Halling 9212* (BRI, NY); Fraser Island, Lake Boomanjin, 25°33'25"S, 153°03'37"E, 68 m, 25 Mar. 2010, *Halling 9276* (BRI, NY); Fraser Island, 4.8 km along Woralie Road, 25°13'07"S, 153°13'23"E, 171 m, 18 May 2010, *Halling 9314* (BRI, NY); Fraser Island, between Pile Valley and Lake McKenzie, 25°28'19"S, 153°04'20"E, 33 m, 24 May 2010, *Halling 9367* (BRI, NY); Fraser Island, Northern Road, 6.8 km N

of Cornwells Road, 25°23'26"S, 153°04'54"E, 129 m, 26 May 2010, *Halling 9388* (BRI, NY); Fraser Island, road from Eurong to Central Station, 25°30'01"S, 153°06'19"E, 51 m, 17 May 2011, *Halling 9484* (BRI, NY); Cooloola, near Frankis Gulch, 26°02'52"S, 153°04'58"E, 68 m, 11 May 2012, *Halling 9690* (BRI, NY); Cooloola, Vic. Camp Milo, eastern firebreak, 25°59'38"S, 153°04'30"E, 58 m, *Halling 9836* (BRI, NY); vicinity Rainbow Beach, on sand track between rubbish tip and Rainbow Beach Road, 25°55'00"S, 153°05'04"E, 16 m, 28 Feb. 2013, *Halling 9847* (BRI, NY); Cape Hillsborough National Park, Twin Beach Lookout Track (Andrews Point track), 20°55'40"S, 149°03'06"E, 100 m, 13 Feb. 2014, *Halling 9893* (BRI, NY). Western Australia. Vicinity of Denmark, 25 May 2011, *K. Syme* (NY); Denmark, Heritage Trail W of McLeod Road, 34°58'48.3", 117°14'2"E, 3 June 2013, *K. Syme 2852* MEL2372698, NY).

# Boletellus deceptivus Halling & Fechner, sp. nov. (Figs 2C, 3C, 4B, 5A–C, 6)

# Diagnosis

Recalling others in sect. *Boletellus* but with deep red, erect squamules then coarsely squamose pileus, with tips of squamae usually whitish to pale ochraceous early on; stipe with scattered pale pink tones, soon white, with flesh barely yellow and cyanescent above, white and rufescent below; spores longitudinally ribbed with cross-striae; associated with Myrtaceae, *Allocasuarina*.

Fig. 5. A–C. *Boletellus deceptivus* (Halling 9786). A, B. Pleurocystidia.
C. Cheilocystidium. D–F. *B. reminiscens* (Halling 9063), D. Cheilocystidia.
E. Caulocystidia. F. Pileipellis. G, H. *B. sinapipes* (Halling 9043),
G. Pileipellis. H. Caulocystidia. I, J. *B. sinapipes* (Halling 9592).
I. Pleurocystidia. J. Cheilocystidia. Scale bar: 10 μm for all, except 5 μm for F and G.

TYPE: AUSTRALIA. Queensland. Main Range National Park, Queen Mary Falls, 28°20'29.5"S, 152°22'16.9"E, 877 m, 14 Feb. 2013, *Halling 9786* (holotype: BRI; isotype: NY).

# Etymology

Deceptivus – deceptively similar to other coarsely squamose species in sect. *Boletellus*.

Mycobank number: MB 811399

Pileus (2.5–)7–9 cm broad, hemispheric to convex to planoconvex, dry, medium to coarsely erect squamulose at first, coarsely squamose scaly, subpyramidal on disc, subrepent to repent towards margin, sometimes with flattened large scales, deep red to raspberry red to gravish-red to ruddy red to pinkishred (9,10B-C6,5,4), soon becoming dull ochraceous tan, or sometimes with scale tips dull and pale olive coloured, frequently retaining the reddish pigment between the scales or at the base of the scales, or with dull yellow between scales, with a sterile marginal veil covering tubes when young, breaking and shrinking with age, often with the scales continuous onto outer surface of veil, with a white band at edge of young veil; appendiculate veil remnants hanging from margin. Flesh yellow to pale yellow, immediately cyanescent when exposed, then appearing white and blue, with mild odour and taste. Tubes deeply depressed to adnate, bright yellow to greenish-yellow to olive brown (4E7), up to 2.5 cm deep, cyanescent, with pores



**Fig. 6.** *Boletellus deceptivus* (holotype, Halling 9786). Context shows cyanescence masking yellow pigment in pileus (arrow). Stipe base context shows rufescence. Scale bar: 1 cm.

1 mm broad, concolourous becoming yellowish-brown (5E7) to dark brownish-red with age. Stipe 4-8(-12) cm long, (4–) 10–15 mm broad, equal, often bulbous at base, strict or curved, dry, pink to pale red to red at apex, white to tan to pale grayish-brown or with scattered pinkish to pale red tints below, white at base, fibrillose striate to finely appressed fibrillose, with interior pale yellow at apex, whitish below, cyanescent in upper one-third, white below to base with a slower orangish-brown oxidation, rarely with some immediate cyanescence in base, often basal cyanescence disappearing with orange–brown prevailing.

Basidiospores dark olive brown in deposit, 15.4-17.5  $(-18.9) \times 7-7.7(-8.4) \ \mu\text{m}, \quad n = 20, \quad x = 16.59 \times 7.32 \ \mu\text{m},$ O = 2.27, subfusoid to fusoid to ellipsoid, longitudinally ribbed and cleft, shallowly cleft at apex, with ribs continuous or attenuated, flattened on edges, cross-striate, melleous in KOH, inamyloid. Basidia  $25-32 \times 7-10 \mu m$ , clavate, four-sterigmate. Pleurocystidia uncommon, scattered, up to 50 µm long, fusoid to fusoid-ventricose, hyaline or rarely with granular melleous contents. Cheilocystidia 30-40 µm long, subclavate to clavate, hyaline, thin-walled. Hymenophoral trama bilateral, of the Boletus-subtype, with cells 4-9.8 µm broad, hyaline, with lateral strata gelatinised with age. Pileus trama interwoven with elements hvaline, smooth and thin-walled, 7-12 um broad, inamyloid. Pileipellis hyphae suberect to repent, with elements composed of cylindrical hyphae in long chains, 7-17.5 µm broad, smooth, thinwalled, with pale red to pink plasmatic pigment, dissolving in KOH. Stipitipellis vertically oriented, hyaline, thin-walled, with rare, recurved, short endcells. Clamp connections absent.

# Habit, habitat and distribution

Solitary to gregarious, in soil or sand or sometimes at base of living trees, in wet or dry sclerophyll forests, under *Eucalyptus* L.'Hér., *Syncarpia* Ten., *Lophostemon* Schott., *Leptospermum* J.R.Forester & G.Forster, *Allocasuarina* L.A.S. Johnson, *Acacia* Mill. So far, occurring in New South Wales to northern Queensland.

# Commentary

It is quite possible that this taxon has been confused with *B. emodensis* and *B. dissiliens* in Australia. It is also possible that it has been misidentified as *B. ananas*. However, the scales on the pileus are coarser than in *B. emodensis* and the stipe flesh is white and rufescent. Although, *B. dissiliens* (see next) has rufescent, white flesh in the stipe, the pileus is not red and has felt-like patches. Finally, *B. ananas* appears almost wholly restricted to the Americas and has a pinkish-coloured pileus that soon loses its colour.

### Additional material examined

AUSTRALIA. New South Wales. Tweed Shire, Route 97 (Nerang–Murwillumbah road), near New South Wales–Queensland border,  $28^{\circ}16'14''S$ ,  $153^{\circ}14'26''E$ , 160 m, 2 June 2007, *R.E. Halling 8978* (BRI, NY). Queensland. Atherton Shire, Atherton State Forest, Mount Baldy Road,  $\pm 3.6 \text{ km}$  from Atherton–Herberton highway,  $17^{\circ}19'58''S$ ,  $145^{\circ}25'3''E$ , 1140 m, 21 Mar. 2007, *R.E. Halling 8937* (BRI, NY); 21 Feb. 2012, *R.E. Halling 9634* (BRI, NY); Wide Bay District, Great Sandy National Park, Fraser Island, Central Station,  $25^{\circ}28'34''S$ ,  $153^{\circ}3'20''E$ , 90 m, 7 Feb. 2009, *R.E. Halling 9017* (BRI,

NY); Springbrook National Park, Apple Tree Park,  $28^{\circ}9'52''S$ ,  $153^{\circ}15'38''E$ , 547 m, 2 Mar. 2012, *R.E. Halling* 9653 (BRI, NY); Main Range National Park, Queen Mary Falls,  $28^{\circ}13'21''S$ ,  $152^{\circ}30'52''E$ , 340 m, 14 Feb. 2013, *R.E. Halling* 9785, 9786 (BRI, NY); Mareeba Shire, Davies Creek National Park, Davies Creek Road, Davies Creek Falls car park, 8.6 km from Kennedy Highway,  $17^{\circ}0'35''S$ ,  $145^{\circ}34'6''E$ , 620 m, 1 Feb. 2015, *R.E. Halling* 9975 (BRI, NY); Davies Creek National Park, Davies Creek Road,  $\sim 12$  km from Kennedy Highway,  $17^{\circ}1'32.3''S$ ,  $145^{\circ}35'55.7''E$ , 678 m, 3 Feb. 2015, *R. E. Halling* 9983 (BRI, NY).

# Boletellus dissiliens (Corner) Pegler & T.W.K. Young, Trans. Brit. Mycol. Soc. 76: 113 (1981) (Figs 2D, 3D, 4C)

### $\equiv$ Boletus dissiliens Corner, Boletus in Malaysia 98 (1972).

# Mycobank number: MB 112138.

The species was well described and illustrated by Corner (1972), on the basis of material collected in Singapore. The distinctive features include the virtual lack of scales on the pileus and lack of red pigmentation in the pileus, although it can become dull pinkish-tan. In fact, the pileus is nearly white and matted subtomentose at first, but breaks up into felt-like patches or rarely low, repent squamae. It is not squamose to squamulose like that of B. ananas, B. ananiceps, B. deceptivus or B. emodensis. Red pigments are absent, although, on rare occasions (in the Australian material examined), some very pale pinkish tints can appear with age. A further distinction is the colour of the unoxidised context of pileus and stipe. In B. dissiliens, the pileus context is yellow and immediately cyanescent. This bluing will quickly mask the true colour, making it appear white. The yellow pigments (and cyanescence) can appear in the upper-third of the stipe context. However, more commonly, the stipe context is white, not cyanescent, but rather rufescent to brownish-orange. This latter oxidation is slower to appear than the cyanescence and will typically appear on the stipe surface as well. Chiu's (1948) description of B. ananas from Yunnan, especially in terms of the colours of the pileus and stipe, recalls B. dissiliens.

# Habit, habitat and distribution

Solitary to gregarious in sand, or rarely on living tree bark or dead wood with *Eucalyptus pilularis* Sm., *E. racemosa* Cav., *E. signata* F.Muell., *Melaleuca* L., *Leptospermum* sp., *Acacia* sp., *Allocasuarina* sp., *Syncarpia glomulifera* (Sm.) Nied., *S. hillii* F. M.Bailey, *Lophostemon confertus* (R.Br.) Peter G.Wilson & J.T. Waterh., *Callitris* sp. and *Xanthostemon* sp. in Australia; under *Quercus* in Singapore.

# Commentary

As noted by Corner (1972, p. 3), type specimens of his Malaysian boletes were retained at Cambridge, and whenever possible, a duplicate set of types were deposited at Kew. Pegler and Young (1981) provided two SEM micrographs from a specimen at Kew, which they cited as a type. This specimen (cited above) is marked type-duplicate on the packet. Horak (2011), however, cited a specimen from the same locality with same date, but with a number (*Corner 26B*) that is lodged in Edinburgh (E-00084564) and noted that it is the holotype preserved in alcohol–formalin. It may well be the holotype, because Corner (1972, p. 98)

specifically states '(typus, Corner *s.n.* 26 March 1931, C & E)' in the protologue. Presumably, 'C' is Cambridge and 'E' is Edinburgh.

Pegler and Young (1981) gave the spore size as  $13-18.5 \times 5.5-7$  um, O = 2.4, and indicated the presence of fine striae. These measurements are in line with Corner's observations (14–16(–17)  $\times$  5.5–7 µm). Although Corner did not note the presence of cross-striae, he stated the spores are '... as in B. ananas but not so strongly ridged' (Corner 1972, p. 98). Horak's (2011) measurements fall within the ranges given by Corner and Pegler and Young, but he commented that there are '...numerous longitudinal and well-defined clefts (not ridges!)....' (p. 191) and cross-striae are absent. One of us (R. E. Halling) examined the Kew isotype and observed that when the spores are not so strongly ribbed they lack crossstriae; these are seemingly young. Spores that do possess crossstriae have broader ribs (with clefts in between), and with the following statistics: (14–) 4.7–17.5(–18.2)  $\times$  (4.9–)5.6–8.4 µm,  $(n = 11, x = 15.9 \times 7.1 \ \mu m, Q = 2.23)$ . See Figs 3D, 4C.

### Material examined (from among 29 in NY)

AUSTRALIA. Queensland. Atherton State Forest, Mount Baldy Road,  $\pm 5.8$  km from Atherton–Herberton highway, 17°18′55″S, 145°24′15″E, 1027 m, 21 Mar. 2007, *R.E. Halling 8943* (BRI, NY); Wide Bay District, Great Sandy National Park, Fraser Island, 3 km W of Central Station, Wanggoolba Creek Road, 25°28′16″S, 153°02′10″E, 24 m, 18 Feb. 2011, *R.E. Halling 9435* (BRI, NY); Fraser Island, road from Pile Valley to Lake McKenzie, 25°26′42″S, 153°02′59″E, 107 m, 6 Mar. 2011, *R.E. Halling 9466* (BRI, NY); Cooloola, Freshwater Road, 25°57′40″S, 153°08′25″E, 129–130 m, 11 Mar. 2012, *R. E. Halling 9688* (BRI, NY); Mareeba Shire, Mareeba, Davies Creek National Park, Davies Creek Road, Campsite #5-6, 17°01′36″S, 145°35′23″E, 670 m, 15 Feb. 2012, *R.E. Halling 9624* (BRI, NY); S of Canungra, along Beechmont Road, near Rhoades Road, 28°03′43″S, 153°11′28″E, 161 m, 12 Feb. 2013, *R. E. Halling 9768* (BRI, NY). SINGAPORE. Reservoir Jungle, 26 Mar. 1931, *Corner* (isotype: K(M)141488).

# Boletellus emodensis (Berk.) Singer, Ann. Mycol. 40: 19 (1942) (Figs 2E, 3E, 4D)

- ≡ Boletus emodensis Berk., Hooker's J. Bot. Kew Gard. Misc. 3: 48 (1851). INDIA. Sikkim, Darjeeling, Hooker 100 (Lectotype hic designatus: K(M)164373).
- = Strobilomyces annamiticus Pat., Bull. Soc. Mycol. France 25: 6 (1909).
- ≡ Boletellus annamiticus (Pat.) E.-J. Gilbert, Bolets 107 (1931)
- = *Strobilomyces pallescens* Cooke & Massee in Cooke, *Grevillea* 18: 5 (1889).
- $\equiv$  Boletellus pallescens (Cooke & Massee) E.-J. Gilbert, Bolets 107 (1931).

### Mycobank number: MB 472279.

Zeng and Yang (2011) provided a detailed, modern description, with updated nomenclature and illustrations of Chinese materials referable to *B. emodensis*. The material cited below coincides in macro- and micromorphology. As noted by Zeng and Yang (2011), the species is well characterised on a macroscopic level by the purplish red to dull crimson colours of both pileus and stipe and a yellow context in both pileus and stipe, the true colour of which is quickly masked by the rapid

cyanescence. The scales on the pileus are quite fine from the beginning and sometimes become somewhat coarser with age, although not to the degree seen in B. deceptivus or B. ananiceps. According to Hooker's notes (Berkeley 1851), the stipe is reddish-brown in the base, white or pinkish above, and slightly changing to blue when cut. One of us (R. E. Halling) has observed that the amount of yellow (and intensity of cyanescence) in the stipe flesh decreases with age and degree of freshness. However, because of the rapidity with which the cyanescence occurs, the yellow colour is easily masked and appears white. In addition, as with closely related taxa (B. ananas, B. ananiceps, B. deceptivus and B. dissiliens), there is a slower but distinctive rufescence or brownish-orange oxidation that occurs in the base of the stipe. In contrast, the latter four species have a white context in the stipe that lacks the cyanescence (except rarely in the upper-third), and the brownish-orange oxidation is more pronounced upward from the base. All except B. ananiceps exhibit the transverse striations on the longitudinal ribs of the spores when viewed with a compound light microscope (Fig. 4).

# Habit, habitat and distribution

Solitary to gregarious on soil, in sand or rarely on living tree bark or dead wood; with *Pinus*, Dipterocarpaceae Blume (Thailand); with Fagaceae (China); with *Eucalyptus*, *Acacia*, *Allocasuarina littoralis* (Salisb.) L.A.S. Johnson, *Leptospermum*, *Syncarpia*, *Lophostemon* (Australia).

# Commentary

There are three separate packets at Kew (K(M)141491, K(M) 164372, K(M)164373) that would appear attributable to Hooker's type collection from India. K(M)164373 is in the best shape of the three, with a pileus 8.2 cm broad, with easily visible fine squamules; the stipe is curved,  $8.5 \times 1.5$  cm, and equal in width. The herbarium sheet is stamped in blue ink 'Herbarium Hookerianum 1867' inside a circle. Penciled on the sheet is 'Boletus Emodensis, Berk. no. 100 Sikkim'. This latter specimen has been designated as the lectotype (above). The spores are costate with cross-striae (Fig. 4D) and some of the ribs appear not to be united at the apex (Fig. 3E) (smooth and rounded *fide* Pegler and Young 1981).

Horak (2011) also lists *Boletus paradoxus* Massee, *Boletus porphyrius* Pat. & C.F.Baker, and *Boletellus floriformis* Imazeki as synonyms of *Boletellus emodensis*. The first two were described from Singapore and the last from Japan.

### Material examined (among 43 specimens in NY)

AUSTRALIA. New South Wales. Boonoo State Forest, Junction Colongon Road and Lindrook Road, 28°53'38"S, 152°13'8"E, 833 m, 2 Mar. 2011, *R.E. Halling 9459* (BRI, NY). Queensland. Mareeba, Davies Creek National Park, Davies Creek Road, 17°1'53"S, 145°36'23"E, 700 m, 4 Feb. 2006, *R.E. Halling 8734* (BRI, NY); Davies Creek Road, 17°1'31"S, 145°35'52"E, 720 m, 9 Feb. 2006, *R.E. Halling 8742* (BRI, NY); 11 Mar. 2007, *R.E. Halling 8904* (BRI, NY); Brisbane, *Bailey 744* (isotype: *Strobilomyces pallescens*, NY); Brisbane, Ashgrove suburb, Ithaca Creek and Cooper's Camp Road, 27°27'6"S, 152°58'42"E, 41 m, 8 Mar. 2012, *R.E. Halling 9676* (BRI, NY); Rainbow Beach, Bullock Point, near Innskip Point, 25°49'43"S, 153°3'55"E, 23 m, 13 Mar. 2012, *R. E. Halling 9701* (BRI, NY); Girraween National Park, road from Bald Rock

Creek to Dr Roberts Waterhole, 28°49'39"S, 151°58'2"E, 979 m, 15 Feb. 2013, *R.E. Halling 9789* (BRI, NY); Wide Bay District. Great Sandy National Park, Fraser Island, Central Station, 25°28'34"S, 153°3'20"E, 90 m, 24 Mar. 2010, *R.E. Halling 9260* (BRI, NY); Cooloola, Freshwater Road, 25°56'48"S, 153°7'44"E, 133 m, 28 Feb. 2013, *R.E. Halling 9850* (BRI, NY). THAILAND. Chiang Mai Province, km 22 on Highway 1095, 19°7'30"N, 98°45'47"E, 731 m, 11 June 2006, *R.E. Halling 8808* (MFLU, NY).

# **Boletellus reminiscens** Halling & Fechner, *sp. nov.* (Figs 2F, 3F, 5D–F)

# Diagnosis

Recalling *B. obscurecoccineus*, but lacking fine scabers on the stipe, with cyanescent context, and costate spores often discontinuously tuberculate.

# Туре

AUSTRALIA. Queensland. Wide Bay District, Great Sandy National Park, Fraser Island, Pile Valley Walking track, from Central Station to Pile Valley, 25°28'34"S, 153°3'20"E, 100 m, 12 Feb. 2009, *R.E. Halling 9063* (holotype: BRI; isotype: NY).

# Etymology

Reminiscens, past participle of reminisci, to remember or recall, in reference to *B. obscurecoccineus*.

Mycobank number: MB 811400.

Pileus 2.5–4.5 cm broad, convex to plano-convex, dry, deep dark red, finely subtomentose to subvelutinous, mottled slightly with small dark yellow spots, becoming finely areolate and a pale olive when fading. Flesh pale yellow, cyanescent then obscurely white, with mild odour and taste. Tubes adnexed, bright yellow to dark yellow to greenish-yellow, cyanescent, with pores cyanescent then eventually pale brown. Stipe 3.5–5 cm long, 6–9 mm broad, strict or curved, equal or slightly broader below, rarely tapering to a blunt point, dry, whitish to pale greenish-yellow at apex, otherwise uniformly deep red with a white base, heavily pruinose to subpruinose above, fibrillose striate below, cyanescent, with interior yellow, cyanescent, deep reddish around larval tunnels.

Basidiospores 12.6–14.7 × 4.2–5.6  $\mu$ m, n = 20,  $x = 13.3 \times$ 5.04  $\mu$ m, Q = 2.64, finely ribbed with ribs shallowly and moderately spaced, with rounded edges and longitudinally intermittent-turberculate, melleous in KOH, inamyloid. Basidia  $24-30 \times 10-12.6 \ \mu\text{m}$ , clavate, 4-sterigmate, hyaline. Pleurocystidia rare, ventricose rostrate, more abundant near pores. Cheilocystida  $20-45 \times 10-12 \ \mu m$ , ventricose rostrate to subfusoid, hyaline, thin-walled. Hymenophoral trama bilateral, of the Boletus subtype, with cells 4-9.8 µm broad, hyaline. Pileus trama interwoven with elements hyaline, smooth and thin-walled, 7-14 µm broad, inamyloid. Pileipellis an erect trichodermium of cylindrical to slightly inflated subcylindrical elements, 7-21 µm broad, smooth thin-walled, inamyloid with pale red plasmatic pigment. Stipitipellis vertically oriented, hyaline, thin-walled, with recurved end cells, occasional isolated basidia, or clusters of clavate to nearly short-subfusoid caulocystidia, 20-25 µm long, usually with melleous contents. Clamp connections absent.

# Habit, habitat and distribution

Solitary to scattered in sand under *Syncarpia*, *Lophostemon*, *Eucalyptus*, *Allocasuarina* and *Leptospermum*. Currently, only seen on Fraser Island in the Great Sandy National Park of south-eastern Queensland.

# Commentary

At present, *Boletellus reminiscens* appears to be uncommon and restricted to the sand habitats of Fraser Island. Despite the deep red colours, it differs from *B. obscurecoccineus* in the cyanescent oxidation reaction, lack of scales on the stipe, and yellow context. Microscopically, the spores of *B. reminiscens* are finely longitudinally ridged as in *B. obscurecoccineus*, but have narrower costae that are sometimes discontinuous, forming isolated tubercules or short pustulate ribs (Fig. 3F).

### Additional material examined

AUSTRALIA. Queensland. Wide Bay District, Great Sandy National Park, Fraser Island, Lake Garawongera Scenic Drive, W of lake, 25°20'24"S, 153°9'8"E, 200 m, 27 Mar. 2010, *R.E. Halling 9284* (BRI, NY); Fraser Island, Wanggoolba Creek Road, 25°28'46"S, 153°2'45"E, 35 m, 29 Mar. 2010, *R.E. Halling 9305* (BRI, NY).

# Boletellus sinapipes Fechner, K.Syme, R.Rob., & Halling, sp. nov. (Figs 5G–J, 7B, D, 8A)

# Diagnosis

Characterised by the brown colours nearly overall, with bright yellow, cyanescent tubes, and mustard brown-coloured tomentum on the stipe base.

# Туре

AUSTRALIA. Queensland. Fraser Island, road from Central Station to Lake McKenzie, 25°28′40″S, 153°2′51″E, 109 m, 9 Feb. 2009, *R.E. Halling 9040* (holotype: BRI, isotype: NY).

# Etymology

Sinapi- = mustard, + -pes = foot, in reference to the mustard brown-coloured tomentum at the base of the stipe.

Mycobank number: MB 811401.

Pileus 3–5(–9.4) cm broad, convex to plano-convex to plane, dry, brown (5F4) to dark brown with a subtle hint of red, velutinous, subtomentose to matted subtomentose, becoming minutely to finely areolate or sometimes cracked at margin. Flesh pale yellow and cyanescent at first and then appearing white under the cyanescence, entirely red with age, with mild odour and taste. Tubes adnexed, bright yellow (2A5) to near chrome yellow (3A7,6), cyanescent, with pores bright yellow and cyanescent then slowly brown, becoming dark olive brown with age. Stipe 5-9(-10.5) cm long, (4-)7-15(-20) mm broad, strict, sometimes curved at base, equal to slightly broader below, dry, finely and obscurely subpruinose to finely subtomentose at apex, fibrillose streaked to matted fibrillose below, bright yellow at apex, dull pinkish-brown to dull brownish-red to dull red below, eventually entirely red, with conspicuous mustard brown (5E7) velvety tomentum at base, with interior pale yellow to dull yellow at apex, whitish below, brownish-yellow in the base, intensely cyanescent at first, completely red with age.

Basidiospores olive brown in deposit,  $11.9-15.4 \times 5.6-7 \mu m$ , n = 20,  $x = 13.13 \times 5.99 \mu m$ , Q = 2.19, subfusoid to fusoid to ellipsoid, longitudinally ribbed and cleft, sometimes shallowly cleft at apex, with ribs continuous or attenuated, rarely not continuous and isolated between apex and base, broadly rounded at first then flattened on edges, lacking cross-striae, melleous in KOH, inamyloid. Basidia  $31-42 \times 13-17 \mu m$ , short-clavate, hyaline to melleous, 4-sterigmate. Pleurocystidia up to 65  $\mu m$  long, fusoid to fusoid-ventricose, hyaline. Cheilocystidia  $30-40 \mu m$  long, subclavate to clavate or sometimes subrostrate, hyaline,

thin-walled. Hymenophoral trama bilateral, of the *Boletus* subtype, with cells 4–9.8  $\mu$ m broad, hyaline, fleeting amyloid. Pileus trama interwoven with elements hyaline, smooth and thin-walled, 7–14  $\mu$ m broad, inamyloid. Pileipellis hyphae a tangled trichodermium, with elements composed of cylindrical hyphae, 5–10  $\mu$ m broad, with scattered, rusty brown, plaque-like encrusting pigment (in water and Melzer's), thin-walled, with amorphous plasmatic melleous pigment, dissolving in KOH. Stipitipellis vertically oriented, hyaline, thin-walled, with recurved, short end cells or clusters of short-clavate to short-subfusoid caulocystidia, 20–25  $\mu$ m long. Clamp connections absent.



Fig. 7. A. *Heimioporus australis* (Halling 9288). B. *Boletellus sinapipes* (Halling 9310). C. *H. fruticicola* (Halling 9775). D. *B. sinapipes* (Halling 9040). E. *H. fruticicola* (Halling 8970). F. *H. cooloolae* (Halling 9845). Scale bar: 1 cm.

# Habit, habitat and distribution

Solitary to gregarious in sand, or rarely on living tree bark (Queensland) with *Eucalyptus, Syncarpia* and *Lophostemon*; or soil (Western Australia) with *Eucalyptus patens* Benth., *E. diversicolor* F.Muell., *E. marginata* Donn ex Sm., *Corymbia calophylla* (R.Br.) K.D.Hill & L.A.S.Johnson, *Allocasuarina decussata* (Benth.) L.A.S.Johnson and *Agonis flexuosa* (Wild.) Sweet.

# Commentary

The most distinctive feature of *B. sinapipes* is the mustard brown-coloured tomentum at the base of the stipe. Fig. 7B depicts a young basidiome, but even as the basidiomes mature (Fig. 7D) and become dark brown to reddish-brown, the colour of the basal tomentum is consistent. There is some biogeographic structure present here with western (REH 9592, 25511) and eastern (REH 9018, REH 9408) Australian components.

# Additional material examined

AUSTRALIA. Queensland. Wide Bay District, Great Sandy National Park, Fraser Island, Central Station, 25°28′34″S, 153°3′20″E, 100 m, 7 Feb. 2009, *R.E. Halling 9018* (BRI, NY); Fraser Island, near Central Station, 25°28′34″S, 153°3′20″E, 90 m, 9 Feb. 2009, *R.E. Halling 9043* (BRI, NY); Fraser Island, road from Central Station to Lake Birrabeen, 25°29′42″S, 153°3′52″E, 160 m, 13 Feb. 2009, *R.E. Halling 9071* (BRI,

NY); 25°29'36"S, 153°3'10"E, 113 m, 25 Mar. 2010, *R.E. Halling 9278* (BRI, NY); Fraser Island, Pile Valley, 25°28'28"S, 153°4'20"E, 87 m, 30 Mar. 2010, *R.E. Halling 9310* (BRI, NY); Fraser Island, road from Eurong to Central Station, 25°28'40"S, 153°04'9"E, 77 m, 15 Feb. 2011, *R.E. Halling 9408* (BRI, NY). Western Australia. Denmark Shire, vicinity of Denmark, 25 May 2011, *K. Syme s.n.* (NY), 34°59'17.9"S, 117°16'35.5"E, Heritage Trail W of Lights Road, 21 May 2013, *K. Syme 2836* (MEL2371699, NY); Denmark Shire, Digby & Lyn Mercer property, Loc 3298 off Denmark–Nornalup Heritage Trail, 34°58'48"S, 117°14'03"E, 17 July 2011, *R.E. Halling 9592* (NY); Denmark, Heritage Rail Trail W of Lights Road, 34°59'17.9"S, 117°16'35.5"E, 21 May 2013, *K. Syme 2836* (MEL2371699, NY).

# Heimioporus australis Fechner & Halling, sp. nov. (Figs 7A, 8B)

# Diagnosis

Differs from other species in *Heimioporus* by the shallowly lacerate-ridged stipe with a dense red pruina.

# Туре

AUSTRALIA. Queensland. Wide Bay District, Great Sandy National Park, Fraser Island, Kingfisher Bay, 25°23'35.7"S, 153°01'50.7"E, 8 m, 28 Mar. 2010, *R.E. Halling 9288* (holotype: BRI; isotype: NY).



Fig. 8. Scanning electron micrographs – Basidiospores. A. *Boletellus sinapipes* (holotype, Halling 9040). B. *Heimioporus australis* (holotype, Halling 9288). C. *H. cooloolae* (Halling 9446). D. *H. fruticicola* (Halling 9811). Scale bar: 5 µm.

# Etymology

Australis- adjective, southern.

Mycobank number: MB 811402.

This taxon was previously thought to be *Heimioporus japonicus* (Hongo) E.Horak by Halling and Fechner (2011*a*). After a careful re-evaluation of specimens, we now know that the surface morphology of the stipe surface on the specimen from Fraser Island was misinterpreted; it is shallowly lacerate-ridged with a dense pruina, but it is not beset with a true reticulum possessed by *H. japonicus* (Imazeki *et al.* 1988, Masai 2014). Illustrations and a full description are in Halling and Fechner (2011*a*).

### Habit, habitat and distribution

Solitary to gregarious under *Eucalyptus, Allocasuarina, Leptospermum* and *Melaleuca*; so far known only from south-eastern Queensland.

### Additional material examined

AUSTRALIA. Queensland, Caloundra, Queens Street, Ben Bennett Bushland Reserve, 26°47′50″S, 153°7′20″E, 29 Jan. 2015, N. Fechner, *AQ905831* (BRI, NY).

# Heimioporus cooloolae Fechner & Halling, sp. nov. (Figs 7F, 8C, 9A–E)

### Diagnosis

Differing from other species of *Heimioporus* by a dull to pale red pileus; a white- or olive-coloured tomentum near the stipe base;



Fig. 9. A–E. *Heimioporus cooloolae* (Halling 9852). A. Pleurocystidia. B. Cheilocystidia. C, D. Caulocystidia. E. Pileipellis. Scale bar: 10 µm.

basidiospores with rugulose surface, a subtruncate, eroded apex, and crater-like pits.

# Туре

AUSTRALIA. Queensland, Wide Bay District, Great Sandy National Park, Cooloola, Freshwater Road, 25°56′47.7″S, 153°7′44″E, 133 m, 28 Feb. 2013, *R.E. Halling 9852* (holotype: BRI, isotype: NY).

### Etymology

Cooloola- + e, genitive, of the Cooloola Sandmass.

Mycobank number: MB 811403.

Pileus 3.5-5(-7.5) cm broad, convex to plano-convex, becoming plane, dry, subtomentose to finely matted subtomentose, pink or with red tones mixed with yellow, but not quite orange, with even margin that slightly projects a sterile flap. Flesh white with some yellow beneath pileus surface, erratic light bluing near tubes, with mild odour and taste. Tubes adnexed, greenish-yellow to olive, unchanging or with some subtle cyanescence, with pores developing some pale brown. Stipe 7-9 cm long, 1-1.5 cm broad, curved, equal and tapered at base, dry, yellow to bright yellow at apex, dull yellow to red below and rhubarb red towards base, subfloccose at apex, fibrillose striate below, with low ridges at first, becoming more pronounced with age and then finely red subscabrous, with some scattered olive-mustard-coloured tomentum at base, otherwise white at base, with interior white to pale yellow at apex, more yellow below and a bit of red in base, with subtle cyanescence in mid-portion.

Basidiospores 9.8–13.3  $\times$  4.9–7 µm, n = 20,  $x = 11.2 \times$ 6.16, Q = 1.82, broadly ellipsoid to oblong-ovate to broadly subfusoid or somewhat amygdaliform in profile, rugulose with scattered crater-like pits, often with a subtruncate, eroded apex, honey brown in KOH, imamyloid (rarely slightly dextrinoid). Basidia  $25-31 \times 8-11 \mu m$ , clavate, hyaline, 4-sterigmate. Pleurocystidia rare, up to 55 µm long, fusoid to fusoidventricose. Cheilocystidia 30-45 µm long, subclavate to clavate, hyaline, thin-walled. Pileus trama interwoven with elements hyaline, smooth and thin-walled, 7-12 µm broad, fleeting amyloid. Hymenophoral trama bilateral, of the Boletus subtype, with cells 4-9.8 µm broad, hyaline, fleeting amyloid. Pileipellis a trichodermium collapsing with age, with cells 5-10 µm broad, cylindrical to narrowly clavate-subcapitate, smooth, thin-walled, inamyloid, hyaline or with granular golden vellow content in KOH, with an amorphous, soluble, reddish, plasmatic pigment. Stipitipellis hyphae hyaline, smooth, thin-walled, with caulocystidia forming an interrupted hymeniform layer of clavate elements,  $28-36 \times 12-16 \,\mu m$ broad. Clamp connections absent.

### Habit, habitat and distribution

Solitary to subcespitose in sand under *Eucalyptus*, *Allocasuarina* and *Leptospermum*; so far known only from the Great Sandy National Park (Cooloola section) in Queensland.

### Commentary

This species has a type of spore ornamentation similar to that of *H. fruticicola* (rugulose with crater-like holes, subtruncate,

eroded apex), but the spores are generally shorter in length and with fewer craters. Also, *H. cooloolae* differs in the paler colours and an occasional presence of a scattered, olivecoloured tomentum at the stipe base. Sand grains adhering to the stipe base will obscure features in this area. Watling and Gregory's (1986) analysis of *Austroboletus* sp. 2 may well be this species.

### Additional material examined

AUSTRALIA. Queensland. Wide Bay District, Great Sandy National Park, Cooloola, Freshwater Road, 25°56'43"S, 153°5'5"E, 34 m, 20 Feb. 2011, *R.E. Halling 9446* (BRI, NY); Cooloola, Freshwater Road, 25°57'16"S, 153°5'51"E, 144 m, 22 Feb. 2013, *R.E. Halling 9817* (BRI, NY); Cooloola, Camp Milo, eastern firebreak, 25°59'44"S, 153°4'31"E, 58–68 m, 27 Feb. 2013, *R.E. Halling 9845* (BRI, NY).

# Heimioporus fruticicola (Berk.) E.Horak, Sydowia 56: 240 (2004) (Figs 7C, E, 8D)

 $\equiv$  Boletus fruticicola Berk., London J. Bot. 7: 574 (1848).

- $\equiv$  Suillus fruticicola (Berk.) Kuntze, Rev. Gen. Pl. 3(2): 535 (1898).
- ≡ Austroboletus fruticicola (Berk.) E.Horak, Sydowia 33: 76 (1980)
- $\equiv$  *Heimiella fruticicola* (Berk.) Watling & Hollands, *Notes Roy. Bot. Gard. Edinburgh* 46: 420 (1990).

Mycobank number: MB 368304.

The species has been described and illustrated previously by Halling and Fechner (2011*a*). It differs from *H. cooloolae* in brighter red colours, lack of a coloured tomentum on the stipe base, and larger spores with more prominent crater-like pits. Fig. 7C depicts material from southern Queensland, and Fig. 7E from northern Queensland.

# Material examined

AUSTRALIA. Tasmania. Penquite, Gunn 1775 (holotype: K). Queensland. Mareeba, Davies Creek National Park, Davies Creek Road, 19 Feb. 1992, Halling 6837 (PERTH E4709); 17°01'36"S, 145°35'23"E, 670 m, 22 Mar. 2007, R.E. Halling 8958 (BRI, NY); 17°00'35"S, 145°34'56"E, 620 m, 24 Mar. 2007, R.E. Halling 8970 (BRI, NY); Kuranda, Black Mountain Road, 5.3 km N of Kuranda, 16°47′04″S, 145°37′25″E, 450 m, 23 Mar. 2007, R.E. Halling 8962 (BRI, NY); Cooloola, Mutyi, 17 Sep. 1982, BRIP 9148 (BRI); 17 Sep. 1982, BRIP 9150 (BRI); near Rainbow Beach, 17 Sep. 1982, BRIP 9149 (BRI); Cooloola, on Fig Tree Point track, 14 Apr. 1966, leg. C. Sandercoe & J. Milne (JECA 86/66 = BRIP 19814) det. R. Watling (BRI); Springbrook National Park, Apple Tree Park, 28°9'52"S, 153°15'38"E, 547 m, 12 Feb. 2013, R.E. Halling 9775 (BRI, NY); Freshwater National Park, W of Deception Bay, 27°10'52"S, 152°59'48"E, 19 m, 21 Feb. 2013, R.E. Halling 9811 (BRI, NY). Victoria. Grampians. Victoria Range, Cultivation Creek, near Buandik camping ground, 37°15'S, 142°17'E, 1 Nov. 1992, May 816 (MEL 2030279).

# Heimioporus retisporus (Pat. & C.F. Baker) E. Horak, Sydowia 56: 239 (2005)

- $\equiv$  Boletus retisporus Pat. & C.F. Baker, J. Straits Branch Roy. Asiatic Soc. 78: 72 (1918).
- *≡ Boletellus retisporus* (Pat. & C.F. Baker) E.-J. Gilbert, *Bolets*, 108 (1931).

*≡ Heimiella retispora* (Pat & C.F. Baker) Boedijn, *Sydowia* 5: 217 (1951).

Mycobank number: MB 369467.

The type specimen from Singapore (*leg.* C.F. Baker, no. 5000, FH) has been well studied by Singer (1945) and Horak (1968, 2011). The species is also well described and illustrated by Corner (1972). Updated distribution provided by Horak (2011) includes Singapore, Malaysia, China, Indonesia, Papua New Guinea, but doubtfully in Australia. So far, we have not seen any *bona fide* specimens from Australia.

The following new combinations and a new name are supported by the phylogenetic analysis.

Aureoboletus mirabilis (Murrill) Halling, comb. nov.

 $\equiv$  Ceriomyces mirabilis Murrill, Mycologia 4: 98 (1912).

- $\equiv$  Boletus mirabilis (Murrill) Murrill, Mycologia 4: 217 (1912).
- $\equiv$  Xerocomus mirabilis (Murrill) Singer, Rev. Mycol. (Paris) 5: 6 (1940).
- ≡ Boletellus mirabilis (Murrill) Singer, Farlowia 2: 129 (1945).
- $\equiv$  Heimioporus mirabilis (Murrill) E.Horak, Sydowia 56: 240 (2004).

Mycobank number: MB 811404.

*Aureoboletus projectellus* (Murrill) Halling, comb. nov.

- ≡ Ceriomyces projectellus Murrill, Mycologia 30: 524 (1938).
- $\equiv$  Boletus projectellus (Murrill) Murrill, Mycologia 30: 525 (1938).
- $\equiv$  Boletellus projectellus (Murrill) Singer, Farlowia 2: 129 (1945).

Mycobank number: MB 811405.

Hemileccinum subglabripes (Peck) Halling, comb. nov.

- ≡ Boletus subglabripes Peck, Bull New York St. Mus. 2: 112 (1889), nom. nov. for Boletus flavipes Peck, Ann. Rep. New York St. Mus. 39: 42 (1887), non Berk., Hooker's J. Bot. Kew Gard. Misc 6: 135 (1854).
- $\equiv$  Suillus subglabripes (Peck) Kuntze, Rev. Gen. Pl. 3: 536 (1898).
- $\equiv$  Ceriomyces subglabripes (Peck) Murrill, Mycologia 1:153 (1909).
- $\equiv$  Leccinum subglabripes (Peck) Singer, Mycologia 37: 799 (1945).

Mycobank number: MB 811406.

Xerocomus tenax Nuhn & Halling, nom. nov.

≡ Boletus tenax A.H. Sm. & Thiers, Boletes of Michigan 249 (1971), nom. illeg., non Lightfoot, Fl. Scot. 2: 1031 (1777), non Bolton, Hist. Fung. Halifax 2: 75 (1788).

Mycobank number: MB 811407.

# Discussion

The results presented here are consistent with those of previous studies (with more limited sampling) that suggested that *Boletellus sensu* Singer (1986) is not monophyletic (Dentinger *et al.* 2010; Li *et al.* 2011; Nuhn *et al.* 2013; Wu *et al.* 2014). The expected division between the previously sampled species *Aureoboletus projectellus, A. mirabilis, B. russellii* and *B. shichianus* was recovered, but the majority of *Boletellus* species and *Heimioporus* are placed in a new lineage that remains weakly supported. Alternative-topology tests do not reject monophyly of

the core Boletellus clade plus B. chrysenteroides, B. badiovinosus, B. reminiscens, B. sinapipes, B. betula and B. elatus. Indeed, the SH and AU tests found that the likelihood of the constrained topology (forcing monophyly of most Boletellus species) was marginally higher than that of the initially recovered topology. These results do not warrant new generic placements for B. chrysenteroides, B. badiovinosus, B. reminiscens and B. sinapipes, but they do indicate that more data from additional genes or genomes of exemplar taxa are needed to increase confidence in the higher-level relationships between Boletellus and Heimioporus. Alternative placements of B. russellii and B. shichianus were not evaluated, but the optimal topologies suggest that these species will need generic re-evaluation. Likewise, further data for B. betula and B. elatus are needed to assess their relationships to Heimioporus and Boletellus. For example, the former taxon was placed in Heimioporus with H. punctisporus (Corner) E. Horak because of the perforated spore wall (Horak 2004). A unique spore morphology for B. elatus was first documented by Nagasawa (1984) and verified by Halling and Ortiz-Santana (2009) to also include B. jalapensis (Murrill) Singer.

The core *Boletellus* clade is composed of two strongly supported sister clades, one including *B. obscurecoccineus* and the other containing the rest of the core *Boletellus* species. The *B. obscurecoccineus* clade is further divided into two groups, one from northern Queensland and one from southern Queensland, which suggests that there may be discrete taxa. Our concept used here for Australian *B. obscurecoccineus* is illustrated in Grey and Grey (2005) and Fuhrer (2005). That concept is the one employed for documenting the taxon as a Fungimap target species in Australia (Grey and Grey 2005). The images offered by Zeng and Yang (2011) illustrate a slenderer taxon from China, with a coarser hymenophore. Other than the type specimen from Java (in FH), recent material from that area is not available for molecular analysis. Clearly, expanded sampling is desired (Fig. 1).

Most of the collections in the remainder of the core Boletellus clade are from Australia, including four collections identified as B. ananiceps, six collections of B. dissiliens, three collections of B. emodensis, and five collections of B. deceptivus, each of which is strongly supported as monophyletic. One collection from Thailand was identified as *B. emodensis* and is weakly supported as the sister group of the Australian B. emodensis group (Fig. 1). The presence of the Thai collection nested within the Australian clade is not unprecedented; other recent analyses (Halling et al. 2012a, 2012b) have shown similar results for South-east Asian exemplars. Even though the morphological concept for austral B. emodensis used in the present study compares favourably to one from China (Zeng and Yang 2011), recent exemplars for molecular analysis from the Himalaya (type locality) are lacking. A Wallacean land-bridge hypothesis put forth by Halling et al. (2008) can be considered here, and future studies may well show additional phylogenetic distinction. Obviously, continued sampling on both sides of Wallacea is needed to test this hypothesis further.

New World specimens in the core *Boletellus* clade include two collections of *B. singeri* Gonz.-Velázq. & R.Valenz. from Mexico, and five collections of *B. ananas* from Belize (one collection), Costa Rica (three collections) and the USA (Georgia; one collection). The latter is close to the type locality of B. ananas, the type species of Boletellus. A collection questionably identified as B. ananas from Thailand is sister to the New World B. ananas materials. There is also one collection of *Boletellus* sp. from Zambia that is placed as sister to the clade containing B. ananas, the Australian species, and the Thai B. emodensis collection. Similar complex patterns of relationships among collections from New World tropics and temperate regions, South-east Asia, Australia and Africa were also observed in Sutorius and the Chromapes group (Halling et al. 2012a, 2012b). This repeated pattern could suggest that members of Boletaceae were widely distributed on Pangaea before its fragmentation began ~100 million years ago (Halling et al. 2008). However, knowledge of tropical species of Boletellus and other Boletaceae, particularly in Africa, is too limited at this time to warrant strong historical biogeographic inferences. Additional sampling could also help refine the limits of hypothetically broadly distributed taxa, such as *B. ananas*, B. emodensis and B. obscurecoccineus.

The oxidation reaction of exposed context needs to be observed much more closely than previously considered. The rapid cyanescence will obscure the true colour of the unoxidised context (Fig. 6, arrow). Also, the localisation of oxidations, to include a rufescence in stipe contexts (Fig. 6, base of stipe context), is another feature of macroscopic distinction.

The presence and type of spore ornamentation has been a traditional microscopic feature for distinguishing genera in the Boletaceae (e.g. Pegler and Young 1981; Singer 1986). Recent molecular phylogenetic studies have suggested that spore ornamentation appears less reliable on its own merits than in the past (Nuhn et al. 2013; Wu et al. 2014; the present study); however, these studies have not resolved this question in regard to the longitudinally ridged and reticulate ornamentation characteristic of Boletellus and Heimioporus because of sparse taxon sampling and low phylogenetic resolution for these genera. Osmundson (2009) presented phylogenetic evidence that Boletellus and Heimioporus are distinct clades, although sampling was limited with all of the included taxa belonging to the core clades of these genera. In the present study, we include a significantly larger taxon sample than in any previous phylogenetic study of these genera, including taxa formerly included in Boletellus but with doubtful status on the basis of their spore morphology (e.g. A. projectellus, A. mirabilis). In our analyses (Fig. 1), B. singeri fits within the core Boletellus clade, whereas B. elatus is allied to B. betula in a distinct clade, suggesting that the presence of longitudinal ridges is homoplasious. In the alternative-topology tested and found to be statistically slightly better to that in Fig. 1 (inset), B. elatus would be closer to the core Boletellus clade; however, the placement of B. russellii would still suggest homoplasy for the presence of longitudinally ridged basidiospores. Therefore, it can be concluded that basidiospore ornamentation is an important characteristic for identifying members of the core Boletellus clade, yet does not appear to be a synapomorphy for a monophyletic genus Boletellus. In addition, acute observation of ridges, costae, intercostal space, edges of costae and apical morphology of spores is still important for distinguishing entities at the species level (e.g. B. elatus and B. singeri in Halling and Ortiz-Santana 2009).

The presence of longitudinally ridged basidiospores, while homoplasious, appears to be restricted to only a very few taxa, all of which appear to be closely related to *Boletellus*. Therefore, although convergent evolution of this character is possible, it may also be the case that longitudinally ridged basidiospores represent the ancestral character state of the common ancestor of *Boletellus*, *Heimioporus*, and allied taxa; our analysis did not attempt to distinguish between these two hypotheses.

Among core *Boletellus* taxa with squamose pilei, only B. ananiceps appears to lack cross-striae on the costae of the spores when viewed with the light microscope (Fig. 4E); they are very rarely present. These striae are invisible on the costae when spores are examined with the SEM for the other taxa (Fig. 3A, C-E). Perreau-Bertrand (1967) provided transmission electron micrographs (TEM) of sections through spores of B. chrysenteroides, illustrating that within the costae, there are electron-transparent lacunae. In Fig. 3C (arrow), there are holes on the sides of the costae, indicating the presence of lacunae in spores of B. deceptivus. There are indentations or holes evident in Fig. 3A (B. ananas), Fig. 3D (B. dissiliens) and Fig. 3E (B. emodensis), but absent in Fig. 3B (B. ananiceps). The lacunae would cause refraction patterns when spores are viewed with transmitted light and appear as the cross-striate bands on spore costae. Such patterns are especially evident for B. ananas, B. deceptivus, B. dissiliens and B. emodensis, whereas they are not apparent in *B. ananiceps* (Fig. 4).

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