

Affinities of the *Boletus chromapes* group to *Royoungia* and the description of two new genera, *Harrya* and *Australopilus*

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Abstract. *Harrya* is described as a new genus of Boletaceae to accommodate *Boletus chromapes*, a pink-capped bolete with a finely scabrous stipe adorned with pink scabers, a chrome yellow base and a reddish-brown spore deposit. Phylogenetic analyses of large-subunit rDNA and translation elongation factor 1 α confirmed *Harrya* as a unique generic lineage with two species, one of which is newly described (*H. atriceps*). Some Chinese taxa were recently placed in a separate genus, *Zangia*, supported by both morphology and molecular data. Multiple accessions from Queensland, Australia, support the synonymy of at least three species in a separate Australian clade in the new genus, *Australopilus*. The truffle-like *Royoungia* is also supported as a separate lineage in this clade of boletes. Even though it lacks stipe characters, it possesses the deep, bright yellow to orange pigments in the peridium. Additional collections from Zambia and Thailand represent independent lineages of uncertain phylogenetic placement in the *Chromapes* complex, but sampling is insufficient for formal description of new species. Specimens from Java referable to *Tylopilus pernanus* appear to be a sister group of the *Harrya* lineage.

Additional keywords: biogeography, Boletineae, boletes, evolution, phylogeny, ribosomal DNA, translation elongation factor 1 α .

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Boletus chromapes was described by Frost (1874) from Vermont, USA. Since then, this distinctive bolete with pink colours on the pileus, pink scabers on the stipe surface and a chrome yellow to cadmium yellow stipe base has been placed in *Ceriumyces* (Murrill 1909), *Krombholzia* (Singer 1942), *Leccinum* (Singer 1947) and *Tylopilus* (Smith and Thiers 1968). Because Murrill's concept of *Ceriumyces* is a mixture of several modern genera, subsequent placement of *B. chromapes* has been based primarily on either colour of the spore deposit or the type of surface ornamentation of the stipe. Thus, Smith and Thiers (1968,

1971) were inclined to consider the spore colour (reddish-brown) more nearly like that of a *Tylopilus*, whereas Singer (1947, 1986) judged that the stipe ornamentation was of a scabrous nature as in a *Leccinum*. Furthermore, the unique, striking morphology of *B. chromapes* elicited further distinction in a monotypic section *Roseoscabra* in *Leccinum* (Singer 1947) or in a subgenus of *Tylopilus* (Smith and Thiers 1971).

To anchor the name to a specimen, Halling (1983) designated a lectotype from among original Frost specimens in Herbarium

VT and noted that descriptions published by Snell and Dick (1970) as a *Leccinum*, and Smith and Thiers (1971) and Grund and Harrison (1976) as a *Tylopilus* adequately describe and illustrate the characters of the taxon. Treatments by Bessette *et al.* (2000) and Roody (2003) as a *Tylopilus* provide colour photographs and updated descriptions.

Wolfe and Bougher (1993) published a revision of *Tylopilus* subg. *Roseoscabra*, adding four new species from Australia, one from Costa Rica, one from Japan and three from China. They noted that *T. chromapes* had been reported additionally from China (Zang and Chen 1990), Korea (Lee and Hong 1985) and Japan (Hongo 1960, 1980). Also, hypotheses were offered for the origin of the subgenus as well as direction of co-migration with mycorrhizal hosts and subsequent divergence of populations. More recently, critical morphological and molecular analyses of Chinese materials supported recognition of a new genus with six species, distinct from *T. chromapes*, and supported the transfer of *T. chlorinosmus* Wolfe & Bougher to *Zangia* (Li *et al.* 2011). Currently, *Zangia* is known to occur only in southern China.

The aim of our studies of multiple accessions morphologically assignable to *Tylopilus* subg. *Roseoscabra* from the Americas, Australia, Indonesia, Thailand and Zambia, with support from phylogenetic inference, was to investigate the limits of genera and species in the *Boletus chromapes* group. On the basis of phenetic similarities, specimens identified as *Tylopilus pernanus* (Pat. & C.F. Baker) Watling from Java, Indonesia and *T. virens* (W.F. Chiu) Hongo from Zambia and Thailand, were included. The morphological and molecular data published by Li *et al.* (2011) prompted the inclusion of *Zangia*, and analyses of Osmundson (2009) indicated that material of *Royoungia* would be appropriate in our analyses as well.

Materials and methods

Morphological datasets

Macromorphological data were derived from fresh specimens. General colour terms are approximations, and the colour codes (e.g. 7D8) are page, column and grid designations from Kernerup and Wanscher (1983); colour names of the form 'Cadmium Yellow' (in quotation marks) are from Ridgway (1912). All microscopic structures were observed with an Olympus BHS compound microscope equipped with Nomarski differential interference contrast optics, and measured from dried material revived in 3% KOH. The letter abbreviation *Q* refers to the mean length : width ratio and *x* refers to the mean length $\times$ mean width, derived from measurements of *n* basidiospores. Herbarium codes (Thiers 2012) are cited for all collections from which morphological features were examined.

Molecular datasets

Sixty-nine sequences were newly generated from 38 collections overall. Forty-six were from 25 collections (Table 1) identified as *Tylopilus chromapes* using morphology, or were closely related to *T. chromapes* based on previous publications (Wolfe and Bougher 1993; Osmundson 2009; Li *et al.* 2011). Sequences included nuclear large-subunit rDNA (nuc-lsu) and translation elongation factor 1 α (*tefl*). Twenty-two publically available nuc-lsu and *tefl* sequences representing five species of *T. chromapes*-like taxa were used as well. Eight nuc-lsu and

eight *tefl* sequences from eight collections (Table 1) were newly generated from species identified as not being closely related to *T. chromapes*. An additional 66 nuc-lsu and *tefl* sequences already available were used to represent the major lineages of the Boletaceae (e.g. *Boletus*, *Tylopilus*, *Leccinum*, *Xerocomus*) (Binder and Hibbett 2006; Binder *et al.* 2010; Li *et al.* 2011; Halling *et al.* 2012). The final dataset consisted of 158 sequences from 82 collections. Newly generated sequences were deposited in GenBank (JX889643–JX889712; Table 1).

DNA extraction, PCR amplification, sequencing and alignments

DNA was extracted from herbarium specimens following Lee and Taylor (1990). Samples were inspected to avoid contaminated or infected tissue, up to 20 mg of pileus context tissue was ground using liquid nitrogen and the homogenised samples were resuspended in 3% sodium dodecyl sulfate extraction buffer. The cell lysate was cleaned after 45 min at 65°C by adding 0.6 mL phenol–chloroform (1 : 1), followed by an isopropyl alcohol and 3 M sodium acetate precipitation and a wash set of 100% ethanol. DNA samples were resuspended in 50 μ L Tris–EDTA buffer. PCR and sequence methods follow Halling *et al.* (2012), with the exception that *tefl* was sequenced using primers 983F, 1577F, 1567R and 2218R (Rehner and Buckley 2005). The nuc-lsu and *tefl* nucleotide datasets were aligned on the MAFFT server 6 (<http://mafft.cbrc.jp/alignment/server>, accessed May 2012) by using the G-INS-i strategy for nuc-lsu and I-INS-i for *tefl*. Both alignments were manually adjusted in MacClade 4.05 (Maddison and Maddison 2005) and concatenated into a single dataset. The alignments have been deposited in TreeBASE (#13452, <http://purl.org/phylo/treebase/phyloids/study/TB2:S13452>, accessed September 2012).

Phylogenetic analyses

The individual nuc-lsu and *tefl* datasets were analysed using maximum likelihood (ML) methods with 100 replicates to estimate the bootstrap support (BS) values by using the RAxML blackbox server (<http://phylobench.vital-it.ch/raxml-bb/>, accessed May 2012) (Stamatakis *et al.* 2008). To assess whether there was strong conflict, we performed separate analyses of the two genes; finding none, we combined the data. The combined datasets were split into a core dataset, in which every taxon had both nuc-lsu and *tefl* sequences, and an extended dataset, which had six individuals represented only by nuc-lsu (see Table 1). Thus, the concatenated datasets are highly homogeneous. The combined datasets were analysed using ML and the BS values were estimated using RAxML 7.2.6 under the GTR model parameters and 1000 rapid bootstrap inferences. Likelihood of the final tree was optimised using gamma model parameters (Stamatakis *et al.* 2005).

Posterior probability (PP) values for internodes in the combined datasets (core and extended) were estimated with MrBayes version 3.2 under the GTR model, with gamma distribution-rate setting (Ronquist *et al.* 2012). The core and extended datasets were analysed with two runs by using four MCMC chains of 14 and 30 million generations respectively, with sampling every 100 generations and an approximate 30% burn-in. Convergence of Bayesian analysis for both the core and

Table 1. Voucher information and GenBank accession numbers (new submissions in bold) for the specimens studied
 Australia: Qld, Queensland. USA: CA, California; MA, Massachusetts; NH, New Hampshire; NY, New York. See text for definition of nuc-lsu and *tefl*

Species	Collector	Isolate ID	Location	Date	nuc-lsu	<i>tefl</i>
<i>Royoungia boletoides</i>	R. Halling	8851 NY	Victoria, Australia	2 July 2006	JX889678	JX889710
<i>Royoungia boletoides</i>	J. Trappe	27546 OSC	New South Wales, Australia	23 October 1999	JX889655	JX889696
<i>Royoungia boletoides</i>	T. Lebel <i>et al.</i>	AWC4137 OSC	Victoria, Australia	28 May 2001	DQ534663	JX889700
<i>Royoungia</i> sp.	R. Halling	8774 NY	Atherton, Qld	22 February 2006	JX889660	JX889701
<i>Royoungia</i> sp.	R. Halling	9483 NY	Fraser Island, Qld	17 May 2011	JX889656	JX889697
<i>Royoungia</i> sp.	R. Halling	9544 NY	Cooloola, Qld	23 May 2011	JX889658	JX889699
<i>Royoungia</i> sp.	R. Halling	9558 NY	Cooloola, Qld	25 May 2011	JX889657	JX889698
<i>Harrya chromapes</i>	T. Osmundson	TWO996 NY	San Gerardo, Costa Rica	15 June 2004	JX889680	JX889712
<i>Harrya chromapes</i>	M. Binder	MB 03-019 CUW	Rutland, MA	17 July 2003	JX889665	JX889705
<i>Harrya chromapes</i>	M. E. Nuhn	MEN11-034 CUW	Chesterfield, NH	11 August 2011	JX889663	JX889703
<i>Harrya chromapes</i>	M. E. Nuhn	MEN11-034B CUW	Chesterfield, NH	11 August 2011	JX889666	JX889706
<i>Harrya chromapes</i>	M. E. Nuhn	MEN11-057 CUW	Chesterfield, NH	3 September 2011	JX889667	JX889707
<i>Harrya chromapes</i>	N. Davoodian	ND 4 NY	North Carolina, USA	23 June 2011	JX889664	JX889704
<i>Harrya chromapes</i>	Z. W. Ge	HKAS49416	Sichuan, China		HQ326930	HQ326863
<i>Harrya chromapes</i>	Z. W. Ge	HKAS59217	Vermont, USA		HQ326931	HQ326864
<i>Harrya chromapes</i>	Z. W. Ge	HKAS59218	New Hampshire, USA		HQ326932	HQ326865
<i>Tylopilus</i> aff. <i>chromapes</i>	D. Arora	01-513 CUW	Mutinondo, Zambia	January 2001	JX889672	JX889682
<i>Tylopilus</i> aff. <i>chromapes</i>	D. Arora	01-549 CUW	Mutinondo, Zambia	January 2001	JX889671	
<i>Harrya atriceps</i>	R. Halling	7403 NY	Copey Costa Rica	20 October 1994	JX889662	JX889702
<i>Harrya atriceps</i> isotype	R. Halling	7797 NY	Jardin, Costa Rica	1 July 1998	JX889661	
<i>Tylopilus palumanus</i> isotype	R. Halling	6791 NY	Queensland	17 February 1992	JX889650	JX889691
<i>Tylopilus queenslandianus</i> Isotype	R. Halling	6826 NY	Queensland	19 February 1992	JX889649	
<i>Australopilus palumanus</i>	T. Osmundson	TWO1096 NY	Davies Creek, Qld	9 February 2006	JX889679	JX889711
<i>Australopilus palumanus</i>	R. Halling	9275 NY	Fraser Island, Qld	25 March 2010	JX889654	JX889695
<i>Australopilus palumanus</i>	R. Halling	9304 NY	Fraser Island, Qld	29 March 2010	JX889653	JX889694
<i>Australopilus palumanus</i>	R. Halling	9421 NY	Fraser Island, Qld	16 February 2011	JX889675	JX889685
<i>Australopilus palumanus</i>	R. Halling	9433 NY	Fraser Island, Qld	18 February 2010	JX889651	JX889692
<i>Australopilus palumanus</i>	R. Halling	9445 NY	Cooloola, Qld	20 February 2011	JX889652	JX889693
<i>Tylopilus pernanus</i>	R. Halling	8061 NY	Java, Indonesia	14 January 2001	JX889644	
<i>Tylopilus pernanus</i>	R. Halling	8066 NY	Java, Indonesia	16 January 2001	JX889645	
<i>Tylopilus</i> sp.	E. Vellinga	ECV3595 UC, MFLU	Doi Suthep, Thailand	2 July 2007	JX889670	JX889681
<i>Tylopilus</i> aff. <i>virens</i>	D. Arora	01-541 CUW	Mutinondo, Zambia	January 2001	JX889677	JX889687
<i>Tylopilus virens</i>	D. Desjardin	7845 SFSU	Doi Suthep, Thailand	24 June 2005	JX889643	
<i>Aureoboletus thibetanus</i>	Z. L. Yang	AFTOL-450	Kunming, Yunnan, China		AY700189	DQ029199
<i>Austroboletus eburneus</i>	R. Halling	9487 NY	Fraser Island, Qld	17 May 2011	JX889668	JX889708
<i>Austroboletus lacunosus</i>	R. Halling	9146 NY	Fraser Island, Qld	4 June 2009	JX889669	JX889709
<i>Boletellus dissiliens</i>	R. Halling	9435 NY	Fraser Island, Qld	18 February 2011	JX889674	JX889684
<i>Boletellus projectellus</i>	M. Binder	AFTOL-713	Cape Cod, MA	14 September 2003	AY684158	AY879116
<i>Boletellus shichianus</i>	Z. L. Yang	AFTOL-532	Yunnan, China	2003	AY647211	DQ408145
<i>Boletus amygdalinus</i>	B. Neill	112605ba	Mendocino County, CA	26 November 2005	JQ327996	JQ327024
<i>Boletus appendiculatus</i>	J. Schreiner	Bap1	Bavaria, Germany	10 August 1995	AF456837	JQ327025
<i>Boletus bicolor</i> var. <i>borealis</i>	E. Both	2858	Erie County, NY		JQ326998	JQ327021
<i>Boletus calopus</i>	M. Binder	Bcl	Bavaria, Germany	7 September 1994	AF456833	JQ327019
<i>Boletus carminipes</i>	M. Binder	MB 06-061	Erie County, NY	4 August 2006	JQ327001	JQ327022

<i>Boletus edulis</i>	M. Binder	Bel	Bavaria, Germany	14 September 1994	AF050643	JQ327018
<i>Boletus inedulis</i>	M. Binder	MB 06-044	Erie County, NY	3 August 2006	JQ327013	JQ327020
<i>Boletus luridiformis</i>	A. F. S. Taylor	AT2001087	Berkshire, England, UK		JQ326995	JQ327023
<i>Boletus peckii</i>	A. R. Clark, E. Both	3959	Erie County, NY	4 August 1995	JQ326999	JQ327026
<i>Boletus variipes</i> var. <i>fagicola</i>	A. H. Smith	4249	Cheboygan, County, NY	10 August 1968	JQ327014	JQ327017
<i>Buchwaldoboletus lignicola</i>	J. Schreiner	Pul1	Maindreieck, Germany	9 September 1995	JQ326997	JQ327040
<i>Chalciporus piperatus</i>	M. Binder	MB 04-001	Rutland, MA	28 September 2004	DQ534648	GU187690
<i>Fistulinella prunicolor</i>	R. Halling	9502 NY	Fraser Island, Qld	18 May 2011	JX889648	JX889690
<i>Gymnogaster boletoides</i>	R. Halling	9455 NY	SE Qld	1 March 2011	JX889673	JX889683
<i>Hemileccinum impolitum</i>	J. Schreiner	Bim1	Bavaria, Germany	21 September 1995	AF139715	JQ327034
<i>Leccinum albellum</i>	M. Binder	MB 06-040	Erie County, NY	30 July 2006	JQ327007	JQ327038
<i>Leccinum scabrum</i>	M. Binder	Ls1	Austria	14 September 1995	AF139705	JQ327039
<i>Paxillus filamentosus</i>	H. Besl	Pfl	Bavaria, Germany	21 August 1995	AF167680	GU187736
<i>Paxillus vernalis</i>	W. Steglich	Pv2	Canada	31 August 1997	AY645059	DQ457629
<i>Phylloporus pelletieri</i>	M. Kronfeldner	Pp1	Bavaria, Germany	9 September 1996	AF456818	JQ327036
<i>Porphyrellus brunneus</i>	R. Halling	9508 NY	Fraser Island, Qld	19 May 2011	JX889646	JX889688
<i>Porphyrellus brunneus</i>	R. Halling	9527 NY	Fraser Island, Qld	20 May 2011	JX889647	JX889689
<i>Porphyrellus porphyrosporus</i>	M. Binder	MB 97-023	Bavaria, Germany	9 September 1996	DQ534643	GU187734
<i>Sutorius australiensis</i>	R. Halling	9280	Fraser Island, Qld	26 March 2011	JQ327005	JQ327031
<i>Sutorius australiensis</i>	R. Halling	9441	Cooloola, Qld	20 February 2011	JQ327006	JQ327032
<i>Sutorius eximius</i>	T. Osmundson	TWO986	La Chonta, Costa Rica	14 June 2004	JQ327009	JQ327028
<i>Sutorius eximius</i>	T. Osmundson	TWO995	San Gerardo, Costa Rica	15 June 2004	JQ327010	JQ327030
<i>Sutorius eximius</i>	R. Halling	8594	Jardin, Costa Rica	5 June 2004	JQ327008	JQ327027
<i>Sutorius eximius</i>	R. Halling	9400	Ulster County, NY	24 September 2010	JQ327004	JQ327029
<i>Sutorius</i> sp.	E. Vellinga	ECV3603	Bai Mae Sae, Thailand	4 July 2007	JQ327000	JQ327033
<i>Strobilomyces floccopus</i>	J. Enzmann	Sfl	Bavaria, Germany	12 August 1995	DQ534626	JQ327037
<i>Tylopilus balloui</i> s.l.	R. Halling	9467 NY	Fraser Island, Qld	6 March 2011	JX889676	JX889686
<i>Tylopilus felleus</i>	A. F. S. Taylor	AT2001011	Stadsskogen, Uppsala, Sweden	17 September 2001	JQ326993	JQ327015
<i>Tylopilus ferrugineus</i>	M. Binder	MB 06-053	Erie County, NY	3 August 2006	JQ326994	JQ327016
<i>Xerocomus subtomentosus</i>	J. Enzmann, A. Bresinsky	Xs1	Bavaria, Germany	10 August 1995	AF139716	JQ327035
<i>Zangia citrina</i>	Y. C. Li	HKAS52677	Fujian, China	24 August 2007	HQ326940	HQ326871
<i>Zangia citrina</i>	Y. C. Li	HKAS52684	Fujian, China	24 August 2007	HQ326941	HQ326872
<i>Zangia olivacea</i>	Z. W. Ge	HKAS55830	Yunnan, China	26 July 2006	HQ326946	HQ326874
<i>Zangia olivacea</i>	Z. L. Yang	HKAS45445	Yunnan, China	6 July 2004	HQ326945	HQ326873
<i>Zangia olivaceobrunnea</i>	Z. L. Yang	HKAS52275	Yunnan, China	9 September 2007	HQ326947	HQ326875
<i>Zangia olivaceobrunnea</i>	Z. L. Yang	HKAS52272	Yunnan, China	8 September 2007	HQ326948	HQ326876
<i>Zangia roseola</i>	Y. C. Li	HKAS52649	Yunnan, China	8 August 2007	HQ326950	HQ326878
<i>Zangia roseola</i>	Y. C. Li	HKAS51137	Yunnan, China	21 September 2006	HQ326949	HQ326877

extended datasets was checked using Tracer v1.5 (Rambaut and Drummond 2009) and AWTY online (Wilgenbusch *et al.* 2004).

Results

Phylogenetic analyses

The RAxML analysis of the core dataset had 1478 alignment patterns, with 33.82% of positions being completely undetermined or gapped. The extended dataset had 1494 alignment patterns, with 36.31% of positions being completely undetermined or gapped. In total, 290 026 trees, with a burn-in of 80 000, were used to estimate PP values for the core dataset. In total, 613 940 trees, with a burn-in of 160 000, were used to estimate PP values for the extended dataset.

The Boletaceae was supported with 100% BS and 1.0 PP values in both the core (not shown) and extended analyses (Fig. 1). The clade containing *Buchwaldoboletus lignicola* and *Chalciporus piperatus* received 100% BS and 1.0 PP support in both core and extended analyses, and is placed as the sister group to all other Boletaceae species, with 100 BS and 1.0 PP support in both analyses. This relationship has been noted previously (Binder and Hibbett 2006). The clade containing *Boletus chromapes* and relatives (i.e. *Zangia*, *Harrya*, *Australopilus*, *T. virens*, *T. pernanus* and *Royoungia*; hereafter, referred to as the 'Chromapes group') had 100% BS support and PP support of 1.0 in the core analysis and 88% BS and 0.99 PP in the extended analysis, and does not form a close relationship with any other genus in the Boletaceae (e.g. *Aureoboletus*, *Austroboletus*, *Boletellus*, *Boletus*, *Leccinum*, *Sutorius*, *Tylopilus*, *Xerocomus*).

A strong geographical pattern was recovered in the Chromapes group. In both the core and the extended tree, Australian species were recovered as monophyletic. The Australian clade contains typical epigeous boletoid 'Tylopilus' *palumanus*, as well as the gasteroid *Royoungia*, with possibly more than one taxon. In the core analysis, *Royoungia* was paraphyletic, but in the extended analysis *Royoungia* was a monophyletic sister group to the boletoid forms; there was no strongly supported conflict between the core and extended analyses with respect to the monophyly or paraphyly of *Royoungia* (Fig. 1). In the core and extended analyses, the sister clade to the Australian clade contained taxa from Thailand with typical epigeous boletoid basidiomata. The sister-group relationship of the Thai and Australian clades was supported by Bayesian extended analysis, but received low ML bootstrap support (Fig. 1).

The *Harrya* clade contains *Boletus chromapes* s.s. and includes specimens collected ~16 km from the type locality: Brattleboro, Vermont, USA (all MEN11-XXX collections). In addition, the clade contains material from Sichuan, China (HKAS49416, Table 1), Costa Rica, and elsewhere in the USA. In both analyses, specimens from Costa Rica (determined as *T. cartagoensis* on the basis of geographic location, *sensu* Wolfe and Bougher 1993; plus the new species, *H. atriceps*, described below), China and the USA were not recovered in separate clades. In the extended analysis, *Tylopilus pernanus* from Java, Indonesia, is included and is the sister group to the *Harrya* clade, although that placement received weak support. The clade containing *Harrya* and the Indonesian specimens is the sister group of the Australian clade.

The sister group to the clade containing Australian, *Harrya*, and Indonesian specimens is a clade containing three specimens from Zambia. This topology was supported by Bayesian analysis (PP only) in the core analysis, and by both likelihood and Bayesian analyses in the extended analysis. *Zangia* forms a strongly supported clade that is the sister group to all other species of the Chromapes group in both the core and extended analyses (Fig. 1). *Zangia* is morphologically similar to *H. chromapes*, but is separated based on differences in pileipellis structure (ixohyphoepithelium v. trichodermium) and molecular data (Li *et al.* 2011).

Taxonomy

Harrya Halling, Nuhn & Osmundson, *gen. nov.*

Diagnosis

Basidiomata epigeous. Pileus rose pink to brownish-pink to pinkish-grey. Hymenophore tubulose, white, then vinaceous pink. Stipe white above, chrome yellow at base, beset with fine pink scabers either isolated or rarely arranged on a raised reticulum. Spores pinkish- to reddish-brown in deposit, smooth, dextrinoid in Melzer's reagent. Hymenial cystidia present. Pseudocystidia absent. Pileipellis a trichodermium. Clamp connections absent.

Typus: *Boletus chromapes* Frost, *Bull. Buffalo Soc. Nat. Sci.* 2: 105. 1874.

Etymology: *Harry* + *a* (fem.) in honour of Harry D. Thiers, American boletologist, teacher and mentor.

Mycobank number: MB 801434.

Harrya chromapes (Frost) Halling, Nuhn, Osmundson, & Manfr. Binder, *comb. nov.* (Fig. 2)

Boletus chromapes Frost, *Bull. Buffalo Soc. Nat. Sci.* 2: 105. 1874.

Ceratomyces chromapes (Frost) Murrill, *Mycologia* 1: 145. 1909.

Krombholzia chromapes (Frost) Singer, *Ann. Mycol.* 40: 34. 1942.

Leccinum chromapes (Frost) Singer, *Amer. Midl. Nat.* 37: 124. 1947.

Tylopilus chromapes (Frost) A.H.Sm. & Thiers, *Mycologia* 60: 949. 1968.

Tylopilus cartagoensis Wolfe & Bougher, *Austral. Syst. Bot.* 6: 191. 1993.

Leccinum cartagoense (Wolfe & Bougher) Halling & G.M.Muell., *Kew Bull.* 54: 747. 1999.

Mycobank number: MB 801438.

As noted above, *H. chromapes* has been sufficiently described and well illustrated. Coker and Beers (1943) noted that the pink scabers are sometimes distributed on a reticulum (see below under *A. palumanus* and Fig. 2A). According to Wolfe and Bougher (1993), the holotype of *T. cartagoensis* differed from *H. chromapes* (as *Tylopilus*) in the pigmentation of the pileus and several microscopic features, evaluated by numerical taxonomic methods. Originally described from one collection on the western slope of Volcan Irazu, Halling and Mueller (2005) illustrated and described *T. cartagoensis* (in *Leccinum*) as occurring routinely in the Cordillera Talamanca and at Volcan Poas in Costa Rica, and noted that it was often smaller in stature,

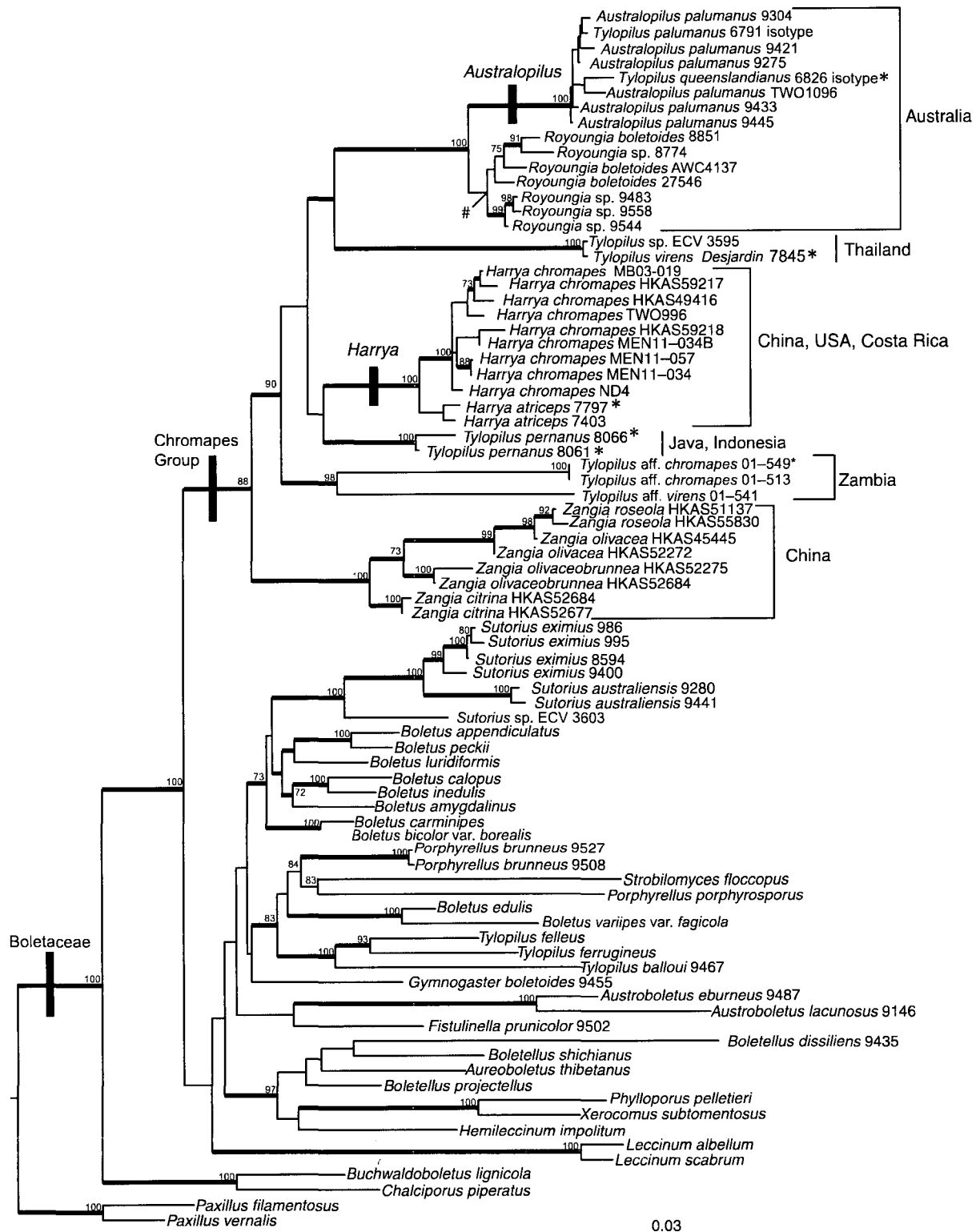


Fig. 1. Phylogenetic relationships and placement of the Chromapes group within the Boletaceae, inferred from the combined nuclear large-subunit rDNA (nuc-18S) and translation elongation factor 1 α (*tef1*) 'extended' dataset (3112 bp) using RAXML and MrBayes. The tree topology corresponds to the optimal maximum likelihood (ML) tree. Support values $\geq 70\%$ bootstrap support (BS) are shown, posterior probability (PP) values ≥ 0.95 are depicted as bold branches on the phylogeny. The node labelled with a hash sign (#) collapses in the optimal tree obtained in the ML analysis of the 'core' dataset. Individuals that lack *tef1* sequences are indicated by an asterisk (*).

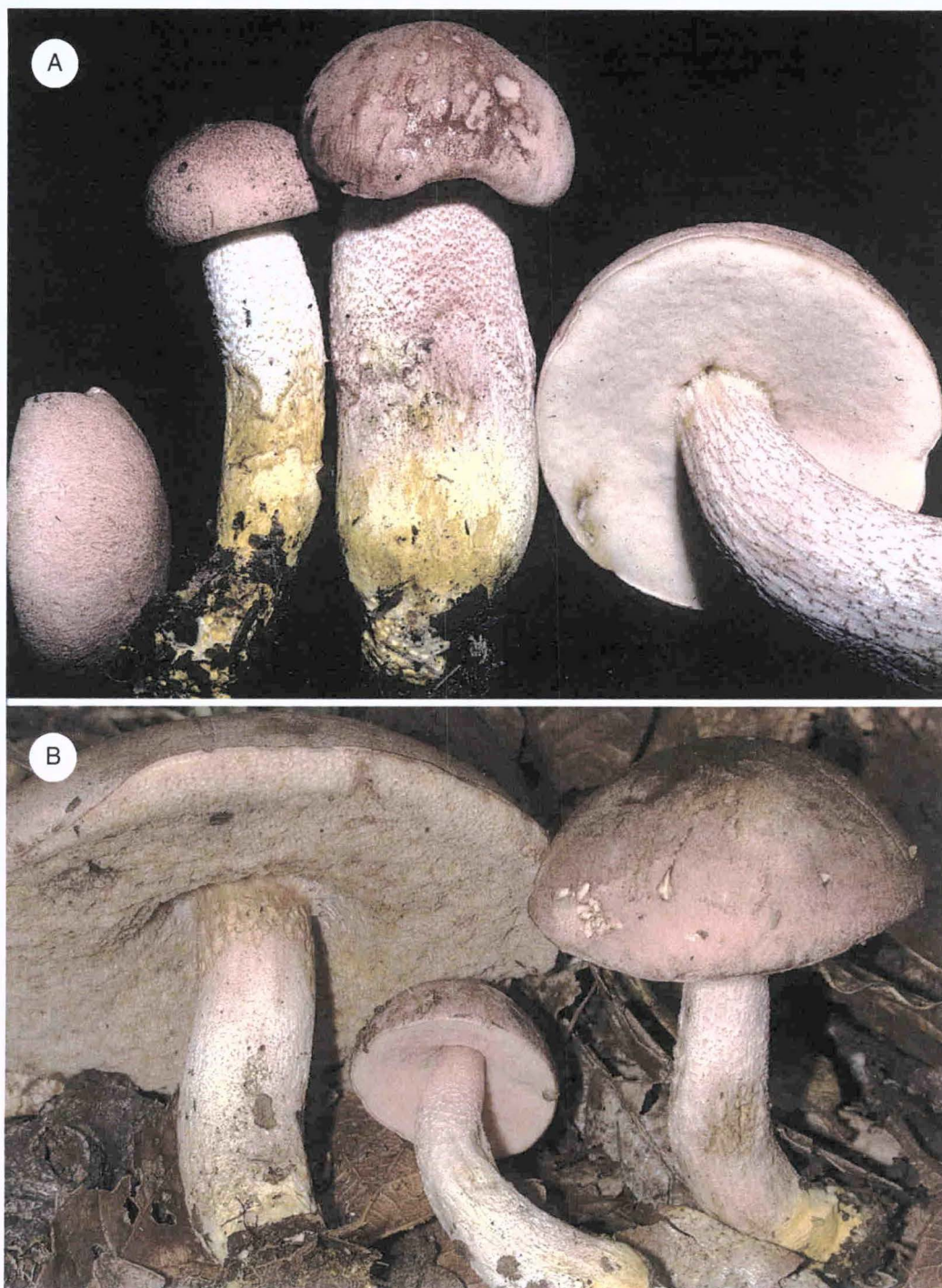


Fig. 2. *Harrya chromapes*. A. Habit. Halling 2173 $\times$ 1. B. Habit. Halling 8254 $\times$ 1.

sometimes less pink in the pileus, but otherwise scarcely differed from *H. chromapes*. The fine pink scabers on the stipe surface, the intense chrome yellow stipe base and spore features are the same. In our analyses, a specimen from Costa Rica originally determined as *T. cartagoensis* (TWO996) is nested among several of *H. chromapes*, and that specimen fits the circumscription as outlined by Wolfe and Bougher (1993), but pseudocystidia are never present. Thus, we accept *T. cartagoensis*

as a synonym of *H. chromapes* and as another example of clinal variation as noted by Halling *et al.* (2012), and in papers cited therein. Analogous results were observed with Costa Rican materials of *Sutorius eximius*.

Habit, habitat, distribution

Reported or observed among litter, on soil in forests associated with conifers, Betulaceae and *Quercus* in North America,

including eastern Canada south to Georgia, Alabama, west to Michigan and Mississippi. In Costa Rica with *Quercus*, in the Cordillera Talamanca, Poas and Irazu volcanoes. In China with the Fagaceae and Pinaceae.

Material examined

USA. [Three from among 81 specimens in NY] New York. Hamilton County: Raquette Lake, Long Point, 20 July 1983, T.J. Baroni (NY); North Carolina. Swain County: Great Smokey Mountains National Park, Indian Creek, 20 August 1992, Halling 6912 (NY); Massachusetts. Hampshire County: Depot Road, between Whately and Williamsburg, 24 July 1977, R.E. Halling 2173 (NY). COSTA RICA. [Four from among 19 specimens in NY, duplicated in USJ] San José Province: Dota, San Gerardo, Albergue de la Montaña, ± 5 km SW of Cerro de la Muerte, $9^{\circ}33'2''\text{N}$, $83^{\circ}48'27''\text{W}$, 2350 m, 19 October 1994, Halling 7386 (NY, USJ); 15 June 2004, Osmundson 996 (NY, USJ); Cartago Province: El Guarco, Tapanti, Parque Nacional Tapanti, Macizo de la Muerte, Area de Conservación La Amistad Pacifico, $9^{\circ}41'6''\text{N}$, $83^{\circ}52'30''\text{W}$, 2600 m, 6 July 2001, Halling 8258 (NY, USJ); Alajuela Province: Grecia, Bosque del Niño, $10^{\circ}9'4''\text{N}$, $84^{\circ}14'42''\text{W}$, 1900 m, 31 May 1996, Halling 7592 (NY, USJ).

Comments

On the basis of the phylogram in Fig. 1, the *Harrya* clade is supported by molecular data, but less so by geography. A distinct, second taxon from Costa Rica is also supported and described below.

Harrya atriceps Halling, G.M.Muell., & Osmundson,
sp. nov. (Figs 3, 4)

Diagnosis

Basidiomata epigeous. Pileus dry, black. Hymenophore tubulose, becoming pinkish-vinaceous. Stipe white, finely scabrous with a chrome yellow base. Spores smooth, subfusoid. Pileipellis a trichodermium. Clamp connections absent.



Fig. 3. *Harrya atriceps*. Habit. Halling 7797 $\times 1$.

Holotype: Costa Rica. San José Province: Dota, Jardín, ± 3.5 km W of Interamerican Highway at Empalme, $9^{\circ}42'52''\text{N}$, $83^{\circ}58'28''\text{W}$, 2220 m, 1 July 1998, Halling 7797 (USJ; Isotype: NY).

Mycobank number: MB 801437.

Pileus 2–5 (–6) cm broad, convex to plano-convex then plane, dry, subtomentose to tomentose, black to dark grey to dark grayish brown, rarely with any hints of pink and if so only at the margin. Flesh white, sometimes with violet red tint under pileus surface, unchanging, 5–10 mm thick, with mild odour and taste. Tubes adnexed to depressed around stipe, 1 cm deep, white to off-white, soon with pale pinkish flesh colours, with pores up to 1 mm broad, concolorous, unchanging or staining pale brown. Stipe 5–9 cm long, 5–10 mm broad, subclavate, dry, straight to curved, pinched at base, yellow overall or $\pm$ white overall and deep chrome to 'Cadmium Yellow' at the base, obscurely longitudinally ridged; surface scabers pallid to dull pale yellow (not pink or brown, even in age), confined to ridges, with interior white above, chrome yellow to cadmium orange to orange at base and rarely with some isolated light blue green staining.

Basidiospores $9.1\text{--}11.9 \times 4.2\text{--}6.3 \mu\text{m}$ ($n=20$, $x=10.7 \times 5.2 \mu\text{m}$, $Q=2.03$), smooth, fusoid to subfusoid, pale melleous in KOH, dextrinoid in Melzer's reagent. Basidia $28\text{--}42 \times 10\text{--}12 \mu\text{m}$, clavate, hyaline, four-sterigmate. Hymenial cystidia $35\text{--}48 \times 4\text{--}7 \mu\text{m}$, scattered and uncommon, thin-walled, hyaline, broadly fusoid to subcylindric. Tube trama boletoid and divergent, with melleous central strand; the lateral strata elements hyaline, $3.5\text{--}10 \mu\text{m}$ wide, subgelatinous with age. Pileipellis hyphae forming a trichodermium, with elements $5\text{--}11 \mu\text{m}$ wide, inamyloid, often containing a dark brown to black plasmatic pigment, rarely with external hyaline encrustations. Pileus trama interwoven, hyaline, rarely with oleiferous elements, consisting of inamyloid, thin-walled hyphae $3\text{--}10 \mu\text{m}$ wide. Stipitipellis hyphae vertically oriented, parallel, giving rise to clusters of caulocystidia and sometimes caulobasidia; caulocystidia subfusoid to clavate or short subfusoid with elongated rostrum, often with melleous content, $23\text{--}67 \times 7\text{--}12 \mu\text{m}$. Stipe trama hyphae parallel, cylindrical, hyaline, thin-walled, inamyloid, $5\text{--}11 \mu\text{m}$ wide. Clamp connections absent.

Habit, habitat, and distribution

Solitary to gregarious; Costa Rica, Cordillera Talamanca, under *Quercus copeyensis* and *Q. seemannii*.

Material examined

COSTA RICA. San José Province: Dota, Jardín, ± 3.5 km W of Interamerican Highway at Empalme, $9^{\circ}42'52''\text{N}$, $83^{\circ}58'28''\text{W}$, 2220 m, 1 July 1998, Halling 7797 (Holotype: USJ. Isotype: NY); 12 km S of Copey on road to Providencia, $9^{\circ}35'19''\text{N}$, $83^{\circ}53'3''\text{W}$, 2800 m, 20 October 1994, Halling 7403 (NY, USJ).

Comments

This is a rarely encountered taxon, having been found only twice over a period of 10 years of biannual collecting. *H. atriceps* was illustrated previously by Halling and Mueller (2005), although not formally described. It is easily distinguished from the sympatric, more routinely encountered *H. chromapes* by virtue

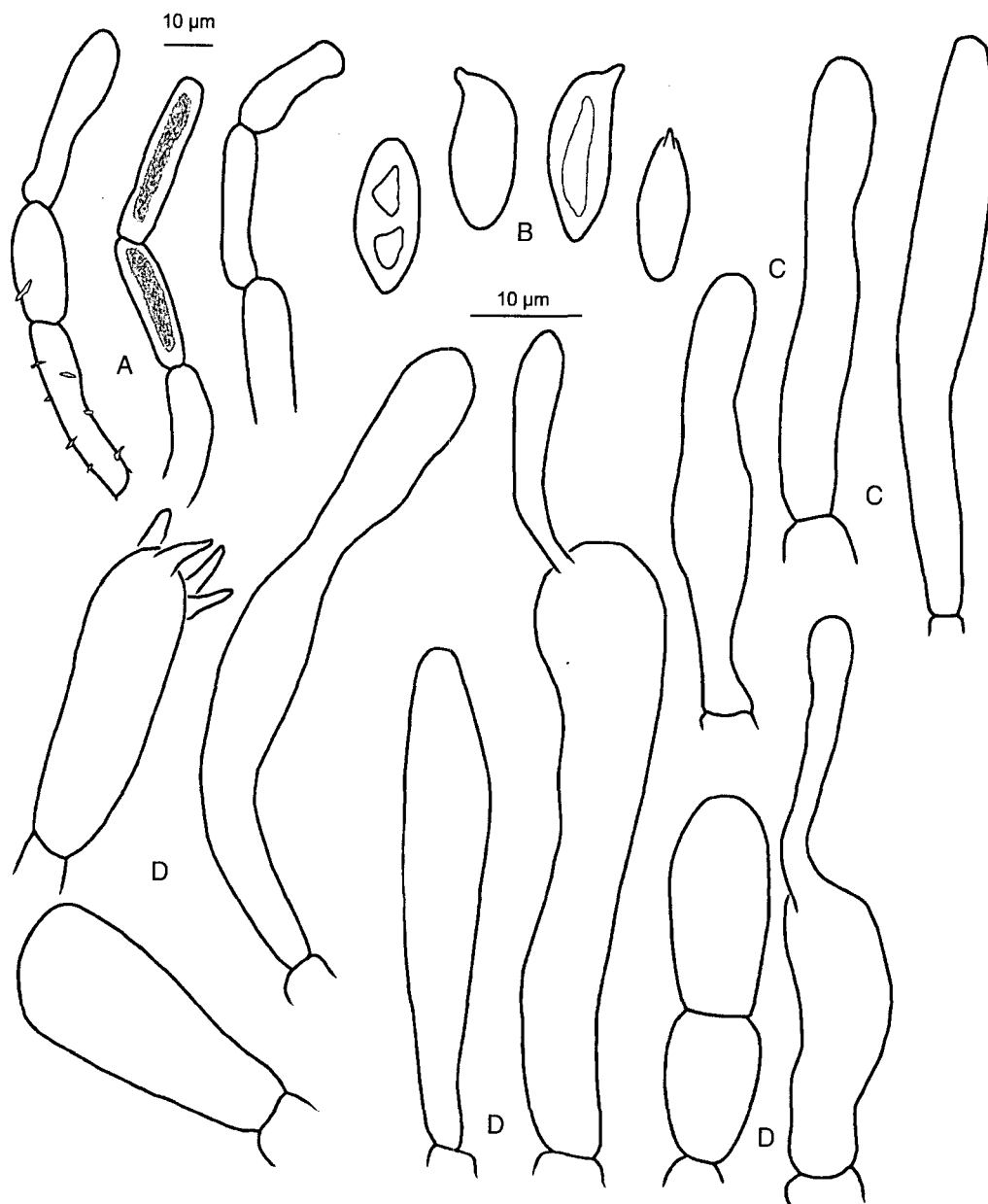


Fig. 4. Microscopic features of *Harrya atriceps*. A. Pileipellis elements. B. Basidiospores. C. Hymenial cystidia. D. Caulocystidial and caulobasidial elements (Halling 7797, holotype).

of the black pileus and a lack of pink scabers. Scabers are present, however, along with the chrome yellow to cadmium orange pigmentation in the stipe base.

Australopilus Halling & Fechner, *gen. nov.*

Diagnosis

Basidiomata epigeous. Pileus grey to dark grey, sometimes pink to deep pink pigments present. Hymenophore tubulose, white then vinaceous pink. Stipe white above, chrome yellow at base, beset with either fine isolated pink scabers or these often arranged in a well- or ill-defined raised reticulum, sometimes scattered on

low longitudinal ridges. Spores pinkish- to reddish-brown in deposit, smooth, fusoid. Pileipellis a trichodermium. Hymenial cystidia present. Pseudocystidia absent. Clamp connections absent.

Typus: *Tylopilus palumanus* Wolfe & Bougher, *Austral. Syst. Bot.* 6: 198. 1993.

Etymology: *australo* – southern, *pilus* – cap (m.).

Mycobank number: MB 801435.

Australopilus palumanus (Wolfe & Bougher) Halling & Fechner, *comb. nov.* (Fig. 5)

Tylopilus palumanus Wolfe & Bougher, *Austral. Syst. Bot.* 6: 198. 1993.



Fig. 5. *Australopilus palumanus*. A. Habit. Halling 9275 $\times 1$. B. Habit. Osmundson 1096 $\times 1$.

Tylopilus queenslandianus Wolfe & Bougher, *Austral. Syst. Bot.* 6: 200. 1993.

Tylopilus propriorichromapes Wolfe & Bougher, *Austral. Syst. Bot.* 6: 201. 1993.

?*Tylopilus subchromapes* Wolfe & Bougher, *Austral. Syst. Bot.* 6: 194. 1993.

?*Tylopilus parachromapes* T.-H.Li & Watling, *Edinburgh J. Bot.* 56: 147. 1999.

Mycobank number: MB 801436.

Original descriptions of macro- and microscopic features are given in Wolfe and Bougher (1993), but see *Comments* below.

Habit, habitat and distribution

Solitary to gregarious; Queensland, Australia, under *Acacia*, *Allocasuarina*, *Eucalyptus*, *Leptospermum*, *Melaleuca*, *Syncarpia* and *Xanthostemon*.

Material examined

AUSTRALIA. Queensland: Davies Creek National Park, Davies Creek Road, Campsites 5, 6, 17°1'36"S, 145°35'23"E, 670 m, 19 February 1992, *Halling 6826* (Holotype: *Tylopilus queenslandianus*, BRI; Isotype: NY); 9 February 2006, *Osmundson 1096* (BRI, NY); 14 March 2007 *Halling 8918* (BRI, NY); near Paluma, 17 February 1992, *Halling 6791* (Holotype: *Tylopilus palumanus*, BRI; Isotype: NY); Cooloolo (National Park), 26°2'S, 153°6'E, 10 May 1988, *N. Bougher* (ACIAR E4026, Holotype: *Tylopilus propriorichromapes*, BRI), Freshwater Road, 25°56'42.7"S, 153°5'5.4"E, 34 m, 20 February 2011, *Halling 9445* (BRI, NY); Fraser Island, track from Central Station to Eurong, 25°29'29"S, 153°6'14"E, 133 m, 5 June 2009, *Halling 9168* (BRI, NY), ±4 km W of Valley of the Giants, 25°22'2"S, 153°6'10"E, 20 m, 6 June 2009, *Halling 9174* (BRI, NY), Wanggoolba Creek Road, 25°28'47.1"S, 153°2'45.5"E, 78 m, 24 March 2010, *Halling 9258* (BRI, NY), 25°27'35"S, 153°1'11"E, 35 m, 29 March 2010, *Halling 9304* (BRI, NY), 25°28'16.3"S, 153°2'9.6"E, 24 m, 18 February 2011, *Halling 9433* (BRI, NY), road from Central Station to Lake Birraheen, 25°29'35"S, 153°3'9"E, 113 m, 25 March 2010, *Halling 9275* (BRI, NY), road from Central Station to Eurong, ±1 km W of Eurong, 25°29'S, 153°6'E, 26 March 2010, *Halling 9279* (BRI, NY), road from Eurong to Central Station, 25°29'59.4"S, 153°6'11.8"E, 90 m, 15 February 2011, *Halling 9405* (BRI, NY), Kingfisher Bay, 25°23'35.7"S, 153°1'50.7"E, 8 m, 16 February 2011, *Halling 9421* (BRI, NY).

Comments

In navigating the key to species offered by Wolfe and Bougher (1993) for *Tylopilus* subg. *Roseoscabra*, we were confronted with two obstacles. First, the user needed to ascertain whether a specimen has a raised reticulum or not. A reticulum can manifest itself (see *A. palumanus* Fig. 5A); however, it may or may not be present in the same species in any given collection (e.g. see *H. chromapes* Fig. 2A; also noted by Coker and Beers 1943) and, therefore, is not a reliable feature for taxon distinction. Second, the presence or absence of pseudocystidia had to be assessed. We have not been able to confirm the presence of pseudocystidia in any of the type material or in any recent accessions from Queensland. T. Baroni (pers. comm.) kindly examined the isotypes of *T. queenslandianus* and *T. palumanus* and could not locate any of those diagnostic sterile cells either. Pseudocystidia were reported to be present in *T. queenslandianus* and absent in *T. palumanus*. Both are supposed to possess a raised reticulum (but see Fig. 5B, *Osmundson 1096*, from within 10 m of the collecting site of the holotype for *T. queenslandianus*). The holotype specimen of *T. propriorichromapes* Wolfe & Bougher is mostly in bad condition and this is not because of poor storage or curation. Rather, it appears to have been maggot-riddled when dried. The scabers are clearly confined to a reticulum. A recent collection, *Halling 9445*, from the type locality (Cooloolo, Queensland) possesses pink scabers on a reticulum and also on the stipe surface between the ridges of the reticulum. Considering the ambiguity in distinguishing the three separate species as originally conceived, and based on the inconclusive distinction from phylogenetic inference from multiple accessions including the types, we treat these taxa as a single species. We have not seen the type of *T. subchromapes* Wolfe & Bougher and so mark it with a '?' in the nomenclator above. On the basis of basidiome size, distribution of scabers on the stipe, and statistical analyses from one specimen, we would suggest that it is conspecific as well. Li and Watling (1999) and Watling and Li (1999)

described one valid (*T. parachromapes*) and one provisional (*T. viscidichromapes*) species in this consortium, and those descriptions fit our concept of *A. palumanus*. We place the valid name in the nomenclator with a '?' also.

Royoungia boletoides Castellano, Trappe, & Malajczuk, *Austral. Syst. Bot.* 5: 614. 1992 (Fig. 6)

Castellano *et al.* (1992) described a new truffle-like fungus from Queensland, Australia, based on a single species from a single collection that they hypothesised to be a bolete. The authors stated that affinities to *Gastroboletus* were suggested because of the shape and size of the spores, the divergent trama, *Boletus*-like basidia and a yellowish pigment leaching from the peridium. Phylogenetic analyses by Osmundson (2009) showed the rather surprising result that *Royoungia* is closely related to epigeous species in the *Chromapes* group. Our current results have provided further support for this phylogenetic hypothesis by using an expanded taxon sample, refining our understanding by showing *Royoungia* to be closely related to *Australopilus*. Solely on the basis of nuc-*lsu*, Li *et al.* (2011) showed an accession (DQ534663 from Binder and Hibbett 2006) determined by one of us (J. M. Trappe) as *Royoungia boletoides*, to be sister to *Zangia*. This accession, AWC4137 (incorrectly labelled in GenBank as ACW4137) was based on a field number assigned by Andrew W. Claridge from coastal sands in Victoria, Australia. Contrary to the protologue, AWC4137 and the other accessions cited here do not have rufescent columella tissue, including other collections from the type locality (H4130, isotype in OSC; Cooloolo, Queensland), nearby sand habitats with comparable vegetation (Fraser Island) and southern Victoria (Fig. 6). Not all sequenced vouchers occur in sandy soils, however.

Phylogenetic analyses (Fig. 1) suggested that the several accessions of *Royoungia* from different regions of eastern Australia may represent more than one species. However, our goal here is not to revise *Royoungia*, but rather to show that our molecular phylogeny indicates its position among epigeous forms in Boletaceae. *Royoungia* was resolved as either monophyletic or paraphyletic in respective analyses of the extended and core datasets, but neither topology was strongly supported. Therefore, the results presented here do not reject monophyly of *Royoungia*. Moreover, a paraphyletic *Royoungia* implies a very unparsimonious scenario for morphological evolution, requiring several origins of the hypogeous-sequestrate habit (v. only one) or an even less plausible reversal to a boletoid form from a gasteroid ancestor.

Material examined

AUSTRALIA. Queensland: Davies Creek National Park, Davies Creek Road, 17°1'31"S, 145°35'52"E, 720 m, 9 February 2006, *Halling 8747* (BRI, NY); Mount Baldy Road, ±8 km from Atherton-Herberton Highway, 17°18'32"S, 145° 23'53"E, 1000 m, 22 February 2006, *Halling 8774* (BRI, NY); Wide Bay District, Great Sandy National Park, Fraser Island, ±4 km W of Valley of the Giants, 25°22'2"S, 153°6'10"E, 20 m, 6 June 2009, *Halling 9173* (BRI, NY), road from Eurong to Central Station, 25°30'1.2"S, 153°6'18.8"E, 51 m, 17 May 2011, *Halling 9483* (BRI, NY), road from Central Station to Lake Birraheen, 25°29'19.2"S, 153°4'11.4"E, 84 m, 19 May 2011, *Halling 9516* (BRI, NY), Bennet Road, 25°26'39"S, 153°2'33"E, 43 m, 20 May

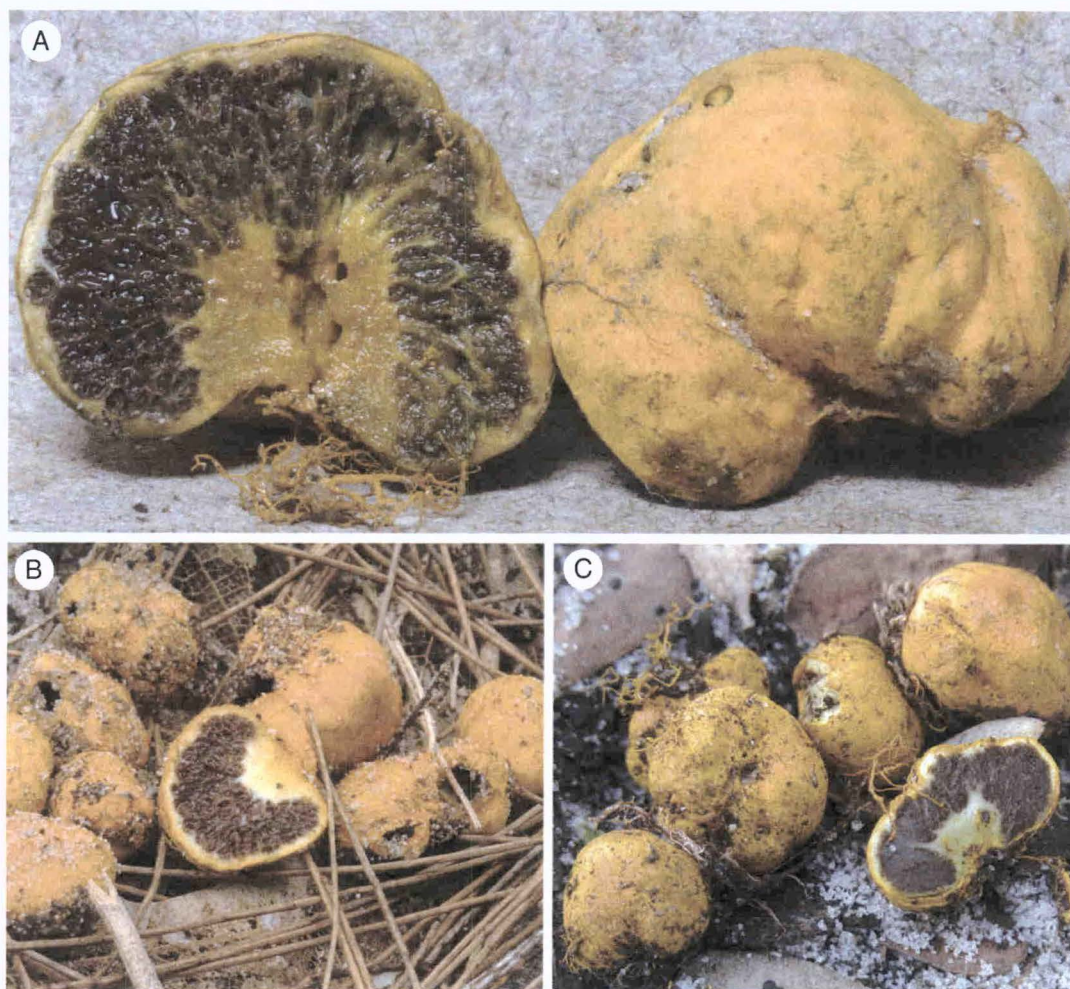


Fig. 6. *Royoungia* sp. A. Habit. Halling 9544 $\times$ 3. B. Habit. Halling 9483 $\times$ 1.5. C. Habit Halling 8851 $\times$ 2.

2011, Halling 9524 (BRI, NY); Cooloola, Freshwater Road, 25°56'36.8"S, 153°7'24.2"E, 154 m, 23 May 2011, Halling 9544 (BRI, NY), 25°57'4"S, 153°8'5.3"E, 150 m, 25 May 2011, Halling 9558 (BRI, NY). Victoria: East Gippsland, Cape Conran, $\pm$ 20 km E of Marlo, 37°47'57"S, 148°44'26"E, 16 m, 2 July 2006, Halling 8851 (MEL, NY).

Discussion

Inconclusive chemosystematical findings and convergent morphological evolution have hampered a precise placement of *H. chromapes* and allies so far. Nevertheless, the major secondary metabolites responsible for the conspicuous colorations in stipe and cap have been identified and characterised. The chrome yellow base of the stipe mainly includes atromentic acid, isoxerocomic acid, isoxerocomic acid methylester and two acetophenone derivatives (Gruber 2002, and references therein), and these components occur throughout the Boletales. The pileipellis contains the prevalent variegatorubin, which is responsible for red colours, although its precursor variegatic acid is not accumulated. This unusual finding indicates a rapid enzymatic oxidation of variegatic acid (Gruber 2002) and explains the absence of blue discoloration when

fruiting bodies are bruised. The blue discoloration is present in many *Zangia* species (Li *et al.* 2011) and it could therefore be another character that separates the genera. In addition, a pallid yellow pigment called chromaped acid has been isolated from the pileipellis (Gruber 2002). The oxidised product of chromaped acid described as 'derivative 85' has also been found in *Aspergillus nidulans* and is thought to stimulate the root growth of certain trees (Gruber 2002). Further studies are required to determine whether chromaped acid and its derivatives are useful markers for the whole Chromapes group.

The taxa treated in the present study are one of two species groups originally described in *Boletus* that have been placed in either *Tylopilus* or *Leccinum* by different authors, but do not fit comfortably in either genus. One of these, the *Sutorius* clade (= *Leccinum eximium* s.l., *Tylopilus eximius* s.l.), was recently treated by Halling *et al.* (2012). Similarly, alternative classifications have been proposed for the Chromapes group (i.e. *Zangia*, *Harrya* and *Australopilus*, but excluding *Royoungia*), depending on character-weighting judgments by different authors, such as *Tylopilus* based on the pink-tinted hymenophore and pinkish spore deposits (Smith and Thiers 1968, 1971), and *Leccinum* based on the presence of scabers

on the stipe surface (Singer 1947, 1986). As in *Sutorius*, molecular phylogenetic analyses showed that the Chromapes group exhibits phylogenetic affinity to neither *Tylopilus* nor *Leccinum*, but rather represents a distinct phylogenetic lineage warranting formal description. One entity in this group, *Zangia* – composed of *Boletus chromapes*-like taxa from southern China – was previously recognised as a distinct lineage (Li *et al.* 2011); our results showed that accessions from Australia, USA and China, Thailand and Zambia are phylogenetically distinct. Here, we formally recognise two well supported clades, composed of Australian (*Australopilus*) and American and Chinese (*Harrya*) taxa. Although the two genera are morphologically similar, the close relationship of *Australopilus* and the hypogeous *Royoungia* provides justification for separate recognition of the two epigeous lineages.

Molecular data, combined with a reassessment of morphological characters, elucidated the species-level taxonomy of the Chromapes group. In the case of the species placed in synonymy (e.g. *T. cartagoensis*), our data suggested that previously described distinguishing features either represent phenotypic variants that are not congruent with species-level entities, or are unreliable when assessed via additional examination of the type specimens and additional collections. In the case of *H. atriceps*, our results supported the recognition of a species formerly considered to be a morphological variant of *H. chromapes*; in this case, the morphological distinguishing features are congruent with molecular phylogenetic results. *H. atriceps* appears to be a rare taxon, observed only twice over a period of 10 years; formal recognition of this taxon, therefore, has implications for the biogeography and potential conservation importance of this species. Our results confirmed the relationship of the Australian Chromapes group clade (*Australopilus*) to the gasteroid genus *Royoungia*. Although differing in overall morphology, the bright yellow to bright orange pigments in the peridium of *Royoungia* and the stipe base of the epigeous taxa may represent a homologous character, although chemical evidence for homology is presently lacking.

Three additional clades in the Chromapes group have been identified with strong or maximum support values. The accessions from Thailand appear in a clade sister to the Australian clade (*Australopilus* and *Royoungia*) in our analyses; however, this placement received low bootstrap support and one accession was lacking *tef1*. Lacking strong phylogenetic evidence for the position of this clade and lacking additional specimens, we conclude that it would be premature to formally recognise this clade. Similarly, the positions of the Zambian and Indonesian clades were not well supported by phylogenetic results (three accessions lack *tef1* data) and few collections are available for each of these lineages, so we do not provide formal taxonomic recognition of these clades at present. The Zambian and Thai clades include morphological entities identified as, or recalling *Tylopilus virens*. Originally described from China (Chiu 1948), *T. virens* was not included by Li *et al.* (2011) in their analyses, and although we were able to generate some data, more collections and molecular analyses are needed.

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