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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 confirm *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.

Key words: biogeography, Boletineae, boletes, evolution, phylogeny, ribosomal DNA, translation elongation factor 1 α

Boletus chromapes was described by Frost (1874) from Vermont, USA. Since then, this distinctive bolete with pink colors on the pileus, pink scabers on the stipe surface and a chrome yellow to cadmium yellow stipe base has been placed in *Cerionomyces* (Murrill 1909), *Krombholzia* (Singer 1942), *Leccinum* (Singer 1947), and *Tylopilus* (Smith & Thiers 1968). As Murrill's concept of *Cerionomyces* is a mixture of several modern genera, subsequent placement of

B. chromapes has been based primarily on either color 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 color (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 & 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*, 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 color 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 & Chen 1990), Korea (Lee & 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 only known to occur 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. Based on 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 color terms are approximations, and the color codes (e.g., 7D8) are page, column, and grid designations from Kornerup and Wanscher (1983); color 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. – Fifty-three sequences were newly generated from 30 collections (Table I) that were identified as *Tylopilus chromapes* using morphology, or were closely related to *T. chromapes* based on previous publications (Wolfe & Bougher 1993, Li et al. 2011). Sequences include nuclear large subunit ribosomal DNA (nuc-lsu) and translation elongation factor 1 α (*tef1*). Twenty-two publically available nuc-lsu and *tef1* sequences representing five species of *T. chromapes*-like taxa were used as well. Eight nuc-lsu and eight *tef1* sequences from eight collections (Table I) were generated from species identified as not being closely related to *T. chromapes*. An additional 82 nuc-lsu and *tef1* sequences already available were used to represent the major lineages of the Boletaceae (e.g. *Boletus*, *Tylopilus*, *Leccinum*, *Xerocomus*) (Binder & Hibbett 2006, Binder et al. 2010, Halling et al. 2012, Li et al. 2011). The final dataset consisted of 158 sequences from 82 taxa. Newly generated sequences were deposited in GenBank (JX889643–JX889712, Table I).

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 homogenized samples were resuspended in 3% SDA extraction buffer. The cell lysate was cleaned after 45 minutes 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% EtOH. 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 & Buckley 2005). The nuc-lsu and *tefl* nucleotide datasets were aligned on the MAFFT server 6 (<http://mafft.cbrc.jp/alignment/server>) 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 & 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>).

Phylogenetic analyses. – The individual nuc-lsu and *tefl* datasets were analyzed using maximum likelihood methods with 100 replicates to estimate the bootstrap support values (BS) using the RAxML blackbox server (<http://phylobench.vital-it.ch/raxml-bb/>) (Stamatakis et al. 2008). To assess if 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 I). Thus, the concatenated datasets are highly homogeneous. The combined datasets were analyzed using maximum likelihood and the bootstrap support values were estimated using RAxML 7.2.6 under the GTR model parameters and 1000 rapid bootstrap inferences. Likelihood of the final tree was optimized using gamma model parameters (Stamatakis, et al. 2005).

Posterior probability (PP) values for internodes in the combined datasets (core and extended) were estimated with Mr. Bayes version 3.2 under the GTR model with gamma distribution rate setting (Ronquist et al. 2012). The core and extended datasets were analyzed with two runs 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 extended datasets was checked using Tracer v1.5 (Rambaut & 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 completely undetermined or gapped. The extended dataset had 1494 alignment patterns with 36.31% of positions completely undetermined or gapped. 290,026 trees with a burn-in of 80,000 were used to estimate PP values for the core dataset. 613,940 trees with a burn-in of 160,000 were used to estimate PP values for the extended dataset.

The Boletaceae is 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* receives 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 & 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”) has 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 is recovered in the Chromapes group. In both the core and the extended tree, Australian species are 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* is paraphyletic, but in the extended analysis *Royoungia* is a monophyletic sister group to the boletoid forms; there is 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 contains taxa from Thailand with typical epigeous boletoid basidiomata. The sister group relationship of the Thai and Australian clades is supported by Bayesian extended analysis, but receives low ML bootstrap support (Fig 1).

The *Harrya* clade contains *Tylopilus chromapes* s.s. and includes specimens collected about 16 km from the type locality: Brattleboro, Vermont, USA (all MEN11-XXX collections). In addition, the clade contains material from Sichuan, China (HKAS49416, Table I), Costa Rica,

and elsewhere in the USA. In both analyses, specimens from Costa Rica (determined as *T. cartagoensis* based on geographic location, *sensu* Wolfe & 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, though that placement receives 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 is 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 Chromapes group species 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 vs. trichodermium) and molecular data (Li et al. 2011).

Taxonomy

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

Diagnosis: Basidiomata epigeous. Pileus rose pink to brownish pink to pinkish gray. 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 honor of Harry D. Thiers, American boletologist, teacher, and mentor.

Mycobank number: MB 801434

- Harrya chromapes* (Frost) Halling, Nuhn, T. Osmundson, & Manfr. Binder *comb. nov.* Figs. 2,3
- Boletus chromapes* Frost, *Bull. Buffalo Soc. Nat. Sci.* 2: 105. 1874.
- Ceromyces 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 2). 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 with 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, 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: eastern Canada south to Georgia, Alabama, west to Michigan and Mississippi. In Costa Rica with *Quercus*, in the Cordillera Talamanca, Poas and Irazu volcanoes.

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

Commentary: Based on 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., & T. Osmundson *sp. nov.* Figs 4,5

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. Holotype: Costa Rica. San José Prov: Dota, Jardín, ±3.5 km W of Interamerican Highway at Empalme, 9°42'52"N, 83°58'28"W, 2220 m, 1 Jul 1998, *Halling 7797* (USJ; Isotype: NY).

Mycobank number: MB 801437

279
280 Pileus 2–5 (–6) cm broad, convex to plano-convex then plane, dry, subtomentose to tomentose,
281 black to dark gray to dark grayish brown, rarely with any hints of pink and if so only at the
282 margin. Flesh white, sometimes with violet red tint under pileus surface, unchanging, 5–10 mm
283 thick, with mild odor and taste. Tubes adnexed to depressed around stipe, 1 cm deep, white to
284 off-white, soon with pale pinkish flesh colors, with pores up to 1 mm broad, concolorous,
285 unchanging or staining pale brown. Stipe 5–9 cm long, 5–10 mm broad, subclavate, dry, straight
286 to curved, pinched at base, yellow overall or ±white overall and deep chrome to “Cadmium
287 Yellow” at the base, obscurely longitudinally ridged; surface scabers pallid to dull pale yellow
288 (not pink or brown, even in age), confined to ridges, with interior white above, chrome yellow to
289 cadmium orange to orange at base and rarely with some isolated light blue green staining.

290
291 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
292 subfusoid, pale melleous in KOH, dextrinoid in Melzer’s reagent. Basidia $28\text{--}42 \times 10\text{--}12 \mu\text{m}$,
293 clavate, hyaline, four-sterigmate. Hymenial cystidia $35\text{--}48 \times 4\text{--}7 \mu\text{m}$, scattered and uncommon,
294 thin-walled, hyaline, broadly fusoid to subcylindric. Tube trama boletoid and divergent, with
295 melleous central strand; the lateral strata elements hyaline, $3.5\text{--}10 \mu\text{m}$ wide, subgelatinous with
296 age. Pileipellis hyphae forming a trichodermium, with elements $5\text{--}11 \mu\text{m}$ wide, inamyloid, often
297 containing a dark brown to black plasmatic pigment, rarely with external hyaline encrustations.
298 Pileus trama interwoven, hyaline, rarely with oleiferous elements, consisting of inamyloid, thin-
299 walled hyphae $3\text{--}10 \mu\text{m}$ wide. Stipitipellis hyphae vertically oriented, parallel, giving rise to
300 clusters of caulocystidia and sometimes caulobasidia; caulocystidia subfusoid to clavate or short
301 subfusoid with elongated rostrum, often with melleous content, $23\text{--}67 \times 7\text{--}12 \mu\text{m}$. Stipe trama
302 hyphae parallel, cylindrical, hyaline, thin-walled, inamyloid, $5\text{--}11 \mu\text{m}$ wide. Clamp connections
303 absent.

304
305 *Habit, habitat, and distribution:* solitary to gregarious; Costa Rica, Cordillera Talamanca, under
306 *Quercus copeyensis*, *Q. seemannii*.

307
308 *Material examined:* COSTA RICA. San José Prov: Dota, Jardín, ±3.5 km W of Interamerican
309 Highway at Empalme, $9^{\circ}42'52''\text{N}$, $83^{\circ}58'28''\text{W}$, 2220 m, 1 Jul 1998, *Halling 7797* (Holotype:

310 USJ, Isotype: NY); 12 km S of Copey on road to Providencia, 9°35'19"N, 83°53'3"W, 2800 m, 20
311 Oct 1994, *Halling* 7403 (NY, USJ).

312
313 *Commentary:* This is a rarely encountered taxon, having been found only twice over a period of
314 10 years of biannual collecting. *Harrya atriceps* was illustrated previously by Halling and
315 Mueller (2005) but not formally described. It is easily distinguished from the sympatric, more
316 routinely encountered *H. chromapes* by virtue of the black pileus and a lack of pink scabers.
317 Scabers are present, however, along with the chrome yellow to cadmium orange pigmentation in
318 the stipe base.

319
320 ***Australopilus*** Halling and Fechner, *gen. nov.*

321
322 *Diagnosis:* Basidiomata epigeous. Pileus gray to dark gray, sometimes pink to deep pink
323 pigments present. Hymenophore tubulose, white then vinaceous pink. Stipe white above, chrome
324 yellow at base, beset with either fine isolated pink scabers or these often arranged in a well-
325 defined or ill-defined raised reticulum, sometimes scattered on low longitudinal ridges. Spores
326 pinkish to reddish brown in deposit, smooth, fusoid. Pileipellis a trichodermium. Hymenial
327 cystidia present. Pseudocystidia absent. Clamp connections absent.

328
329 Typus: *Tylopilus palumanus* Wolfe & Bougher, *Austral. Syst. Bot.* 6: 198. 1993.

330
331 Etymology: *australo* – southern, *pilus* – cap (m.).

332
333 Mycobank number: MB 801435

334
335 ***Australopilus palumanus*** (Wolfe & Bougher) Halling & Fechner, *comb. nov.* Figs 6,7

336
337 *Tylopilus palumanus* Wolfe and Bougher, *Austral. Syst. Bot.* 6: 198. 1993.

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

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

340 ?*Tylopilus subchromapes* Wolfe and 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 *Commentary* below.

Habit, habitat, and distribution: solitary to gregarious; Queensland, Australia, under *Acacia*, *Allocasuarina*, *Eucalyptus*, *Leptospermum*, *Melaleuca*, *Syncarpia*, *Xanthostemon*.

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

Commentary: 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 if a specimen has a raised reticulum or not. A reticulum can manifest itself (see *A.*

palumanus Fig 6), but it may or may not be present in the same species in any given collection (e.g., see *H. chromapes* Fig 2; also noted by Coker & 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 com) 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 7, Osmundson 1996, 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 (Cooloola, Queensland) possesses pink scabers on a reticulum but 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. Based on basidiome size, distribution of scabers on the stipe, and statistical analyses from one specimen, we would suggest it is conspecific as well. Watling and Li (1999a,b) have described one valid (*T. parachromapes*) and one provisional species (*T. viscidichromapes*) 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. Figs 8–10

Castellano et al (1992) described a new truffle-like fungus from Queensland, Australia based on a single species from a single collection that they hypothesized 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 provide further support for this phylogenetic hypothesis using an expanded taxon sample, refining our understanding by showing *Royoungia* to be closely related to *Australopilus*. Based solely on nucleotide sequence, Li et al. (2011) showed an accession (DQ534663 from Binder & Hibbett 2006) determined by one of us (JMT) as *Royoungia boletoides*, to be sister to *Zangia*. This accession, AWC4137 (incorrectly labeled 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; Cooloola, Queensland), nearby sand habitats with comparable vegetation (Fraser Island), and southern Victoria (Figs 8–10). Not all sequenced vouchers occur in sandy soils however.

Phylogenetic analyses (Fig. 1) suggest 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* is resolved as either monophyletic or paraphyletic in analyses of the extended and core datasets, respectively, but neither topology is 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 (vs. 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 Rd, 17°1'31"S, 145°35'52"E, 720 m, 9 Feb 2006, *Halling* 8747, (BRI, NY); Mt. Baldy Rd, ±8 km from Atherton-Herberton Highway, 17°18'32"S, 145° 23'53"E, 1000 m, 22 Feb 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 Jun 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 Birrabreen, 25°29'19.2"S, 153°4'11.4"E, 84 m, 19 May 2011, *Halling* 9516 (BRI, NY), Bennet Rd, 25°26'39"S, 153°2'33"E, 43 m, 20 May 2011, *Halling* 9524 (BRI, NY); Cooloola, Freshwater Road, 25°56'36.8"S, 153°7'24.2"E, 154 m, 23

434 May 2011, *Halling 9544* (BRI, NY), 25°57'4"S, 153°8'5.3"E, 150 m, 25 May 2011, *Halling 9558*
435 (BRI, NY). Victoria. East Gippsland, Cape Conran, ±20 km E of Marlo, 37°47'57"S,
436 148°44'26"E, 16 m, 2 Jul 2006, *Halling 8851* (MEL, NY).

437
438 *Discussion:* Inconclusive chemosystematical findings and convergent morphological evolution
439 have hampered a precise placement of *H. chromapes* and allies so far. Nevertheless, the major
440 secondary metabolites responsible for the conspicuous colorations in stipe and cap have been
441 identified and characterized. The chrome yellow base of the stipe mainly includes atromentic
442 acid, isoxerocomic acid, isoxerocomic acid methylester, and two acetophenone derivatives
443 (Gruber 2002 and references therein), and these components occur throughout the Boletales. The
444 pileipellis contains the prevalent variegatorubin, which is responsible for red colors, but its
445 precursor variegatic acid is not accumulated. This unusual finding indicates a rapid enzymatic
446 oxidation of variegatic acid (Gruber 2002) and explains the absence of blue discolorations when
447 fruiting bodies are bruised. The blue discoloration is present in many *Zangia* species (Li et al
448 2011) and it could therefore be another character that separates the genera. In addition, a pallid
449 yellow pigment called chromaped acid has been isolated from the pileipellis (Gruber 2002). The
450 oxidized product of chromaped acid described as 'derivative 85' has also been found in
451 *Aspergillus nidulans* and is thought to stimulate the root growth of certain trees (Gruber 2002).
452 Further studies are required to determine if chromaped acid and its derivatives are useful markers
453 for the whole Chromapes group.

454
455 The taxa treated in this study are one of two species groups originally described in *Boletus* that
456 have been placed in either *Tylopilus* or *Leccinum* by different authors, but do not fit comfortably
457 in either genus. One of these, the *Sutorius* clade (= *Leccinum eximium* s.l., *Tylopilus eximius* s.l.),
458 was recently treated by Halling et al. (2012). Similarly, alternative classifications have been
459 proposed for the Chromapes group (i.e., *Zangia*, *Harrya* and *Australopilus*, but excluding
460 *Royoungia*), depending upon character weighting judgments by different authors: *Tylopilus*
461 based on the pink-tinted hymenophore and pinkish spore deposits (Smith & Thiers 1968, 1971),
462 and *Leccinum* based on the presence of scabers on the stipe surface (Singer 1947, 1986). As in
463 *Sutorius*, molecular phylogenetic analyses show that the Chromapes group exhibits phylogenetic
464 affinity to neither *Tylopilus* nor *Leccinum*, but 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 recognized as a distinct lineage (Li et al., 2011); our results show that accessions from Australia, North America and China, Thailand, and Zambia are phylogenetically distinct. Here, we formally recognize two well-supported clades, composed of Australian, and American and Chinese taxa, as *Australopilus* and *Harrya*, respectively. 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, elucidate the species-level taxonomy of the Chromapes group. In the case of the species placed in synonymy (*T. cartagoensis* etc.), our data suggest 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 *Harrya atriceps*, our results support 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. *Harrya 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 confirm 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, though 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 receives low bootstrap support and one accession is lacking *tefl*. Lacking strong phylogenetic evidence for the position of this clade and lacking additional specimens, we conclude that it would be premature to formally recognize this clade. Similarly, the positions of the Zambian and Indonesian clades are not well-supported by phylogenetic results (three accessions lack *tefl* 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 while we were able to generate some data, more collections and molecular analyses are needed.

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Literature Cited

Bessette, AE, Roody WC, Bessette AR. 2000. North American Boletes: A Color Guide to the Fleshy Pored Mushrooms. Syracuse University Press. 396 p.

- 527 Binder M, Hibbett DS. 2006. Molecular systematics and biological diversification of Boletales.
528 Mycologia 98: 971–981.
529
- 530 ———, Larsson K-H, Matheny PB, Hibbett DS. 2010. Amylocorticiales ord. nov. and Jaapiales
531 ord. nov.: early-diverging clades of Agaricomycetidae dominated by corticioid forms. Mycologia
532 102: 865–880.
533
- 534 Castellano MA, Trappe JM, Malajczuk N. 1992. Australasian Truffle-like Fungi. III. *Royoungia*
535 gen. nov. and *Mycoamaranthus* gen. nov. (Basidiomycotina). Australian Systematic Botany 5:
536 613–616.
537
- 538 Chiu WF. 1948. The boletes of Yunnan. Mycologia 40: 199–231.
539
- 540 Coker WC, Beers AH. 1943. The Boletaceae of North Carolina. University of North Carolina
541 Press, Chapel Hill. 96 p.
542
- 543 Frost CC. 1874. Catalogue of boleti of New England, with descriptions of new species. Bulletin
544 Buffalo Society of Natural Science 2:100–105.
545
- 546 Gruber G. 2002. Isolierung und Strukturaufklärung von chemotaxonomisch relevanten
547 Sekundärmetaboliten aus höheren Pilzen, insbesondere aus der Ordnung der Boletales.
548 Dissertation, Ludwig-Maximilians-Universität München, Munich, Germany.
549
- 550 Grund DW, Harrison KA. 1976. Nova Scotian boletes. Bibliotheca Mycologica 47:1–283.
551
- 552 Halling, RE. 1983. Boletes described by Charles C. Frost. Mycologia 75:70–92.
553
- 554 ———, Mueller GM. 2005. Common Mushrooms of the Talamanca Mountains, Costa Rica. The
555 New York Botanical Garden Press, Bronx. 195 p.
556

- 557 ———, Nuhn M, Fechner NA, Osmundson TW, Soyong K, Arora D, Hibbett DS, Binder M.
558 2012. *Sutorius*: A new genus for *Boletus eximius*. Mycologia 104:951–961.
559
- 560 Hongo, T. 1960. The Agaricales of Japan I-2. Rhodophyllaceae, Paxillaceae, Gomphidiaceae,
561 Boletaceae and Strobilomycetaceae. Acta Phytotaxonomica et Geobotanica 18:97–112.
562
- 563 ———. 1980. *Tylopilus* of western Japan. Memoirs Shiga University 30:63–67.
564
- 565 Kornerup A, Wanscher JH. 1983. Methuen handbook of colour. Ed. 3. Eyre Methuen, Ltd.,
566 London.
567
- 568 Lee J-Y, Hong S-W. 1985. Illustrated flora and fauna of Korea. Vol. 28 Mushrooms. Ministry of
569 Education, Seoul. 962 p.
570
- 571 Lee SB, Taylor JW. 1990. Isolation of DNA from fungal mycelia and single cells. In: Innis MA,
572 Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols, a guide to methods and applications. San
573 Diego, USA: Academic Press. p 282–287.
574
- 575 Li Y-C, Feng B, Yang Z-L. 2011. *Zangia*, a new genus of Boletaceae supported by molecular
576 and morphological evidence. Fungal Diversity 49:125–143.
577
- 578 Maddison DR, Maddison WP. 2005. MacClade 4: Analysis of Phylogeny and Character
579 Evolution. Sinauer Associates, Sunderland, Massachusetts.
580
- 581 Murrill WA. 1909. The Boletaceae of North America – II. Mycologia 1:140–158.
582
- 583 Osmundson TW. 2009. Systematic, biogeographical, and ecological perspectives on the diversity
584 and conservation of microbial obligate symbionts, using mycorrhizal boletes (Fungi) as exemplar
585 taxa. Ph.D. Dissertation, Columbia University, New York, NY.
586

- Rambaut A, Drummond AJ. 2009. Tracer v1.5. Available from
<http://tree.bio.ed.ac.uk/software/tracer>
- Rehner SA, Buckley EP. 2005. Cryptic diversification in *Beauveria bassiana* inferred from nuclear its and ef1- alpha phylogenies. *Mycologia* 97:84–98.
- Ridgway R. 1912. Color standards and color nomenclature. Published by the author, Washington DC. 43 p, 53 pl.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, S. Höhna S, B. Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542
- Roody WC. 2003. Mushrooms of West Virginia and the Central Appalachians. University Press of Kentucky, Lexington. 520 p.
- Singer R. 1942. Das System der Agaricales. II. *Annales Mycologici* 40: 1–132.
- . 1947. The Boletoidae of Florida. The Boletineae of Florida with notes on extralimital species III. *American Midland Naturalist* 37:1–135.
- . 1986. The Agaricales in Modern Taxonomy. Koeltz Scientific Books, Koenigstein, Germany. 981 p.
- Smith AH, Thiers HD. 1968. Notes on boletes—I 1. The generic position of *Boletus subglabripes* and *Boletus chromapes* 2. A comparison of four species of *Tylopilus*. *Mycologia* 60:943–954.
- , ———. 1971. The Boletes of Michigan. University of Michigan Press, Ann Arbor. 428 p.

- 618 Snell WH, Dick EA. 1970. The Boleti of Northeastern North America. Cramer, Lehre. 115 p.
619
- 620 Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web
621 servers. *Systematic Biology* 57:758–758.
622
- 623 ———, Ludwig T, Meier H. 2005. RAxML-III: a fast program for maximum likelihood-based
624 inference of large phylogenetic trees. *Bioinformatics* 21:456–456.
625
- 626 Thiers BM. 2012. [27 June 2012]. Index Herbariorum: A global directory of public herbaria and
627 associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>.
628
- 629 Watling R, Li T-H. 1999a. New taxa and combinations of Australian boletes. *Edinburgh Journal*
630 *of Botany* 56:143–148.
631
- 632 ———, ———. 1999b. Australian boletes: a preliminary survey. Royal Botanic Garden,
633 Edinburgh. 71 p.
634
- 635 Wilgenbusch JC, Warren DL, Swofford DL. 2004. AWTY: A system for graphical exploration
636 of MCMC convergence in Bayesian phylogenetic inference. <http://ceb.csit.fsu.edu/awty>.
637
- 638 Wolfe CB Jr, Bougher NL. 1993. Systematics, mycogeography, and evolutionary history of
639 *Tylopilus* subg. *Roseoscabra* in Australia elucidated by comparison with Asian and American
640 species. *Australian Systematic Botany* 6:187–213.
641
- 642 Zang M, Chen KK. 1990. Ectomycorrhizal fungi associated with alpine conifers from
643 southwestern China. *Acta Mycologica Sinica* 9:128–136.
644

Table 1. Voucher information and GenBank accession numbers (new submissions in boldface) for the specimens studied.

Figure 1. Phylogenetic relationships and placement of the *Chromapes* group within the Boletaceae inferred from the combined nuc-lsu+tef1 “extended” dataset (3112 bp) using RAxML and Mr. Bayes. The tree topology corresponds to the optimal maximum likelihood tree. Support values $\geq 70\%$ BS are shown, PP values ≥ 0.95 are depicted as bold branches on the phylogeny. The node labeled with a pound 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 (*).

Figures 2–3. *Harrya chromapes*. Habit. 2. *Halling* 2173 $\times 1$. 3. *Halling* 8254 $\times 1$.

Figure 4. *Harrya atriceps*. Habit. *Halling* 7797 $\times 1$.

Figure 5. Microscopic features of *Harrya atriceps*. a. pileipellis elements. b. basidiospores. c. hymenial cystidia. d. caulocystidial elements (*Halling* 7797, holotype).

Figures. 6–7 *Australopilus palumanus*. Habits. 6. *Halling* 9275 $\times 1$. 7. *Osmundson* 1096 $\times 1$.

Figures 8–10. *Royoungia* sp. Habits. 8. *Halling* 9544 $\times 10$. 9. *Halling* 8851 $\times 3$. 10. *Halling* 9483 $\times 3$.

| Table I. Voucher information and GenBank accession numbers (new accessions in boldface) | | | | | | |
|---|-----------------|------------------|--|-------------|----------|-------------|
| Species | Collector | Isolate ID | Location | Date | nuc-lsu | <i>tefl</i> |
| <i>Royoungia boletoides</i> | R. Halling | 8851 NY | Victoria, Australia | 2 Jul 2006 | JX889678 | JX889710 |
| <i>Royoungia boletoides</i> | J. Trappe | 27546 OSC | New South Wales, Australia | 23 Oct 1999 | JX889655 | JX889696 |
| <i>Royoungia boletoides</i> | T. Lebel et al. | AWC4137 OSC | Victoria, Australia | 28 May 2001 | DQ534663 | JX889700 |
| <i>Royoungia</i> sp. | R. Halling | 8774 NY | Atherton, Queensland, Australia | 22 Feb 2006 | JX889660 | JX889701 |
| <i>Royoungia</i> sp. | R. Halling | 9483 NY | Fraser Island, Queensland, Australia | 17 May 2011 | JX889656 | JX889697 |
| <i>Royoungia</i> sp. | R. Halling | 9544 NY | Cooloolo, Queensland, Australia | 23 May 2011 | JX889658 | JX889699 |
| <i>Royoungia</i> sp. | R. Halling | 9558 NY | Cooloolo, Queensland, Australia | 25 May 2011 | JX889657 | JX889698 |
| <i>Harrya chromapes</i> | T. Osmundson | TWO996 NY | San Gerardo, Costa Rica | 15 Jun 2004 | JX889680 | JX889712 |
| <i>Harrya chromapes</i> | M. Binder | MB 03-019 CUW | Rutland, Massachusetts, USA | 17 Jul 2003 | JX889665 | JX889705 |

| | | | | | | |
|---------------------------------|--------------|-------------------|--|-------------|----------|----------|
| <i>Harrya chromapes</i> | M.E. Nuhn | MEN11-034 CUW | Chesterfield, New Hampshire, USA | 11 Aug 2011 | JX889663 | JX889703 |
| <i>Harrya chromapes</i> | M.E. Nuhn | MEN11-034B CUW | Chesterfield, New Hampshire, USA | 11 Aug 2011 | JX889666 | JX889706 |
| <i>Harrya chromapes</i> | M.E. Nuhn | MEN11-057 CUW | Chesterfield, New Hampshire, USA | 3 Sept 2011 | JX889667 | JX889707 |
| <i>Harrya chromapes</i> | N. Davoodian | ND 4 NY | N. Carolina, USA | 23 Jun 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 aff. chromapes</i> | D. Arora | 01-513 | Mutinondo, Zambia | Jan 2001 | JX889672 | JX889682 |
| <i>Tylopilus aff. chromapes</i> | D. Arora | 01-549 | Mutinondo, Zambia | Jan 2001 | JX889671 | |
| <i>Harrya atriceps</i> | R. Halling | 7403 NY | Copey Costa Rica | 20 Oct 1994 | JX889662 | JX889702 |

| | | | | | | |
|---|--------------|---------------|--|-------------|----------|----------|
| <i>Harrya atriceps</i> Isotype | R. Halling | 7797 NY | Jardín, Costa Rica | 1 Jul 1998 | JX889661 | |
| <i>Tylopilus palumanus</i> Isotype | R. Halling | 6791 NY | Queensland, Australia | 17 Feb 1992 | JX889650 | JX889691 |
| <i>Tylopilus queenslandianus</i> Isotype | R. Halling | 6826 NY | Queensland, Australia | 19 Feb 1992 | JX889649 | |
| <i>Australopilus palumanus</i> | T. Osmundson | TWO1096 NY | Davies Creek, Queensland, Australia | 9 Feb 2006 | JX889679 | JX889711 |
| <i>Australopilus palumanus</i> | R. Halling | 9275 NY | Fraser Island, Queensland, Australia | 25 Mar 2010 | JX889654 | JX889695 |
| <i>Australopilus palumanus</i> | R. Halling | 9304 NY | Fraser Island, Queensland, Australia | 29 Mar 2010 | JX889653 | JX889694 |
| <i>Australopilus palumanus</i> | R. Halling | 9421 NY | Fraser Island, Queensland, Australia | 16 Feb 2011 | JX889675 | JX889685 |
| <i>Australopilus palumanus</i> | R. Halling | 9433 NY | Fraser Island, Queensland, Australia | 18 Feb 2010 | JX889651 | JX889692 |
| <i>Australopilus palumanus</i> | R. Halling | 9445 NY | Cooloola, Queensland, Australia | 20 Feb 2011 | JX889652 | JX889693 |
| <i>Tylopilus pernanus</i> | R. Halling | 8061 NY | Java, Indonesia | 14 Jan 2001 | JX889644 | |

| | | | | | | |
|-------------------------------------|--------------|---------------------|--|--------------|----------|----------|
| <i>Tylopilus pernanus</i> | R. Halling | 8066 NY | Java, Indonesia | 16 Jan 2001 | JX889645 | |
| <i>Tylopilus</i> sp. | E. Vellinga | ECV3595 UC, MFLU | Doi Suthep, Thailand | 2 Jul 2007 | JX889670 | JX889681 |
| <i>Tylopilus</i> aff. <i>virens</i> | D. Arora | 01-541 CUW | Mutinondo, Zambia | Jan 2001 | JX889677 | JX889687 |
| <i>Tylopilus virens</i> | D. Desjardin | 7845 SFSU | Doi Suthep, Thailand | 24 Jun 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, Queensland, Australia | 17 May 2011 | JX889668 | JX889708 |
| <i>Austroboletus lacunosus</i> | R. Halling | 9146 NY | Fraser Island, Queensland, Australia | 4 Jun 2009 | JX889669 | JX889709 |
| <i>Boletellus dissiliens</i> | R. Halling | 9435 NY | Fraser Island, Queensland, Australia | 18 Feb 2011 | JX889674 | JX889684 |
| <i>Boletellus projectellus</i> | M. Binder | AFTOL-713 | Cape Cod, MA, USA | 14 Sept 2003 | AY684158 | AY879116 |
| <i>Boletellus shichianus</i> | Z.L. Yang | AFTOL-532 | Yunnan, China | 2003 | AY647211 | DQ408145 |

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| <i>Boletus amygdalinus</i> | B. Neill | 112605ba | Mendocino Co, CA, USA | 26 Nov 2005 | JQ327996 | JQ327024 |
| <i>Boletus appendiculatus</i> | J. Schreiner | Bap1 | Bavaria, Germany | 10 Aug 1995 | AF456837 | JQ327025 |
| <i>Boletus bicolor</i> var. <i>borealis</i> | E. Both | 2858 | Erie Co, NY, USA | | JQ326998 | JQ327021 |
| <i>Boletus calopus</i> | M. Binder | Bc1 | Bavaria, Germany | 7 Sept 1994 | AF456833 | JQ327019 |
| <i>Boletus carminipes</i> | M. Binder | MB 06-061 | Erie Co, NY, USA | 4 Aug 2006 | JQ327001 | JQ327022 |
| <i>Boletus edulis</i> | M. Binder | Be1 | Bavaria, Germany | 14 Sept 1994 | AF050643 | JQ327018 |
| <i>Boletus inedulis</i> | M. Binder | MB 06-044 | Erie Co, NY, USA | 3 Aug 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 Co, NY, USA | 4 Aug 1995 | JQ326999 | JQ327026 |
| <i>Boletus variipes</i> var. <i>fagicola</i> | A.H. Smith | 4249 | Cheboygan, Co, NY, USA | 10 Aug 1968 | JQ327014 | JQ327017 |

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|-----------------------------------|----------------|-----------|--------------------------------------|--------------|----------|----------|
| <i>Buchwaldoboletus lignicola</i> | J. Schreiner | Pul1 | Maindreieck, Germany | 9 Sept 1995 | JQ326997 | JQ327040 |
| <i>Chalciporus piperatus</i> | M. Binder | MB 04-001 | Rutland, MA, USA | 28 Sept 2004 | DQ534648 | GU187690 |
| <i>Fistulinella prunicolor</i> | R. Halling | 9502 NY | Fraser Island, Queensland, Australia | 18 May 2011 | JX889648 | JX889690 |
| <i>Gymnogaster boletoides</i> | R. Halling | 9455 NY | SE Queensland, Australia | 1 Mar 2011 | JX889673 | JX889683 |
| <i>Hemileccinum impolitum</i> | J. Schreiner | Bim1 | Bavaria, Germany | 21 Sept 1995 | AF139715 | JQ327034 |
| <i>Leccinum albellum</i> | M. Binder | MB 06-040 | Erie Co, NY, USA | 30 Jul 2006 | JQ327007 | JQ327038 |
| <i>Leccinum scabrum</i> | M. Binder | Ls1 | Austria | 14 Sept 1995 | AF139705 | JQ327039 |
| <i>Paxillus filamentosus</i> | H. Besl | Pf1 | Bavaria, Germany | 21 Aug 1995 | AF167680 | GU187736 |
| <i>Paxillus vernalis</i> | W. Steglich | Pv2 | Canada | 31 Aug 1997 | AY645059 | DQ457629 |
| <i>Phylloporus pelletieri</i> | M. Kronfeldner | Pp1 | Bavaria, Germany | 9 Sept 1996 | AF456818 | JQ327036 |

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| <i>Porphyrellus brunneus</i> | R. Halling | 9508 NY | Fraser Island, Queensland, Australia | 19 May 2011 | JX889646 | JX889688 |
| <i>Porphyrellus brunneus</i> | R. Halling | 9527 NY | Fraser Island, Queensland Australia | 20 May 2011 | JX889647 | JX889689 |
| <i>Porphyrellus porphyrosporus</i> | M. Binder | MB 97-023 | Bavaria, Germany | 9 Sept 1996 | DQ534643 | GU187734 |
| <i>Sutorius australiensis</i> | R. Halling | 9280 | Fraser Island, Queensland, Australia | 26 Mar 2011 | JQ327005 | JQ327031 |
| <i>Sutorius australiensis</i> | R. Halling | 9441 | Cooloola, Queensland, Australia | 20 Feb 2011 | JQ327006 | JQ327032 |
| <i>Sutorius eximius</i> | T. Osmundson | TWO986 | La Chonta, Costa Rica | 14 Jun 2004 | JQ327009 | JQ327028 |
| <i>Sutorius eximius</i> | T. Osmundson | TWO995 | San Gerardo, Costa Rica | 15 Jun 2004 | JQ327010 | JQ327030 |
| <i>Sutorius eximius</i> | R. Halling | 8594 | Jardín, Costa Rica | 5 Jun 2004 | JQ327008 | JQ327027 |
| <i>Sutorius eximius</i> | R. Halling | 9400 | Ulster Co, NY, USA | 24 Sept 2010 | JQ327004 | JQ327029 |
| <i>Sutorius sp</i> | E. Vellinga | ECV3603 | Bai Mae Sae, Thailand | 4 Jul 2007 | JQ327000 | JQ327033 |

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| <i>Strobilomyces floccopus</i> | J. Enzmann | Sf1 | Bavaria, Germany | 12 Aug 1995 | DQ534626 | JQ327037 |
| <i>Tylopilus balloui</i> s.l. | R. Halling | 9467 NY | Fraser Island, Queensland, Australia | 6 Mar 2011 | JX889676 | JX889686 |
| <i>Tylopilus felleus</i> | A.F.S. Taylor | AT2001011 | Stadsskogen, Uppsala, Sweden | 17 Sept 2001 | JQ326993 | JQ327015 |
| <i>Tylopilus ferrugineus</i> | M. Binder | MB 06-053 | Erie Co, NY, USA | 3 Aug 2006 | JQ326994 | JQ327016 |
| <i>Xerocomus subtomentosus</i> | J. Enzmann, A. Bresinsky | Xs1 | Bavaria, Germany | 10 Aug 1995 | AF139716 | JQ327035 |
| <i>Zangia citrina</i> | Y.C. Li | HKAS52677 | Fujian, China | 24 Aug 2007 | HQ326940 | HQ326871 |
| <i>Zangia citrina</i> | Y.C. Li | HKAS52684 | Fujian, China | 24 Aug 2007 | HQ326941 | HQ326872 |
| <i>Zangia olivacea</i> | Z.W. Ge | HKAS55830 | Yunnan, China | 26 Jul 2006 | HQ326946 | HQ326874 |
| <i>Zangia olivacea</i> | Z.L. Yang | HKAS45445 | Yunnan, China | 6 Jul 2004 | HQ326945 | HQ326873 |
| <i>Zangia olivaceobrunnea</i> | Z.L. Yang | HKAS52275 | Yunnan, China | 9 Sep 2007 | HQ326947 | HQ326875 |

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| <i>Zangia olivaceobrunnea</i> | Z.L. Yang | HKAS52272 | Yunnan, China | 8 Sep 2007 | HQ326948 | HQ326876 |
| <i>Zangia roseola</i> | Y.C. Li | HKAS52649 | Yunnan, China | 8 Aug 2007 | HQ326950 | HQ326878 |
| <i>Zangia roseola</i> | Y.C. Li | HKAS51137 | Yunnan, China | 21 Sep 2006 | HQ326949 | HQ326877 |

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