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Rubinoboletus phaseolisporus (Boletaceae) from Western Australia is a *Tylopilus* with bean-shaped spores

Todd W. Osmundson[1], Neale L. Bougher[2], Richard M. Robinson[3,5] and Roy E. Halling[4,6]

[1]Biology Department, University of Wisconsin-La Crosse, La Crosse, WI, 54601, USA
[2]Western Australian Herbarium, Biodiversity and Conservation Science,
Department of Biodiversity, Conservation and Attractions,
Locked Bag 104, Bentley Delivery Centre, Western Australia 6983, Australia
[3]Department of Biodiversity, Conservation and Attractions,
Manjimup 6258, Western Australia, Australia
[4]Institute of Systematic Botany, New York Botanical Garden,
2900 Southern Blvd, Bronx, NY 10458-5126, USA
[5]Current address: Seven Mile Beach, Tasmania 7170, Australia
[6]Corresponding author, email: rhalling@nybg.org

Abstract

Osmundson, T.W., Bougher, N.L., Robinson, R.M. & Halling, R.E. *Rubinoboletus phaseolisporus* (Boletaceae) from Western Australia is a *Tylopilus* with bean-shaped spores. *Nuytsia* 32: 87–97 (2021). The bolete genus name *Rubinoboletus* Pilát & Dermek has been misapplied in the past to include taxa belonging to several genera including *Tylopilus* P.Karst. In this study, we provide morphological and molecular phylogenetic justification for alignment of *Rubinoboletus phaseolisporus* T.H.Li, R.N.Hilton & Watling in *Tylopilus* with the North American taxon *T. balloui* (Peck) Singer. Thus, a new combination, *Tylopilus phaseolisporus* (T.H.Li, R.N.Hilton & Watling) Osmundson, Bougher, R.Rob. & Halling, is proposed for this brightly-coloured species that is apparently endemic to bushland in south-west Western Australia.

Introduction

In one paper in a series on the Cooloola boletes of Queensland, Watling and Gregory (1989) stated, '*Tylopilus* is very well represented in Australasia and many more undescribed species occur there than in north temperate regions.' A brief diagnosis of the genus was followed by discussion of varying concepts employed by McNabb (1967), Smith and Thiers (1971), Corner (1972), and Pegler and Young (1981). Following Corner (1972), nine alliances were enumerated by Watling and Gregory (1989). These encompassed entities that are likely of restricted distribution in SE Asia (e.g. *Boletus longipes* Massee (=*Ionosporus* Khmeln.), *Boletus nanus* Massee and other seemingly obscure taxa recognized by Corner), some others that recall northern hemisphere species (e.g. *T. alboater* (Schwein.) Murrill), or ones that have been considered generic synonyms of *Tylopilus* P.Karst (*Boletochaete* Singer, *Porphyrellus* E.-J.Gilbert). More detailed evaluations and descriptions were given for taxa that would appear to be aligned morphologically with the type species, *T. felleus* (Bull.) P.Karst. Subsequently, Watling (2001a, b; 2008) has provided more refined discussions on the possible heterogeneous concepts of *Tylopilus*.

Of particular interest to the present study is the species alliance surrounding *Tylopilus balloui* (Peck) Singer, a distinctive bolete from northeastern North America with striking, bright orange pigments

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(Figure 1A). This species, described by Peck (1912) as a *Boletus* L. from southern New York state, has a combination of morphological features that has led to alternative classifications based on different character weighting judgments. *Tylopilus balloui* has been placed in *Gyrodon* Opat. (Snell 1941), *Tylopilus* (Singer 1947), *Rubinoboletus* Pilát & Dermek (Heinemann & Rammeloo 1983), *Chalciporus* Bataille (Klofac & Krisai-Greilhuber 2006), and *Gyroporus* Quél. (Horak 2011). Molecular data have recently proven useful in resolving this taxonomic quandary, and support placement in *Tylopilus* despite *T. balloui* having ellipsoid-ovoid basidiospores that are uncharacteristic for a genus in which the type species and most other species have longer, subfusiform spores (Singer 1947; Osmundson & Halling 2010; Halling *et al.* 2012; Trappe *et al.* 2013). Singer (1947) emphasized that short basidiospores occur in almost all groups of boletes, and *Tylopilus* appears to be no exception. Osmundson and Halling (2010) noted that recent field and laboratory studies in Australasia have revealed a number of taxa morphologically similar to *T. balloui*, though differing in several morphological features as shown by Halling (2018). These data suggest that the name *T. balloui* as commonly ascribed to field and herbarium collections represents a species complex rather than a single widespread species (Halling *et al.* 2008), an observation consistent with that of Watling (2001a, b). Previously, Watling and Gregory (1988), Watling and Li (1999) and Li and Watling (1999) identified specimens of the '*balloui*' group as *Rubinoboletus* species, following Heinemann and Rammeloo's (1983) placement of African species morphologically similar to *T. balloui*. Despite that generic assignment, Watling and Li (1999) nonetheless suggested that *Rubinoboletus* was an unnatural assemblage and that its relationships needed reassessment. Watling (2008) subsequently recommended that the complex around *T. balloui* should be maintained in *Tylopilus* despite some anomalies, and that *Rubinoboletus* should be restricted to its original circumscription (Pilát & Dermek 1969).

According to Singer (1973), the type species of *Rubinoboletus*, *R. rubinus* (W.G.Sm.) Pilát & Dermek, is a *Chalciporus*. In accordance with that placement, Grgurinovic (1997) and Klofac and Krisai-Greilhuber (2006) transferred a number of species of the '*balloui*' group from *Rubinoboletus* to *Chalciporus*, and the latter authors reduced *Rubinoboletus* to subgeneric status within *Chalciporus*. More recently, large molecular datasets also support placement of *R. rubinus* in *Chalciporus* (Binder & Hibbett 2006; Nuhn *et al.* 2013; Wu *et al.* 2014). In contrast, molecular studies place *T. balloui* in *Tylopilus s.s.* with the type species, *T. felleus* (Binder & Hibbett 2006 (Suppl. Fig. 1); Wu *et al.* 2014; Gelardi *et al.* 2019). These results suggest that other taxa morphologically more closely allied to *T. balloui* than to *C. rubinus* (W.G.Sm.) Singer should also be placed in *Tylopilus*. Furthermore, these results indicate that spore shape, when treated as an isolated character, is an unreliable indicator of phylogenetic relationships in boletes.

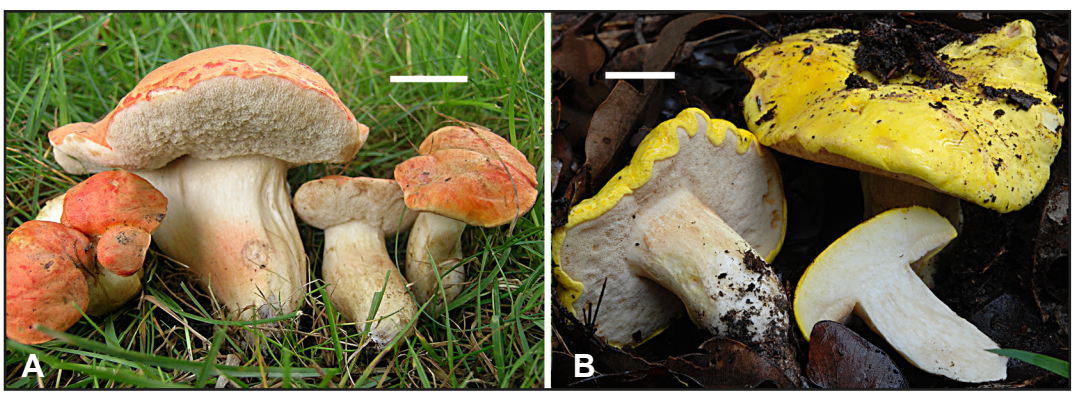

Figure 1. Basidiome habits. A – *Tylopilus balloui* (USA. New York: Bronx, New York Botanical Garden, 29 Sep 2008, *R.E. Halling* 9016, NY 1034441); B – *Tylopilus phaseolisporus* (PERTH 08166390). Scale bars = 2 cm.

Watling and Li (1999) provided an updated description of *R. phaseolisporus* citing two additional specimens (*K. Elson & B. Dell* UWA1835, *A. Saar* UWA2619 cited below), and stated that it is common under jarrah (*Eucalyptus marginata* Sm.) in Western Australia. While they noted that the spore deposit colour strongly suggested placement in *Tylopilus* (as originally suggested in a personal communication from R. Hilton to Watling), the spore shape did not match Corner's (1972) concept of the genus. Watling and Li (1999) further suggested that the bright yellow pileus colour and spore shape suggested affinity to *Gyroporus*; however, because the stipe lacked the circumferential hyphae characteristic of that genus, the authors opted for placement of the new taxon in *Rubinoboletus* as *Rubinoboletus phaseolisporus* T.H.Li, R.N.Hilton & Watling.

Based on extensive field and micromorphological documentation and a molecular analysis that incorporates the most comprehensive published phylogenetic framework for the family Boletaceae, we provide justification for the proper placement of this taxon – thus far known only to occur in south-west Western Australia – in *Tylopilus*. Accordingly, we propose the new taxonomic combination *Tylopilus phaseolisporus*.

Materials and Methods

Morphology

Macromorphological data were obtained from fresh specimens. General colour terms are approximations, and the colour codes (e.g. 7D8) are page, column and grid designations from Kornerup and Wanscher (1983). All microscopic structures were observed and measured with an Olympus BHS compound light microscope equipped with Nomarski Differential Interference Contrast (DIC) optics from dried material revived in 3% KOH. The abbreviation *Q* refers to the mean length/width ratio measured from *n* basidiospores, observed from *p* collections and *x* refers to the mean length × mean width. Light micrographs were obtained via Spot 5.3 Imaging software using a Spot Insight Gigabit digital camera from Diagnostic Instruments. Herbarium codes (Thiers 2021) are cited for all collections from which morphological features were examined. The new taxonomic combination is registered with MycoBank.

DNA isolation, PCR amplification and DNA sequencing

Genomic DNA was extracted from basidiomata tissues preserved in silica desiccant or from dried herbarium specimens for two collections of *T. phaseolisporus* (*R.E. Halling*, *N.L. Bougher & R.* Garvey 8823 and *R.E. Halling* 8827; see *Specimens examined*, below), a collection of *T. balloui* from the New York Botanical Garden (*Osmundson* 1030, NY 02072601), USA, the related species *T. oradivensis* Osmundson & Halling from Costa Rica (Osmundson & Halling 2010), and three collections from Queensland, Australia that we hypothesized to belong to the *T. balloui* group based on macro- and micromorphology (Table 1). Approximately 10 mg of dried tissue was ground using a Bio101/Savant Fast Prep FP120 tissue homogenizer (Qbiogene Inc., Carlsbad, CA, USA), and DNA was extracted using either the Qiagen DNeasy Plant Mini or DNeasy 96 extraction kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's instructions.

Partial sequences from two nuclear loci were obtained for this study: the nuclear ribosomal large subunit (LSU) and nuclear translation elongation factor 1α (*tef1* or EF-1α). PCR amplifications were performed in 25 µL volumes consisting of 2.5 µL 10X PCR buffer, 2.5 µL dNTP mix (0.2 mM each dNTP), 2.5 µL bovine serum albumin, 1 µl each primer (10 µM primer solution), 5 µL PCR additive (Q solution, Eppendorf), 1 unit Taq polymerase, and ddH_20 to reach 25 µL total volume. For *tef1*,

better results were obtained by eliminating Q solution and replacing the volume with ddH_20. PCR primers were LR0R (5'- ACC CGC TGA ACT TAA GC-3') / LR7 (5'- TAC TAC CAC CAA GAT CT -3') (Vilgalys & Hester 1990) for LSU and EF1-526F (5'- GTC GTY GTY ATY GGH CAY GT -3') (S. Rehner unpublished) / 1567R (5'- ACH GTR CCR ATA CCA CCR ATC TT -3') (Rehner & Buckley 2005) for *tef1*. Amplification conditions for LSU were (i) initial denaturation at 95° C for 2 min; (ii) 30 cycles of denaturation at 94° C for 60s, annealing at 50° C for 45s and extension at 72° C for 60s; (iii) final extension at 72° C for 7 min. Amplification of *tef1* was conducted using a modification of the touchdown PCR protocol by Rehner and Buckley (2005): (i) 95° C for 5 min; (ii) 94° C for 60s, 65° C for 60s, decreasing 1° C per cycle for the following 9 cycles, then 72° C for 60s; (iii) 25 additional cycles of 94° C for 60s, 56° C for 60s, and 72° C for 60s; (iv) final extension at 72° C for 5 min. PCR products were purified and sequenced at the High Throughput Genomics Facility, University of Washington (Seattle, WA, USA) using BigDye terminator chemistry (Applied Biosystems, Inc., Foster City, CA, USA). Sequencing primers were identical to the PCR primers, with the addition of internal sequencing primers LR3 (5'- CCG TGT TTC AAG ACG GG - 3') for LSU and EF-ir (5'- GCR TGY TCN CGR GTY TGN CCR TC '3') for *tef1*.

Portions of two additional loci were sequenced for collection *R.E. Halling* 8827: the mitochondrial ATPase subunit 6 (*atp6*) and mitochondrial ribosomal large subunit (mtLSU). These sequences were not used in the analysis for the current study, but previous analyses of *atp6* alone and in combination with LSU support placement of *T. phaseolisporus* in *Tylopilus* (Osmundson 2009). The mtLSU sequence has not been used in any analyses to-date. PCR conditions for *atp6* used the same reaction mixture specified above except for eliminating Q solution, tripling the volumes of the two degenerate primers, and adjusting the water volume accordingly. Amplification of *atp6* was conducted using the primers atp6-1 (5'- ATT AAT TSW CCW TTA GAW CAA TT -3') and atp6-2 (5'- TAA TTC TAN WGC ATC TTT AAT RTA -3'), and the cycling parameters of Kretzer and Bruns (1999). Amplification of mtLSU was conducted using the primers ML5 (5'- CTC GGC AAA TTA TCC TCA TAA G -3') and ML6 (5'- CAG TAG AAG CTG CAT AGG GTC -3') (White *et al*. 1990) and the reaction mixture and thermocycling conditions described for LSU above. A total of 10 new sequences were obtained. GenBank accession numbers for all newly generated sequences and previously-submitted sequences from these samples are listed in Table 1.

Alignment and phylogenetic analyses

Sequences generated for this study were end-trimmed and checked for errors using Geneious Prime 2019 (Biomatters Ltd., Auckland, New Zealand), then aligned with the two-locus LSU/tef1 Boletaceae dataset from Wu *et al*. (2014), representing the most comprehensive multilocus dataset for the family to-date. Alignments for each locus were conducted using MUSCLE 3.8.425 with the maximum number of iterations set to 20, implemented in Geneious Prime 2019. Alignments were curated using GBlocks 0.91b (Castresana 2000; Talavera *et al*. 2007), with the following parameter settings: minimum number of sequences for conserved position = default (50% of the number of sequences + 1); minimum number of sequences for a flank position = default (85% of the number of sequences); maximum number of contiguous nonconserved positions = 8; minimum block length = 10; allowed gap positions = with half. The two individual alignments were concatenated using Geneious Prime 2019, and four data partitions were specified corresponding to *tef1* first + second codon positions, *tef1* third codon positions, *tef1* introns, and LSU. Data were analysed under a maximum likelihood optimality criterion using RAxML 8.2.12 (Stamatakis 2014). A search for the maximum likelihood tree was conducted using the following parameter settings: rate categories = 25; random seed value 12345; estimate proportion of invariable sites = no; alternative runs on distinct starting trees = 100. Multiparametric bootstrapping was conducted with random seed value 12345 and 1000 replicates.

Table 1. Collections of *Tylopilus phaseolisporus* and additional *Tylopilus balloui s.l.* sequenced for this study, with GenBank accession numbers. Asterisks denote sequences submitted for previous studies (Halling *et al*. 2008; Osmundson & Halling 2010).

Taxon	Specimen	LSU	tef1	atp6	mtLSU
Tylopilus phaseolisporus	*R.E. Halling* 8823	MW620812	N/A	N/A	N/A
Tylopilus phaseolisporus	*R.E. Halling* 8827	MW620809	MW620810	MW620811	MW620808
Tylopilus balloui	*T.W. Osmundson* 1030	EU430737*	MW815570	N/A	N/A
Tylopilus oradivensis	*R.E. Halling* 8187	EU430732*	MW815572	N/A	N/A
Tylopilus aff. *balloui*	*T.W. Osmundson* 1105	EU430738*	MW815571	N/A	N/A
Tylopilus aff. *balloui*	*T.W. Osmundson* 1122	EU430742*	MW815573	N/A	N/A
Tylopilus aff. *balloui*	*T.W. Osmundson* 1132	EU430739*	MW815574	N/A	N/A

GBlocks and RAxML were implemented on the CIPRES Science Gateway (www.phylo.org; Miller *et al*. 2010).

An initial analysis was conducted using the full taxon set from Wu *et al*. (2014), with outgroup sequences *Suillus* aff. *luteus* HKAS 57748 and *Suillus* aff. *granulatus* HKAS 57622. Based on the results of this analysis (see Results) and in order to include additional characters that may clarify relationships, a second analysis included only the Boletoideae clade and outgroup sequences *Boletellus dissiliens* REH 9435 (Xerocomoideae), *Retiboletus* aff. *ornatipes* HKAS 63548 (Leccinoideae), and *Retiboletus griseus* HKAS 63590 (Leccinoideae). Tree figures were prepared using the Interactive Tree of Life (iTOL) website (Letunic & Bork 2019).

Results

Molecular analyses

The alignment for the initial analysis with the full taxon set from Wu *et al*. (2014) consisted of 297 sequences and 1076 alignment positions (484 bp tef1; 592 bp LSU), containing 613 distinct alignment patterns (*tef1* positions 1+2: 160; *tef1* position 3: 151; *tef1* introns: 16; LSU: 286). This analysis placed *T. phaseolisporus* and the other '*balloui*' taxa in the Boletoideae clade sensu Wu *et al*. (2014). Relationships within the clade received low bootstrap support (Figure 2), so a Boletoideae-only analysis was conducted to allow inclusion of additional characters that could not be unambiguously aligned with the full Boletaceae dataset.

The alignment for the Boletoideae-only analysis consisted of 79 sequences and 1248 alignment positions (558 bp *tef1*; 690 bp LSU) containing 604 distinct alignment patterns (*tef1* positions 1+2: 104; *tef1* position 3: 150; *tef1* introns: 86; LSU: 264). The results of this analysis placed *T. phaseolisporus* in a well-supported clade (91 percent bootstrap support) with the *T. balloui* specimen from New York, USA and other specimens morphologically allied in a *T. balloui* species complex (Figure 3). Within that clade, the two *T. phaseolisporus* specimens formed a moderately well supported (70 percent) clade with an Australian '*balloui*' collection. A sister group relationship with a North American clade (*T. balloui* from USA and *T. oradivensis* from Costa Rica) rather than to the other Australian collections is indicated in the maximum likelihood tree, though this relationship did not receive strong bootstrap support (35 percent).

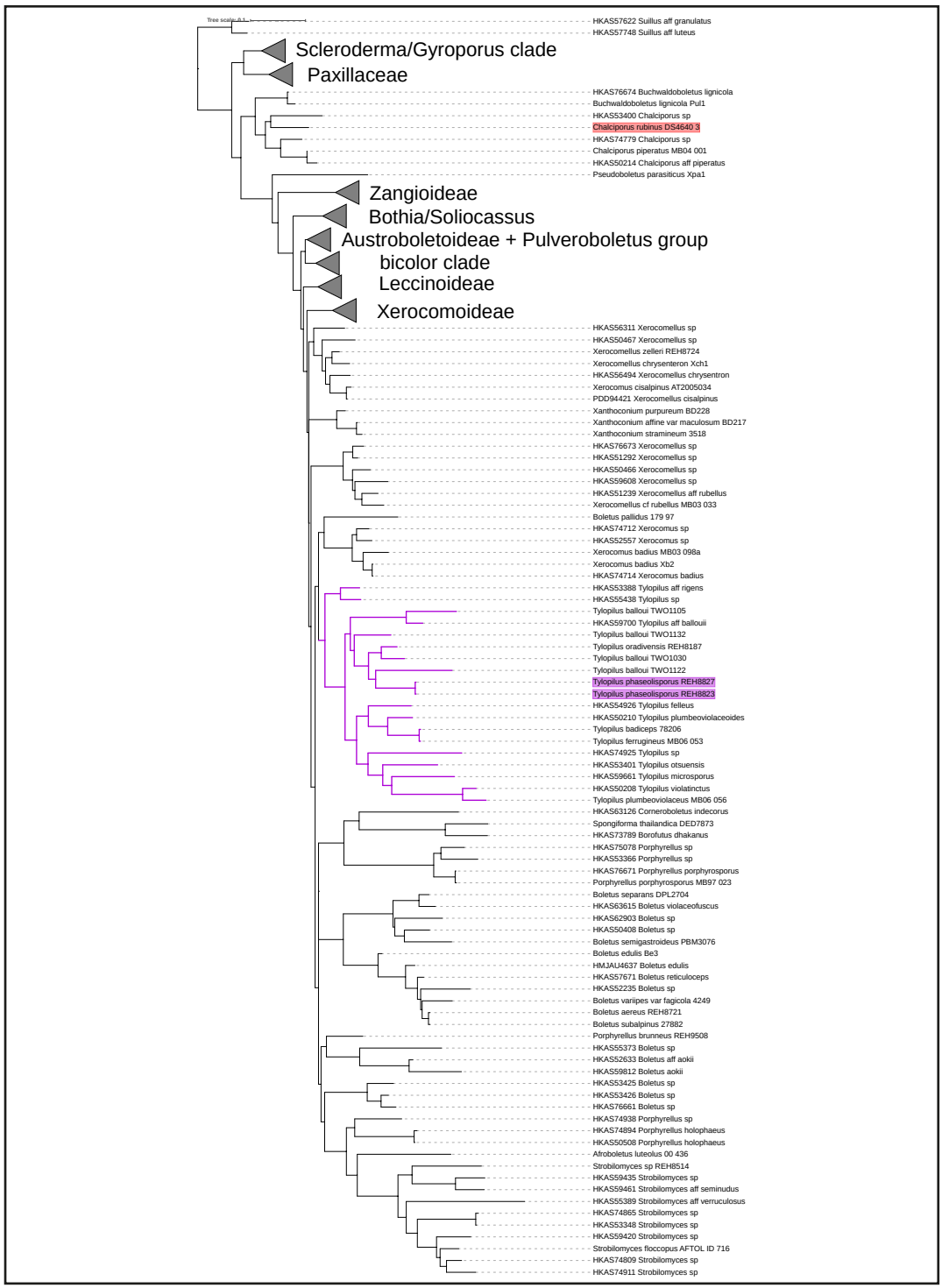

Figure 2. Maximum likelihood phylogram for combined LSU-*tef1* analysis of *Tylopilus phaseolisporus* and other *Tylopilus balloui s.l.* specimens with the full taxon sample of Wu *et al*. (2014). Clades, except for those containing *T. phaseolisporus* and *Chalciporus* (*Rubinoboletus*) *rubinus*, are collapsed, with labels corresponding to the names given to these clades in Wu *et al*. (2014). Purple branches denote *Tylopilus s.s.*, and purple-highlighted labels denote the two specimens of *T. phaseolisporus*; red label denotes *Chalciporus rubinus*, the type species of *Rubinoboletus* (as *Rubinoboletus rubinus*).

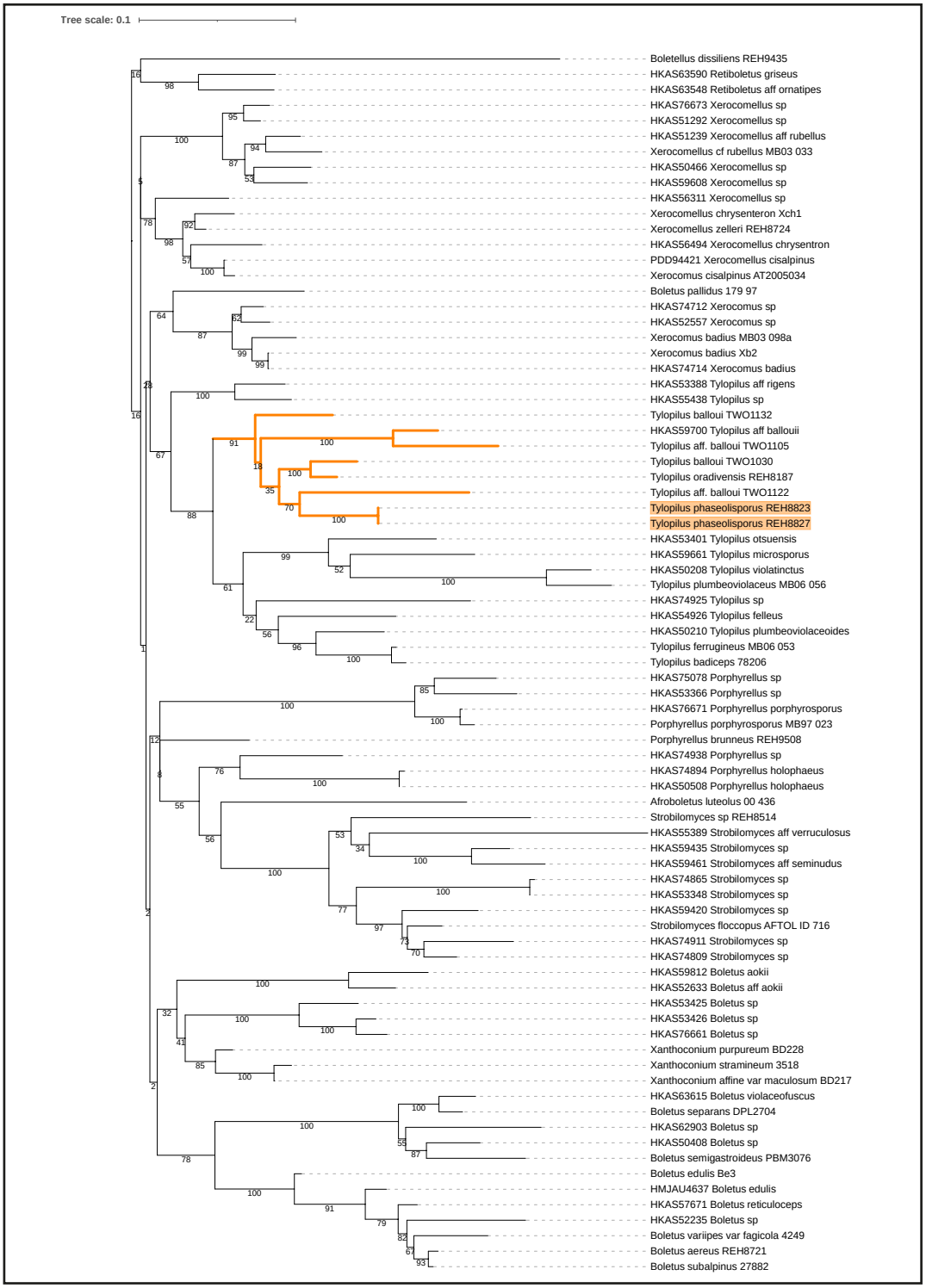

Figure 3. Maximum likelihood phylogram for combined LSU-*tef1* analysis of *Tylopilus phaseolisporus* and other *Tylopilus balloui s.l.* specimens with the reduced taxon sampling restricted to subfamily Boletoideae sensu Wu *et al.* (2014); orange branches denote the *T. balloui* species complex, and orange-highlighted taxon labels denote the two specimens of *T. phaseolisporus* included in the analysis.

Taxonomy

Tylopilus phaseolisporus (T.H.Li, R.N.Hilton & Watling) Osmundson, Bougher, R.Rob. & Halling, *comb. nov.*

Rubinoboletus phaseolisporus T.H.Li, R.N.Hilton & Watling in T.H. Li & R. Watling, *Edinburgh J. Bot.* 56: 146 (1999). *Chalciporus phaseolisporus* (T.H.Li, R.N.Hilton & Watling) Klofac & Krisai, *Österreich Zeit. Pilzk.* 15: 50 (2006). *Type*: Mundaring State Forest, Western Australia, 15 June 1975, *R.N. Hilton* UWA 1990 (*holo*: E00465106!; *iso*: PERTH 00770361!).

MycoBank: MB 839093.

Pileus (3.5–)5.5–8.5(–9) cm broad, convex to plano-convex, with irregular margin, viscid to subviscid, soon drying, glabrous to obscurely finely matted, yellow (3A5, 3A8, 4A7), then pale yellow, dulling to orange yellow (near 5A8), developing pale brownish tone with age; margin incurved, smooth, entire. *Flesh* white, unchanging, with mild odour and taste. *Tubes* adnexed to subdecurrent, pale cream (5A2) at first, becoming pale pinkish buff (5A3), staining cinnamon brown (near 7E8, 8E7), with pores pale pinkish buff staining cinnamon brown. *Stipe* (2–)6.5–8.5 cm long, (1–)1.5–3 cm broad, tapering downward or sometimes clavate, dry to moist, yellow when young, then white and covered with fine, scurfy pruinosity that is pale caramel coloured, stains cinnamon brown and is denser toward the base; context solid, white; basal mycelium white.

Basidiospores pinkish brown in deposit, 4.9–7 × 2.8–3.5 µm, (x = 5.95 × 3.36 µm, Q = 1.77, (n = 40, p = 2), smooth, phaseoliform in profile, subellipsoid in adaxial and abaxial views, hyaline in KOH and Melzer's reagent. *Basidia* clavate, 25–30 × 7–9 µm, hyaline, 4-sterigmate. *Pleurocystidia* common, 35–60 × 8–22 µm, narrowly to broadly fusiform, thin-walled, with oily to granular or coarse orange brown to brownish yellow content, rarely hyaline. *Tube trama* boletoid and divergent, hyaline in KOH and Melzer's reagent, with hyphae 3–7 µm broad. *Pileus trama* inamyloid, hyaline in KOH, with hyphae 3–7 µm broad. *Pileipellis* a collapsed trichodermium embedded in a gelatinous matrix, with elements 2.8–4.2 µm wide. *Stipitipellis* a disrupted hymeniform layer of clavate, subclavate or rarely short subfusiform elements, 20–40 µm long, hyaline, and thin-walled, often intermixed with amorphous golden brown pigment clusters. *Clamp connections* absent. (Figures 1B, 4)

Specimens examined. WESTERN AUSTRALIA: Nannup, Easter Forest Block, Dickson Road (Bridge Spot), 31 May 1983, *N.L. Bougher* E 349 (PERTH 07607113); Murray, Dwellingup, Alcoa Mine, Nettleton Road, 10 June 2002, *N.L. Bougher* E 7120 (PERTH 07649983); Denmark, Nornalup, Valley of the Giants, Old Valley Road, 7 June 1992, *N.L. Bougher & K. Syme* E 4773 (PERTH 07554583); between Jarrahdale and Gleneagle Forest, 1.25 miles along track which turns off opposite Rock, *K. Elson & B. Dell* UWA 1835 (E 00465107, PERTH 00770434); Alcoa Mine, Nettleton Road, Dwellingup, 11 June 2002, *M. Glen, R. Armstead, R. Daniels* E 7145 (PERTH 07650833); Manjimup, 21 June 2006, *R.E. Halling* 8827 (NY 1393472); Nannup, Easter Forest Block, Dickson Road, 21 June 2006, *R.E. Halling*, *N.L. Bougher & R. Garvey* 8823 (NY 1393466, PERTH 08019134); Manjimup, Grid FC55, Lewin Forest block, 100 m E of Arthur Road, access from Eastwin Road, 5 June 2013, *R.M. Robinson*, *P. Anderson*, *& S.J.M. McMullan-Fisher* FC 1880 (PERTH 08166390); Nannup, Layman Forest Block, Plot FC40, Crouch Road, 500 m E of junction with Cul De Sac Road, 15 June 2006, *R.M. Robinson & J.C. Fielder* FC 1015 (PERTH 06660622); Nannup, Barrabup Forest Block, Plot FC43, 1.4 km N off Keene Road on logging track, 21 June 2006, *R.M. Robinson & J. Fielder* FC 1045 (PERTH 06660991); Nannup, St John Forest Block, Plot FC39, 400 m N on boundary of St John Conservation

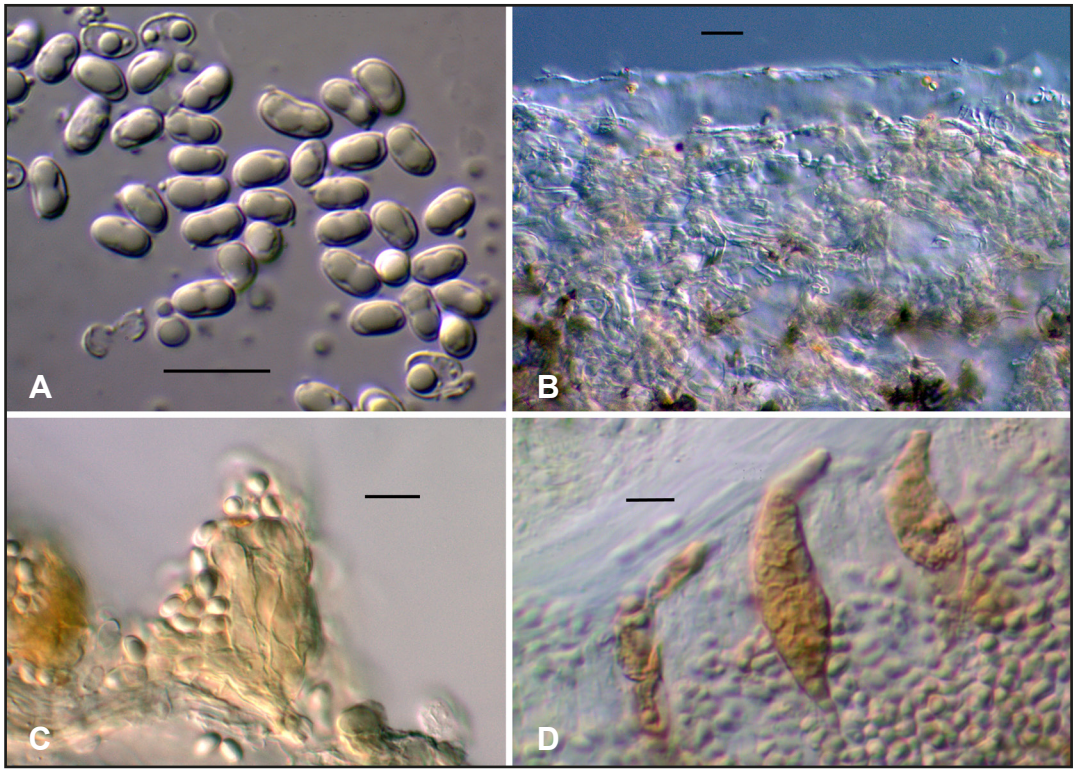

Figure 4. Micromorphology of *Tylopilus phaseolisporus*. A – Basidiospores (NY 1393466); B – Pileipellis (NY 1393472); C – Stipitipellis (NY 1393466); D – Pleurocystidia (NY 1393466). Scale bars = 10 µm (A, C, D); 20 µm (B).

Park off St John Road West, 400 m E of junction with St Luke Road, 21 June 2006, *R.M. Robinson & J. Fielder* FC 1053 (PERTH 06661076); Bridgetown-Greenbushes, Barrabup Forest Block, Plot FC38, St John Road East, 1 km N of junction with Mowen Road, 14 July 2006, *R.M. Robinson & J. Fielder* FC 1208 (PERTH 06662722); Nannup, Barrabup Forest Block, Plot FC46, 100 m N of Keene Road on logging track, 15 June 2012, *R.M. Robinson & C. Newland* FC 1771 (PERTH 08164541); FC19, Dooganally Road, Tumlo Forest Block, 38.4 km from Collie, 8 July 2003, *R.M. Robinson & K. Pearce* FC 589 (PERTH 06437664); Nannup, Forest Check Monitoring Plot 10, near Dickson Tower, Easter Forest Block, 16 June 2004, *R.M. Robinson & R.H. Smith* FC 623 (PERTH 06640532); Forest Check Monitoring Plot 10, near Dickson Tower, Easter Forest Block, 16 June 2004, *R.M. Robinson & R.H. Smith* FC 628 (PERTH 06640486); Boulter Road, Boranup, 26 June 1982, *A. Saar* UWA 2619 (E 00465108, PERTH 00909890).

Distribution and habitat. Scattered to gregarious in litter on soil or sand under a variety of dominant species including *Eucalyptus marginata*, *E. diversicolor*, *E. jacksonii*, *E. guilfoylei*, *Corymbia calophylla*, *Allocasuarina decussata*, *A. fraseriana*, and/or *Acacia pentadenia*. So far, known only from south-west Western Australia.

Discussion

Despite differing in pileus colouration, *T. phaseolisporus* bears a close resemblance both macroscopically (stature, hymenophore colour, and staining reactions) and microscopically (basidiospore shape and size, pleurocystidial shape and contents) to the North American species *T. balloui*. Originally described

by Peck in a formerly more heterogeneous and inclusive *Boletus*, *B. balloui* Peck was transferred to *Tylopilus* by Singer (1947). Although *T. balloui* resembles other *Tylopilus* in terms of spore deposit colour, hymenial colouration and staining, and pleurocystidial shape and contents, its short, phaseoliform basidiospores are unusual for *Tylopilus*. Subsequent placements in *Gyrodon* (Snell 1941) and *Rubinoboletus* (Heinemann & Rammeloo 1983) resulted from placing excessive weight on this character despite the other similarities with *Tylopilus* and the lack of other strong similarities between *T. balloui* and the type species of either *Gyrodon* or *Rubinoboletus*. As a result of *T. balloui* being transferred to *Rubinoboletus*, other species resembling this taxon – including *T. phaseolisporus* – were placed there as well, and some of these were transferred again to *Chalciporus* along with *R. rubinus*. The results of the present study confirm placement of both *T. balloui* and *T. phaseolisporus* in *Tylopilus*. In our analyses, *T. balloui* is represented by a specimen (*T.W. Osmundson* 1030, NY 02072601) collected in Bronx, New York, USA, on the grounds of the New York Botanical Garden in association with native *Fagus grandifolia* trees; this locality is ±67 km west of the type locality at Orient Point on eastern Long Island. The phylogenetic results presented here furthermore indicate that additional taxa resembling *T. balloui* should be described (or retained) in *Tylopilus*, not *Rubinoboletus* or *Chalciporus*.

Our field explorations suggest that Australia is rich in taxa similar to *T. balloui*, only some of which are included in the molecular analyses presented here. We are currently examining the taxonomy and systematics of the *Tylopilus balloui* complex in Australia and on a more global scale.

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