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(Article begins on next page)





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Alessioporus and Pulchroboletus (Boletaceae, Boletineae), two novel genera for Xerocomus ichnusanus and X. roseoalbidus from the European Mediterranean basin: molecular and morphological evidence

Matteo Gelardi, Via Traversa della Selciatella 2B, I-00062 Bracciano (RM), Italy

Giampaolo Simonini, Via Bell'aria 8, I-42121Reggio Emilia, Italy

Enrico Ercole, Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Viale P.A. Mattioli 25, I-10125 Torino, Italy

Alfredo Vizzini, Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Viale P.A. Mattioli 25, I-10125 Torino, Italy

Abstract

Alessioporus and Pulchroboletus are proposed as new monotypic genera to accommodate the thermo-xerophilic European species *Xerocomus ichnusanus* and *X. roseoalbidus* respectively. The present research focused on both morphological features and multigene molecular phylogeny (nrITS, nrLSU, *tef-1* α datasets) to elucidate the taxonomic status of these two rare Mediterranean boletes and delineate a natural classification within the family Boletaceae. Macro- and microscopic descriptions of the two species based on inclusive taxon sampling are provided and supported by line drawings of the main anatomical features. Phylogenetic relationships, ecology, geographical distribution and delimitation from the most closely allied taxa also are highlighted. In addition, epitype specimens are selected for both species.

- Basidiomycota
- biogeography
- Boletales
- Boletus
- epitypification
- European fungi
- molecular phylogeny
- taxonomy

Introduction

Molecular phylogenetic techniques are essential to reassess and resolve traditional fungal taxonomy based on morphological traits (<u>Martin et al. 2011</u>, <u>Yang 2011</u>, <u>Martin and Bonito 2012</u>). DNA-based research has led recently to the establishment of many new segregate boletoid and gasteroid genera within the family Boletaceae (<u>Binder and Bresinsky 2002a; Bresinsky and Besl 2003</u>;

Halling et al. 2007, 2012a, b; Desjardin et al. 2008, 2009; Orihara et al. 2010; Lebel et al. 2011; Li et al. 2011; Hosen et al. 2012; Zeng et al. 2012; Trappe et al. 2013).

Despite the remarkable results obtained over the past few years in evaluating the ecological importance and diversity of the boletes and their closest allies (<u>Agerer 1999</u>, <u>2006</u>; <u>Binder 1999</u>; <u>Binder and Bresinsky 2002b</u>; <u>Binder and Hibbett 2006</u>; <u>Drehmel et al. 2008</u>; <u>Wilson et al. 2011</u>, <u>2012</u>; <u>Nuhn et al. 2013</u>), several boletoid lineages still require careful reappraisal (Feng et al. 2012, Ainsworth et al. 2013, <u>Gelardi et al. 2013</u>, <u>Nuhn et al. 2013</u>, <u>Vizzini et al. 2014</u>).

Among the European boletes of uncertain taxonomic placement, *Xerocomus ichnusanus* Alessio, Galli & Littini and *X. roseoalbidus* Alessio & Littini (Boletaceae, Boletineae Rea emend. E.-J. Gilbert) represent an emblematic example of critical species not yet subjected to molecular investigation. Both taxa were described from Sardinia, Italy, and classified in *Xerocomus* Quél. based on their morphology (Alessio 1984, 1987) but later were recombined by other authors based on the assumption that *Xerocomus* should be reduced to an infrageneric taxon within *Boletus* L. (Oolbekkink 1991, Moreno et al. 1995). Subsequently Klofac (2007) erected sect. *Caespitosi* within *Xerocomus* to accomodate these two boletes. *Boletus* and *Xerocomus*, however, are cumbersome and polyphyletic (Binder 1999, Binder and Hibbett 2006, Drehmel et al. 2008, Šutara 2008, Dentinger et al. 2010, Halling et al. 2012a, Gelardi et al. 2013, Nuhn et al. 2013, Vizzini et al. 2014). In particular, the multigene analyses by Nuhn et al. (2013) showed that *Xerocomus* s.l. divides into at least five distinct clades, that is Xerocomellus clade (type = *X. chrysenteron* [Bull.] Quél.), rubellus clade and badius clade in the Anaxoboletus group, Xerocomus clade (type = *X. subtomentosus* (L.) Quél.) in the Hypoboletus group and *Pseudoboletus parasiticus* (Bull.) Šutara.

As part of a long-term revision of bolete taxonomy, numerous samples of *X. ichnusanus* and *X. roseoalbidus* from different geographic areas were screened and sequenced, leading us to conclude that they cannot be satisfactorily included in either *Boletus* or *Xerocomus* and thus demand separate genera. Accordingly, in view of the obvious morphological and ontogenetic peculiarities and because molecular inference (a combined analysis of nrITS, nrLSU and *tef-1* α regions) unambiguously favors the separation of two natural, independent monophyletic lineages, our goal is to introduce *Alessioporus* and *Pulchroboletus* as new genera for the species under consideration.

Materials and Methods

Collection sites and sampling

Specimens examined, including the holotype of *X. ichnusanus*, were collected at several Italian and French localities in the past 34 y and are mostly deposited in AMB, MCVE, IB and TO (acronyms from <u>Thiers 2014</u>), and "MG" and "RG", the personal herbaria of Matteo Gelardi and Roberto Galli respectively.

Morphological studies

Macroscopical descriptions, macro-chemical reactions (25% NH4OH, 30% KOH, FeSO4), habitat annotations and associated plant communities are based on detailed field notes from fresh basidiomes, and color terminology and alphanumeric codes are those of <u>Kornerup and Wanscher</u> (1978). Micromorphological features were observed from dried material; sections either were rehydrated in water, 5% KOH or in ammoniacal Congo red. Observations of structures and measurements of anatomical features were performed by mounting preparations in ammoniacal Congo red. Colors and amount of pigmentation were described after examination in water and 5%

KOH. Measurements were made at $1000 \times$ with a calibrated ocular micrometer (Nikon Eclipse E200 and Zeiss Jena jenamed Variant optical light microscopes). Spores were measured from spore prints and from the hymenophore of mature basidiomes. Values are given as (minimum) average \pm standard deviation(maximum), Q = average quotient (length/width ratio) \pm standard deviation with minimum and maximum values in parentheses, while average spore volume was approximated as a rotation ellipsoid (V= 4/3*[length/2]*[width/2]*[width] * $\pi/2 \pm$ standard deviation). The notation [n/m/p] indicates that measurements were made on "n" randomly selected spores from "m" basidiomes of "p" collections. Metachromatic, cyanophilic and iodine reactions were tested by staining the spores in brilliant cresyl blue, cotton blue and Melzer's reagent respectively. Line drawings of microstructures were made from rehydrated material and based on photomicrographs.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was isolated from 25 mg dried herbarium specimens (Table I) with the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for ITS barcode amplification (White et al. 1990, Gardes and Bruns 1993), primers LR0R/LR6 (Vilgalys and Hester 1990, www.botany.duke.edu/fungi/mycolab) for LSU rDNA amplification and the primer pair EF1-983FdEF1-2218R (Rehner and Buckley 2005) for amplification of the *tef-1* α fragment. Amplification reactions were performed in a PE9700 thermal-cycler (Perkin-Elmer, Applied Biosystems) following the profiles given by Vizzini et al. (2011) for ITS and LSU and Nuhn et al. (2013) for *tef-1* α . The PCR products were purified with the AMPure XP kit (Beckman) and sequenced by MACROGEN (Seoul, Republic of Korea). Sequence assembly and editing were performed with Geneious 5.3 (Drummond et al. 2010). The sequences are deposited in GenBank (Table I, Fig. 1). Alignments and phylogenetic tree are available at TreeBASE (www.treebase.org, submission number S15675).



BI comb. ITS-LSU-tef-La (*, II, +)

Fig. 1.

Hypoboletus group (Nuhn et al. 2013). Bayesian phylogram obtained from the combined ITS-LSUtef-1 α sequence alignment. Bothia castanella was used as the outgroup taxon. BPP values exceeding 0.75 (in boldface) and MLB values over 50% are shown above clade branches. Newly sequenced collections are in boldface. Numbers (1–8, 1–5) refer to the *A. ichnusanus* and *P. roseoalbidus* collections reported (Table I).

	GenBank accession No.			
Species	ITS	LSU	Tef-1α	Source and country
Alessioporus ichnusanus 1 (epitypus)	KJ729491	KJ729504	KJ729513	AMB 12756, ITALY (Sardinia)
Alessioporus ichnusanus 2	KJ729492	KJ729505	_	TO AVX13, ITALY (Sardinia)
Alessioporus ichnusanus 3	KJ729493	KJ729506	_	MG 549a, ITALY (Lazio)
Alessioporus ichnusanus 4	KJ729494	KJ729507	_	RG XER.ICH. 5, ITALY (Liguria)
Alessioporus ichnusanus 5	KJ729495	KJ729508	_	RG XER.ICH. 6, ITALY (Piedmont)
Alessioporus ichnusanus 6	KJ729496	KJ729509	_	MG420a, ITALY (Lazio)
Alessioporus ichnusanus 7	KJ729497	KJ729510	_	MCVE 17721, ITALY (Emilia Romagna)
Alessioporus ichnusanus 8	KJ729498	KJ729511	_	TO AVX11, FRANCE (Corsica)
Pulchroboletus roseoalbidus 1(epitypus)	KJ729486	KJ729499	KJ729512	AMB 12757, ITALY (Sardinia)
Pulchroboletus roseoalbidus 2	KJ729487	KJ729500	_	MG532a, ITALY (Lazio)
Pulchroboletus roseoalbidus 3	KJ729488	KJ729501	_	MCVE 18217, ITALY (Sardinia)
Pulchroboletus roseoalbidus 4	KJ729489	KJ729502	_	MG416a, ITALY (Lazio)
Pulchroboletus roseoalbidus 5	KJ729490	KJ729503	—	MCVE 17577, ITALY (Emilia Romagna)

Table I.

Collections newly sequenced in this study and their GenBank accession numbers

Sequence alignment, dataset assembly and phylogenetic analysis

Based on BLASTn results, preliminary phylogenetic analysis and outcomes of recent molecular studies on the Boletaceae (Binder and Hibbett 2006, Nuhn et al. 2013), sequences were retrieved from GenBank and UNITE (unite.ut.ee/) for comprehensive phylogenetic analysis. The phylogenetic analysis, based on a combined ITS/LSU/ tef-1a dataset, was focussed on the intergeneric position of Xerocomus ichnusanus and X. roseoalbidus in the Hypoboletus group of the Boletineae, a well supported clade containing members of Aureoboletus Pouzar, Boletellus Murrill and Boletus L. (non-porcini mushrooms), along with species of Hemileccinum Šutara, Phylloporus Quél. and Xerocomus sensu stricto (Nuhn et al. 2013). Alignments were generated for each ITS, LSU and tef-1a dataset with MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. Alignments were imported into MEGA 5.10 (Tamura et al. 2011) for manual adjustment. The best-fit substitution model for each alignment was estimated by both the Akaike information criterion (AIC) and the Bayesian information criterion (BIC) with jModelTest 0.1.1 (Posada 2008) to provide a substitution model for the alignment. gtr + Γ model was chosen for the ITS alignment, while TrNef + Γ was selected for LSU and *tef-1* α alignments. Based on the results of Nuhn et al. (2013), Bothia castanella (Peck) Halling, T.J. Baroni & Manfr. Binder (DQ867110, DQ867117, KF030421) was chosen as outgroup taxon for the combined dataset.

Phylogenetic hypotheses were constructed with Bayesian inference (BI) and maximum likelihood (ML) criteria. The BI was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) with four incrementally heated simultaneous Monte Carlo Markov chains (MCMC) run for 10 000 000 generations, under the selected evolutionary model. Trees were sampled every 1000 generations, resulting in overall sampling of 10 001 trees; the first 2500 trees (25%) were discarded as burn-in. For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP). ML estimation was performed with RAxML 7.3.2 (Stamatakis 2006) with 1000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for optimal topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the "–f a" option of RAxML and "–X 12345" as a random seed to invoke the novel rapid bootstrapping algorithm.

BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al. 2010). Only BPP values exceeding 0.75 and MLB over 50% are reported in the resulting tree (Fig. 1). Branch lengths were estimated as mean values over the sampled trees. Pairwise percent identity values of ITS sequences were calculated with MEGA 5.10 (Tamura et al. 2011).

Results

Molecular analysis

Amplification and sequencing were successful for all specimens selected for molecular study (Table I), with the exception of the holotype of *X. ichnusanus*, which was too old and in poor condition. The combined data matrix (focused on the Hypoboletus group) comprised 82 sequences (including 51 from GenBank and three from UNITE) corresponding to 23 taxa. Both Bayesian and maximum likelihood analyses produced the same topology; therefore only the Bayesian tree with both BPP and MLB values is shown (Fig. 1). The eight newly sequenced collections of *X. ichnusanus* clustered with a sequence of *X. ichnusanus* from UNITE (UDB000464, Italy, Campania Region, MCVE 18253) forming a clade (Alessioporus) with 100% branch support in both MP and BI trees. The pairwise identity value of the ITS sequences of the entire Alessioporus clade is 98.6%. The five newly sequenced collections of *X. roseoalbidus* from UNITE (UDB000486, Italy, Calabria Region, MCVE 18144) forming the well supported Pulchroboletus clade (BPP = 1; MLB = 100). The pairwise identity value of the ITS sequences of the entire Pulchroboletus clade is 99.3%. The Alessioporus clade was sister to a clade consisting of the Pulchroboletus and the Hemileccinum clades.

Taxonomy

Alessioporus Gelardi, Vizzini & Simonini, gen. nov.

MycoBank MB808529

Diagnosis: Basidiomes pileate-stipitate with tubular-poroid hymenophore, epigeal, small to medium-small; pileus tomentose to glabrous, dry, with a wavy-lobed margin when young, ochraceous-brown, dark olive-brown to copper-brown with brownish black fibrils; hymenophore adnate to depressed around the stipe, bright yellow to olive-green; stipe surface reticulate to coarsely ribbed or occasionally smooth, often exhibiting a prominent narrow pseudo-annulus generally in the middle or lower part of the stipe, which is reminescent of the junction point of pileus and stipe resulting from a secondary angiocarpy (mixangiocarpy) at the very early developmental stage, rooting at the base; context whitish to yellowish but darker downward; tissues quickly discoloring dark indigo blue when handled or injured; flavor mild; spore print olive-brown; basidiospores smooth, ellipsoidal to ellipsoidal-fusoid; cystidia cylindrical-fusi-form to ventricose-fusiform or lageniform; pileipellis a trichoderm of interwoven, filamentous hyphae; hymenophoral trama bilateral-divergent of the "*Boletus*-type"; with a lateral stipe stratum of the "boletoid type"; clamp connections absent; growing gregarious to more often caespitose or branched with up to 12 basidiomes arising from a common base and occasionally with secondary fruiting bodies emerging from the stipe of adjacent basidiomes.

Type species: Xerocomus ichnusanus Alessio, Galli & Littini.

Etymology: Named in honor of the late Carlo Luciano Alessio, who first described the species and dedicated most of his mycological studies to the investigation of Italian boletes.

Alessioporus ichnusanus (Alessio, Galli & Littini)

Gelardi, Vizzini & Simonini, comb. nov. Figs. 2-3 MycoBank MB808530



Fig. 2.

Alessioporus ichnusanus. A–D. Fresh basidiomes in nature. A. MG420a. B. MG241a. C. MCVE 20197. D. MCVE 17721. E, F. Velar tissues connecting pileus margin and stipe surface in young basidiomes, MCVE 17721. G, H. Narrow, granulose pseudo-annulus in the middle-lower part of the stipe. G. MG239a. H. MCVE 17721. Bars: A–H = 1 cm.





Alessioporus ichnusanus. Microscopic characters. A. Spores. B. Basidia. C. Cheilo- and pleurocystidia. D. Stipitipellis and lateral stipe stratum. E. Pileipellis. Bars: $A = 15 \mu m$, $B-D = 20 \mu m$, $E = 50 \mu m$. Drawings by M. Gelardi.

Basionym: Xerocomus ichnusanus Alessio, Galli & Littini, in Alessio, Boll. Gruppo Micol. G. Bres. 27(3–4):170. 1984.

- \equiv *Boletus ichnusanus* (Alessio, Galli & Littini) Oolbekkink, Persoonia 14(3):269. 1991.
- = *Boletus pulverulentus* Opat. sensu Cetto, Les Champignons de A à Z. Vol. 1:112–113. 1980.
- = *Boletus pulverulentus* f. *reticulatipes* Cetto, 1000 funghi. Vol. 2:376. 1982 (nom. inval., Art. 39.1).
- = *Boletus pulverulentus* f. *reticolata* Angarano, Boll. Gruppo Micol. G. Bres. 33(3–4/5–6):160. 1990 (nom. inval., Art. 39.1).
- ? = Boletus siculus Inzenga, Funghi Siciliani II:57. 1869. Non *Xerocomus ichnusanus* sensu Pérez-De-Gre-gorio M.À. Bolets de Cataluña XIX, Làmina núm. 950. 2000. = *Boletus fragrans* Vittad.

Holotype: Despite claims in the original diagnosis, no holotype material was located in Alessio's personal herbarium in Turin. In Roberto Galli's herbarium (RG), however, a sample labeled "TYPUS" with the same collection date cited by Alessio (23 October 1980) was found. The locality indicated is "Gonnosfanadiga (OR)", close to the location cited by Alessio, "Arborea". Although not far apart, Gonnosfanadiga and Arborea are two Sardinian municipalities presently in two different provinces, namely Medio Campidano (VS) and Oristano (OR) respectively. Furthermore Roberto Galli (pers comm) indicated that the specimens photographed by Littini (Alessio 1984 p 167) are undoubtedly the same as his type collection. For these reasons we assume Galli's collection is the holotype (now deposited as AMB 12755) and suggest that Alessio mistakenly

reported the wrong locality in the diagnosis and additionally forgot to retrieve the type material from R. Galli, who was the first documented collector of the species.

Unfortunately the holotype consists of only a small slice of pileus with no parts of stipe. Moreover, because of its aged condition and signs of repeated dissection, it did not yield useful molecular data. Accordingly we think it opportune to define an epitype, consisting of recently collected specimens, capable of yielding molecular information and coming from the same places as the original collections.

Epitype (designated here): AMB 12756, topotypic collection, Italy, Sardinia, Medio Campidano, Gonnosfanadiga, 21 Oct 2003, R. Galli, MycoBank MBT 177755, GenBank sequences <u>KJ729491</u> (ITS), <u>KJ729504</u> (LSU) and <u>KJ729513</u> (*tef-1*α). A color photograph of the epitype is found in <u>Galli</u> (2004), front cover, and <u>Galli (2007)</u>, p 134.

Basidiomes small to medium-small. Ontogenetic development secondary angiocarpic. Pileus (2–)3– 11(-13) cm diam, at first hemispherical then convex and finally pulvinate-flattened to vaguely depressed at center, moderately fleshy, firm at the beginning but progressively softer with age; margin initially coalescent with the stipe cortex but soon disrupting and curved downward, typically wavy and lobed until maturity, then progressively expanding and regular or uplifted, not or only slightly extending beyond the tubes; surface matt, dry, finely tomentose to glabrous, not areolate; cuticle ochreous (5C8-5D5), ochraceous-brown (6D8-6E5) to brownish olive-gray (5E8-5F4, 6F8-6F4), sometimes with copper red hues (7–8D7 to 7–8F8), evenly ornamented with radially arranged, brownish or dark brown to nearly blackish brown fibrils; slowly and barely darkening on handling and finally becoming sordid blackish brown (5F2, 5-6F3, 7-8F3 to 7-8F6); subcuticular layer whitish. Tubes at first thin then broader and usually as long as the thickness of the pileus context (up to 1 cm long), adnate to depressed around the stipe apex and shortly decurrent with a tooth, bright yellow (3A7, 3A8) to olive yellow (3B8-3D7) and finally olive green (3E7, 3E8), bruising blue when cut. Pores initially even, later with a convex surface, small at first then gradually wider (up to 0.2 cm diam), simple, roundish to angular, concolorous with tubes, turning blue when bruised and eventually fading to drab brown, at maturity with russet-brown stains at the pore edge. Spore print olive-brown. Stipe $(3-)4-11(-13) \times 1-3(-5)$ cm, as long as or a little longer than the pileus diameter at maturity, central to slightly off-center, solid, firm, dry, straight to curved or sinuous, initially ventricose-fusiform then cylindrical or rarely enlarged downward or faintly clavate but usually attenuate at the apex and always tapering at the base, moderately to strongly rooting; surface covered with a rough and longitudinally stretched reticulum or coarsely ribbed lengthwise or rarely appearing smooth throughout, exhibiting a prominent but narrow granular pseudo-annular zone in the lower or middle part, the latter only occasionally being absent, smooth in the lower quarter; yellowish cream (4A8-4B6) then yellowish brown in age (5-6C8 to 5-6D6) but of a brighter yellow at apex and on ribs (3A7, 3A8), dark red (9D8-9D6), reddish brown (8D8-8D6) to brown (8–9E8 to 8–9E5) in the lower half and gradually darker toward the base, bruising blue then sordid blackish brown when pressed; basal mycelium whitish gray. Context firm and tough when young, later soft in the pileus, up to 2 cm thick in the central zone, a little more fibrous in the stipe, whitish in the pileus (2-3A2) but sometimes pinkish below the cuticle (6-7A2), yellowish (2-3A3 to 2–3B3) in the stipe but gradually darker (2–3A4 to 2–3B4) downward, rhubarb red to brownish black (10-11D8 to 10-11F7) at the base; immediately turning blue throughout after sectioning and finally fading to drab yellowish; yellowish orange to rusty red or brown where eaten by slugs and with pinkish hues where eroded by maggots; subhymenophoral layer pale yellowish; dried material with brownish pileus and hymenophore, dirty yellow to ochraceous elsewhere. Odor faintly fruity, agreeable. Flavor mild.

Macrochemical reactions

NH₄OH: staining rusty brown on hymenophore, no reaction elsewhere. KOH: staining dark red on pileus, dark reddish brown on hymenophore and stipe, pinkish on pileus context, orange on stipe context but reddish brown at the base. FeSO₄: staining pale yellow on context, no reaction elsewhere; weak "fleeting-amyloid" reaction observed with Melzer's solution on hymenophoral trama.

Basidiospores [474/24/14] $(12.0-)13.3 \pm 0.8 (-14.0) \times (5.5-)5.7 \pm 0.3(-6.5) \mu m, Q = (2.19-)2.36 \pm 0.3(-6.5) \mu m$ 0.12(-2.59), V = $226 \pm 34 \,\mu\text{m}^3$, inequilateral, ellipsoidal to ellipsoidal-fusoid or subcylindrical in side view, ellipsoid in face view, smooth, with a pronounced apiculus and shallow suprahilar depression, with rounded apex, moderately thick-walled (0.5–1.0 µm), straw yellow-colored in water and KOH, having one, two or more frequently three large oil droplets when mature, inamyloid to weakly dextrinoid, acyanophilic and showing a faint metachromatic reaction. Basidia $(29-)31-40(-51) \times (8-)10-12$ (-16) µm (n = 30), cylindrical-clavate to clavate, moderately thickwalled (0.5–1.0 µm), predominantly four-spored but also 3-, 2-, or 1-spored, with relatively long sterigmata (3–6 µm), hyaline to pale yellowish and containing straw-yellow oil guttules in water and KOH, without basal clamps; basidioles subclavate to clavate, about the same size as basidia. Cheilocystidia $(35-)37-61(-65) \times 7-11 \mu m$ (n = 17), common, projecting straight to sometimes flexuous, cylindrical-fusiform to ventricose-fusiform or otherwise lageniform, with long neck and rounded tip, smooth, moderately thick-walled $(0.5-1.0 \,\mu\text{m})$, hyaline to pale yellowish or rarely brownish vellow in water and KOH, straw vellow (inamyloid) in Melzer's, without epiparietal encrustations. Pleurocystidia $(38-)45-70(-80) \times 8-13 \mu m$ (n = 29), frequent, color, dimensions, shape and chemical reactions as in cheilocystidia; pseudocystidia not observed. Pileipellis a trichoderm consisting of strongly interwoven, filamentous and sinuous, often branched hyphae not constricted at septa, tending to be repent in the outermost layer and thus turning into a cutis partially embedded in gelatinous matter; terminal elements [182/20/9] (42.0–)46.4 ± 3.9(-54.5) × (7.5–)8.2 ± 0.4(-9/0) µm, Q = (5.30–)5.90 ± 0.57(-7.12), cylindrical, apex rounded-obtuse, usually enlarged and with occasional protrusions, moderately thick-walled (up to 1 μ m), nearly hyaline to pale vellowish in KOH, smooth or seldom ornamented with subtle granular epiparietal encrustations; subterminal elements similar in shape, color and dimensions to terminal elements. Stipitipellis a texture of slender, loosely intermingled and longitudinally running, smooth-walled, adpressed hyphae, 3–11 um across, hyaline in water and KOH; the stipe apex covered by a well developed caulohymenial layer consisting of sterile caulobasidioles, fertile spore-bearing caulobasidia and scattered projecting caulocystidia similar in shape, color, dimensions and chemical reactions to hymenial cystidia. Lateral stipe stratum under the caulohymenium present and well differentiated from the stipe trama, of the "boletoid type" at the stipe apex a 50–130 µm thick layer consisting of divergent, inclined and running toward the external surface, loosely intermingled and unbranched or sparingly branched hyphae remaining separate from each other and embedded in a gelatinous substance; the stratum reducing during development and finally disappearing at maturity. Stipe trama composed of densely arranged, subparallel to loosely interwoven, filamentous, smooth, inamyloid hyphae, 5–15 µm broad, hyaline to pale yellowish in water and KOH. Hymenophoral trama bilateral-divergent of the "Boletus-type", with moderately to distinctly divergent and loosely arranged, gelatinized hyphae, lateral strata hyphae in transversal section remaining separate and (2-)4–7 µm apart, hyaline in water and KOH, inamyloid in Melzer's; lateral strata 30–50 µm thick, mediostratum 15–20 µm thick, consisting of a tightly adpressed, not gelatinized bundle of hyphae, 2-13 µm wide; in Congo red the mediostratum is darker than the lateral strata; oleiferous hyphae present. Basal mycelium consisting of subparallel to loosely intermingled, densely arranged, unbranched, filamentous, sinuous, inamyloid, smooth-walled hyphae, 2-10 µm broad, wall up to 1.0 µm thick, hyaline to yellowish in water and KOH. Clamp connections absent in all tissues. Hyphal system monomitic. Edible.

Material examined: FRANCE: CORSICA, Bastia, 42°42′03″N, 9°27′01″E, with Quercus ilex, 14 Oct 2009, A. Vizzini (TO AVX11). ITALY: CALABRIA REGION, Cosenza, Acri, Cozzo S. Angelo, 39°33'N, 16°25'E, 830 m, with Quercus sp., 4 Jun 1996, C. Lavorato (MCVE 18082); same loc., with Castanea sativa and Quercus sp., 23 Aug 1996, C. Lavorato (MCVE 18081); same loc., 900 m, with C. sativa and Q. frainetto, 29 Aug 1996, C. Lavorato (MCVE 18146); Cosenza, Serra di Zoto, Santa Sofia d'Epiro, 39°33'N, 16°25'E, 550 m, on acidic soil (pH 5.5-6.2) with Quercus sp., Cistus sp. and Populus alba, 8 Sep 1996, C. Lavorato (MCVE 18147); same loc., 600 m, with Q. pubescens, 12 Sep 1997, C. Lavorato (MCVE 18254); CAMPANIA REGION, Avellino, Trevico, 41°03'N, 15°15'E, 850 m, with Quercus sp., 22 Aug 1997, G. Bramini (MCVE 18253); EMILIA ROMAGNA REGION, Modena, Castelfranco Emilia, Villa Sorra Park, 44°36'45"N, 10°01'15"E, 30 m, on basic soil (pH 8.0) with Q. robur, 5 Sep 1987, G. Simonini (MCVE 17340); same loc., with O. robur, 19 Sep 1987, G. Simonini (MCVE 17587); same loc., with Q. robur, 19 Sep 1987, G. Simonini (MCVE 17588); same loc., with Q. robur, 11 Aug 1990, G. Simonini (MCVE 17591); Reggio nell'Emilia, Vetto, Gottano, 44°27'45"N, 10°18'45"E, 620 m, on basic soil (pH 7.6) with Q. pubescens, 30 Jul 1994, G. Simonini (MCVE 17721); LAZIO REGION, Rome, Malagrotta, 41°87′78″N, 12°32′11″E, 45 m, on sandy, calcareous soil in a pure plantation of Q. ilex, 22 Sep 2009, V. Migliozzi (MG266a); same loc., with Q. ilex, 27 Sep 2009, M. Gelardi (MG239a); same loc., in a pure plantation of O. suber, 27 Sep 2009, M. Gelardi (MG240a-MCVE 25599); same loc., in a pure plantation of Q. cerris, 27 Sep 2009, M. Gelardi (MG241a); Manziana (Rome), 42°12′34″N, 12°12′20″E, 370 m, on acidic soil with Q. cerris alongside track, 04 Aug 2011, M. Gelardi and V. Migliozzi (MG420a); Ostia (Rome), Castelfusano Pinewood, 41°72'27"N, 12°30'79"E, 2 m, with O. ilex and Pinus pinea, 5 Sep 2013, M. Gelardi (MG549a); LIGURIA REGION, Genova, Recco, 44°21′51″N, 9°8′17″E, on acidic soil with Q. cerris, Q. pubescens and Erica arborea, 25 Aug 2005, E. Rigoni (RG XER.ICH. 5); PIEDMONT REGION, Alessandria, Miogliola Garbarini, 44°31'N, 8°22'E, 400 m, on acidic soil with Q. pubescens and Corvlus avellana, 6 Sep 2007, R. Galli (RG XER.ICH. 6); SARDINIA, Medio Campidano, Gonnosfanadiga, with Quercus spp. and Cistus spp., 39°51'N, 8°35'E, 23 Oct 1980, R. Galli (AMB 12755); same loc., with Quercus spp. and Cistus spp., 21 Oct 2003, R. Galli (AMB 12756); Medio Campidano, San Gavino Monreale, 39°33'N, 8°48'E, 55 m, Eucalyptus sp., 03 Nov 1983, S. Curreli (IB 1983/0552); Carbonia-Iglesias, Sant' Anna Arresi, 43°57'N, 9°05'E, 12 Nov 1985, Anonymous (Rebaudengo's pers. herb., s.n.); Olbia-Tempio, Tempio Pausania, 43°57'N, 9°05'E, with Q. suber, 3 Oct 1987, F. Fiandri (MCVE 17589); same loc., with Q. suber, 7 Oct 1987, F. Fiandri (MCVE 20197); Nuoro, Belvì, Onitzu, 39°58'N, 9°11'E, 600 m, with Q. suber, 19 Sep 2013, A. Mua (TO AVX13).

Habit, ecology, phenology, distribution: Solitary to gregarious or more often caespitose, sometimes with basidiomes arising from the stipes of adjacent basidiomes, in warm Mediterranean regions, growing in association with *Quercus* spp. (*Q. ilex, Q. suber, Q. coccifera, Q. cerris, Q. robur, Q. pubescens, Q. petraea, Q. pyrenaica, Q. frainetto*) and to a lesser extent *Cistus* spp., rarely with *Castanea sativa* and perhaps with the exotic *Eucalyptus camaldulensis*, also reported with *Pteridium aquilinum* (Tentori 2006), on dry soil, ubiquitous, summer to early autumn. Reported from southern Europe, uncommon to rare.

Commentary: Formally described as a new *Xerocomus* species by <u>Alessio (1984)</u>, *Alessioporus ichnusanus* first was found in Sardinia by the Italian mycologist R. Galli in 1980 and initially reported as an unknown species (<u>Galli 1981</u>). It was since recorded from several different places in Sardinia (<u>Galli 1987</u>; <u>Brotzu 1988</u>, 1993; Foiera et al. 1993; <u>Brotzu and Colomo 2009</u>), Sicily and mainland Italy (<u>Morara 1988</u>; <u>Simonini and Fiandri 1988</u>; <u>Alessio 1991</u>; <u>Migliozzi and Coccia 1991</u>; <u>Redeuilh and Simonini 1995</u>, 1999; <u>Lavorato 1996</u>; <u>Gennari 1997</u>, 2005; <u>Chevtzoff 1998</u>; <u>Galli 1998</u>, 2007, 2013; <u>Ladurner and Simonini 2003</u>; <u>Cazzoli 2006</u>; <u>Tentori 2006</u>; <u>Zuccherelli 2006</u>; <u>Consiglio and Papetti 2009</u>; <u>Gelardi 2010</u>; <u>Illice et al. 2011</u>; <u>Rodà 2012</u>) and from several

European countries, mostly in the Mediterranean, namely France (<u>Chevtzoff 1998</u>, <u>Redeuilh and</u> <u>Simonini 1999</u>, <u>Eyssartier and Roux 2011</u>), Spain, including the Balearic Islands (<u>Águeda et al.</u> 2006, <u>Ruiz Fernández and Ruiz Pastor 2006</u>, <u>Calzada Domínguez 2007</u>, <u>Muñoz et al. 2008</u>), Bulgaria (<u>Assyov and Stoykov 2011b</u>) and Greece, including the Cyclades Islands (<u>Constantinidis 2009</u>; <u>Polemis et al. 2012</u>), with a single finding in Austria (<u>AMS 2009</u>).

Alessioporus ichnusanus is a medium-small species, exhibiting an ochraceous-brown to dark olivaceous-brown fibrillose pileus, sometimes with copper red hues and a wavy margin at least in young specimens, a yellow to olive colored hymenophore and a stout, deeply rooting stipe covered with a rough and darker net that is rarely absent, as reported by <u>Chevtzoff (1998)</u>, <u>Taylor et al.</u> (2001, 2002) and <u>Galli (2007, 2013</u>), bright yellow at the apex, dark red-brown to blackish brown elsewhere and with a whitish gray basal mycelium. The context is whitish in the pileus, yellowish in the stipe with reddish shades, purplish red to brownish black at the base, turns uniformly blue on exposure, as do the external surfaces after injury or bruising. Such strong discoloration, caused by auto-oxidation, is unlike the light blue staining of most *Xerocomus* species (<u>Alessio 1985</u>). The most important morphological character is the narrow, granular ring-like zone in the middle or lower half of the stipe, formed by the remnants of the connection between the pileus margin and the stipe cortex during the primordial stage. Other observations indicate highly variable spore size, sometimes with aberrant basidiospores more than 20 μm or even up to 28–30 μm long (<u>Alessio 1984</u>, 1985; <u>Simonini and Fiandri 1988; Brotzu 1993</u>; <u>Foiera et al. 1993</u>; <u>Chevtz-off 1998</u>; <u>Lannoy and Estadès 2001</u>; <u>Galli 2007, 2013</u>; <u>Brotzu and Colomo 2009</u>).

<u>Lavorato (1991)</u> and Contu (pers comm) suggested that *Boletus siculus* Inzenga (<u>Inzenga 1869</u>) might be an older name for *A. ichnusanus*. Unfortunately no type material was preserved for *B. siculus* and there is no evidence to support their conspecificity.

Alessioporus ichnusanus was misinterpreted as a reticulate phenotype of *Boletus pulverulentus* Opat. (Cetto 1980, 1982; Angarano 1990), and perhaps with *B. poikilochromus* Pöder, Cetto & Zuccherelli (Cetto 1983), in that the same provisional name, *B. pulverulentus* f. *reticulatipes*, has been given to both of them. Apart from the reticulum, *B. pulverulentus* differs from *A. ichnusanus* by its slender, xerocomoid appearance, stronger bluing overall, narrower spores with a Q value of 2.6–2.9 and the nearly sterile stipe (Alessio 1985, Breitenbach and Kränzlin 1991, Lannoy and Estadès 2001, Muñoz 2005, Watling and Hills 2005, Galli 2007, Klofac 2007, Šutara et al. 2011).

Boletus poikilochromus shares with *A. ichnusanus* the boletoid appearance and strongly bluing tissues but differs mainly by the tendency of the basidiomes to fade to cinnamon brown with age, its peculiar odor and noticeably smaller spores ($11.7 \times 4.7 \mu m$ ave.) (Lannoy and Estadès 2001, Muñoz 2005, Galli 2007, Klofac 2007, pers obs).

Boletus rainisii A.E Bessette & O.K. Miller occurs in North America and is discriminated by the dark olivaceous brown pileus, bright yellow smooth stipe with reddish hues at the extreme base, darker blue staining reaction throughout, shorter pileipellis terminal cells that are covered with brown encrustations, sterile stipe surface and its association with conifers (Bessette et al. 2000).

The Chinese *B. sinopulverulentus* Gelardi & Vizzini is readily distinguishable by the smaller size, entirely dark brown stipe that is finely scabrous-fissured radially so as to resemble a zebra pattern, stronger blue staining reaction, non-rooting stipe, nearly sterile stipe surface and smaller spores (Gelardi et al. 2013).

Alessioporus ichnusanus is an edible species but curiously <u>Brotzu and Colomo (2009)</u> refer to it as probably poisonous. It was keyed out in several regional mycotas and bolete monographes, such as

Engel et al. (1996), Estadès and Lannoy (2004), Horak (2005), Muñoz (2005), Klofac (2007), Boccardo et al. (2008), Rödig (2012), and it is undoubtedly a well characterized species, unlikely to be confused with any other taxa. The only incorrect attribution we have found is a misinterpretation as *B. fragrans* Vittad. (<u>Pérez-De-Gregorio 2000</u>). Because of its rarity, *A. ichnusanus* recently was included in the Red List of Italian macrofungi (<u>Rossi et al. 2013</u>).

Pulchroboletus Gelardi, Vizzini & Simonini, gen. nov.

MycoBank MB808531

Diagnosis: Differing from *Alessioporus* by the pastel pink, cream-pinkish to whitish pink or rarely blood red pileus surface, the smooth to densely punctuate stipe surface, rarely with a coarse reticulum, the pseudo-annulus usually located in the upper or middle part of the stipe, the pinkish lilac context of the pileus and unique ITS, LSU and *tef-1* α sequences.

Type species: Xerocomus roseoalbidus Alessio & Littini.

Etymology: the epithet "pulchroboletus" (pulchro = beautiful) is derived from Latin and refers to the dramatic tints of the basidiomes.

Pulchroboletus roseoalbidus (Alessio & Littini) Gelardi, Vizzini & Simonini, comb. nov. <u>Figs. 4</u>–<u>5</u>





Pulchroboletus roseoalbidus. A–C, G, H. Fresh basidiomes in nature. A. AMB 12757, epitype (photo by A. Mua). B. MG416a. C. Caespitose growth, MG533a. D. Velar tissues connecting pileus margin and stipe surface in a young basidiome, MG416a. E. Narrow, granulose pseudo-annulus in the middle-upper part of the stipe, MCVE 27721. F. Stipe cross section of the "ring-zone" granules with remnants of the veil (white arrow), MCVE 17577. G, H. Basidiomes simulating a truly angiocarpic behavior with tendency toward gasteromycetization. G. MCVE18214. H. MCVE 18215. Bars: A–D, G, H = 1 cm, E = 0.5 cm, F = 100 μ m.



Fig. 5.

Pulchroboletus roseoalbidus. Microscopic characters. A. Spores. B. Basidia. C. Cheilo- and pleurocystidia. D. Stipitipellis and lateral stipe stratum. E. Pileipellis. Bars: A, $B = 15 \mu m$; C, $D = 20 \mu m$; $E = 30 \mu m$. Drawings by M. Gelardi.

MycoBank MB808532

Basionym: Xerocomus roseoalbidus Alessio & Littini, in Alessio, Mic. Ital. 16:21. 1987.

- = *Boletus roseoalbidus* (Alessio & Littini) G. Moreno & Heykoop, Doc. Myc. 25:274. 1995.
- = *Pulveroboletus albopruinosus* Cetto & Zuccherelli, I funghi dal vero. Vol. 5:437. 1987.
- = *Xerocomus calañiensis* Ruiz Fernández, Guía Micoló-gica. Tomo IV: suplemento al orden Boletales en España:283. 1997 (nom. inval., Art. 39.1).

Holotype: Unavailable (see DISCUSSION).

Lectotype (designated here): Plate 46 (p 17) in <u>Alessio (1987)</u>, Mic. Ital. 16, MycoBank MBT 177765. Epitype (designated here): AMB 12757, Italy, Sardinia, Cagliari, Sinnai, Mount Cresia, 17 Sep 2009, A. Mua and M. Casula (<u>Fig. 4A</u>), MycoBank MBT 177766, GenBank sequences <u>KJ729486</u> (ITS), <u>KJ729499</u> (LSU) and <u>KJ729512</u> (*tef-1*α).

Basidiomes small to medium small

Ontogenetic development secondary angiocarpic, Pileus (1-)2-10(-12) cm diam, at first hemispherical then persistently convex and finally pulvinate-flattened to vaguely depressed at center, moderately fleshy, firm when young but progressively softer with age; margin initially involute and coalescent with the stipe cortex but soon disrupting and curved downward, then progressively expanded or uplifted, regular to faintly sinuate or lobed, not or only slightly exceeding beyond the tubes (up to 0.1 cm); surface mat, dry but slightly greasy in moist weather, finely tomentose but becoming glabrous in aged specimens, not areolate; cuticle at first pastel pink (7A4-7C3, 8A4-8C3, 9A3-9B2), gradually bleaching to pale pink (10-12A3 to 10-12A2, 13-15A2), cream-pinkish (6A4, 6A3), pinkish beige (6–7B3 to 6–7C2) or whitish pink (5–6–7A2), sometimes with darker pigmented, pinkish purple areas or spots (10-11C5 to 10-11D4), occasionally uniformly blood red or vinaceous red (8–10C8 to 8–10C7, 10–11D8 to 9–11E7); nearly unchangeable or staining deep purple-red when rubbed, bluish when injured; subcuticular layer pinkish purple. Tubes at first thin then increasingly broader and usually longer than pileus context thickness (up to 1.8 cm long), adnate to depressed around the stipe apex and shortly decurrent with a tooth, at times subdecurrent, bright yellow (3A7, 3A8) to olive yellow (3B8–3D7) and finally olive green (3E7, 3E8), bruising blue on cutting. Pores initially forming a flat surface, later convex, small at first then distinctly wider (up to 3 mm diam), simple, roundish to angular, concolorous with tubes, turning blue on bruising and eventually fading to drab brown, at maturity with russet-brown stains at the pore edges. Spore print olive-brown. Stipe $(2-)3-9(-11) \times (0.5-)1-$ 3(-7) cm, as long as or a little longer than pileus diameter at maturity, central to slightly off-center, solid, firm, dry, straight to curved or sinuous, generally narrowing downward, rarely cylindrical, ventricose-fusiform or flattened, occasionally subclavate but always pointed at the base, conspicuously rooting; surface smooth to fibrillose, without reticulum or only sporadically with a rough, elongated net, with a prominent but narrow granular pseudoannular zone in the middle or upper part, such ornamentation being sometimes undetectable; bright yellow at apex (3A7, 3A8), yellowish elsewhere (4A6, 4A3) but covered with fine and scattered, reddish to purple-red punctuations, more pronounced toward the base, reddish brown (8–9D8 to 8–9E7) at the base in old basidiomes, bruising blue then sordid blackish brown on handling; basal mycelium whitish. Context firm and tough in youth, later soft in the pileus (up to 2.3 cm thick in the central zone), more fibrous in the stipe, pinkish lilac (12–14B4 to 12–14B2) in the pileus, particularly bright beneath the cuticle and above the tubes, yellow (2–3A2 to 2–3A3) in the stipe but gradually darker (2–3A4) downward; immediately turning blue in the stipe and above the tubes but nearly unchangeable in the rest of the pileus when exposed to air, after several hours fading to cream-yellowish in the pileus and dirty ochraceous in the stipe; reddish purple where eaten by slugs in the pileus and with pinkish purple hues in tissues eroded by maggots; subhymenophoral layer pinkish lilac; dried material with whitish pink pileus and brownish hymenophore, reddish brown on stipe, dirty yellow to ochraceous on context. Odor faintly fruity, agreeable. flavor mild.

Macrochemical reactions: NH₄OH: staining rusty brown on hymenophore, orange on stipe surface, none elsewhere (the pinkish lilac tint in pileus context is bleached away); KOH: staining dark red to reddish brown on pileus and stipe, dark reddish brown on hymenophore, pinkish on pileus context, orange on stipe context but reddish brown at the base; FeSO₄: staining pale olive-green throughout; weak "fleeting-amyloid" reaction observed with Melzer's solution on hymenophoral trama.

Basidiospores [361/20/12] (13.5–)14.7 \pm 0.8 (–16.0) × (6.5–)6.8 \pm 0.3(–7.5) µm, Q = (1.97–) 2.17 \pm 0.11(–2.30), V = 359 \pm 39 µm³, inequilateral, broadly ellipsoidal to less frequently ellipsoid-fusoid in side view, broadly ellipsoid in face view, smooth, with a pronounced apiculus and shallow to moderately marked suprahilar depression, with rounded apex, moderately thick-walled (0.5–1.0 µm), straw-yellow colored in water and KOH, having one, two or more frequently three large oil

droplets when mature, inamyloid to moderately dextrinoid, acyanophilic and with an ortochromatic reaction. Basidia (23–)30–50 (–53) × (10–)12–15(–19) μ m (n = 25), cylindrical to cylindricalclavate, rarely truly clavate, moderately thick-walled (0.5–1.0 µm), predominantly four-spored but frequently also three-, two-, or one-spored, with relatively long sterigmata (3–6 µm, although 15 µm long sterigmata were observed on one anomalous basidium), hyaline to pale yellowish and containing straw-yellow oil guttules in water and KOH, without basal clamps; basidioles clavate, about the same size as basidia. Cheilocystidia $(32-)38-66(-68) \times 6-12 \mu m$ (n = 20), common, projecting straight to sometimes flexuous, fusiform to subcylindrical or rarely ventricose-fusiform. with long neck and rounded tip, occasionally clavate, smooth, moderately thick-walled (0.5–1.0 μm), hyaline to pale yellowish in water and KOH, straw yellow (inamyloid) in Melzer's, without epiparietal encrustations. Pleurocystidia $(40-)59-76(-80) \times (8-)12-18(-20) \mu m (n = 25)$, color, shape and chemical reactions as in cheilocystidia, but slightly longer and distinctly broader. predominantly ventricose-fusiform (a single cystidium with a diverticulate tip was observed), infrequent; pseudocystidia not observed. Pileipellis a trichoderm consisting of strongly interwoven, filamentous and sinuous, often branched hyphae not constricted at septa, tending to be repent in the outermost layer and thus turning into a cutis partially embedded in gelatinous matter; terminal elements [259/20/10] $(30.0-)44.2 \pm 8.4$ $(-55.5) \times (7.0-)8.0 \pm 0.4(-8.5) \mu m$, Q = $(4.01-)5.67 \pm$ 0.95(-7.14), cylindrical, some short and acorn-shaped or bullet-shaped, occasionally cystidioid, apex rounded-obtuse and enlarged or tapered, moderately thick-walled (0.5–1.0 µm), nearly hyaline to pale yellowish in KOH, smooth or rarely ornamented with subtle granular epiparietal encrustations; subterminal elements similar in shape, color and dimensions with terminal ones. Stipitipellis a texture of slender, subparallel to loosely intermingled and longitudinally running, smooth-walled, adpressed hyphae, $3-11 \mu m$ wide, hyaline in water and KOH; the stipe apex covered by a well developed caulohymenial layer consisting of sterile caulobasidioles, fertile sporebearing caulobasidia and projecting caulocystidia similar in shape, color, dimensions and chemical reactions to the pleurocystidia. Lateral stipe stratum under the caulohymenium present and well differentiated from the stipe trama, of the "boletoid type", at the stipe apex a 40–80 µm thick layer consisting of divergent, inclined, running toward the external surface, loosely intermingled and unbranched or sparingly branched hyphae remaining separate from each other and embedded in a gelatinous substance; the stratum reducing during development and finally disappearing at maturity. Stipe trama composed of densely arranged, loosely to strongly interwoven, filamentous, smooth, inamyloid hyphae, 2–13 µm broad, hyaline to pale yellowish in water and KOH. Hymenophoral trama bilateral-divergent of the "Boletus-type", with moderately to distinctly divergent and loosely arranged, gelatinized hyphae, lateral strata hyphae in transversal section remaining separate and (1– 4-7(-10) µm apart, hyaline in water and KOH, inamyloid in Melzer's; lateral strata (10-)15-40μm thick, mediostratum 15–40 μm thick, consisting of a tightly adpressed, not gelatinized bundle of hyphae, 3–13 µm wide; in Congo red the mediostratum is darker than the lateral strata; oleiferous hyphae present. Basal mycelium consisting of subparallel to loosely intermingled, densely arranged, unbranched, filamentous, sinuous, inamyloid, smooth-walled hyphae, 3-17 µm broad, wall up to 1.0 µm thick, hyaline to pale yellowish in water and KOH. Clamp connections absent in all tissues. Hyphal system monomitic. Edible.

Material examined: ITALY: CALABRIA REGION, Cosenza, S. Demetrio Corone, Castagna Rotonda, 39°33'N, 8°35'E, 650 m, in mixed deciduous woodland, 10 Aug 1994, *C. Lavorato* (MCVE 17986); Cosenza, S. Demetrio Corone, Cozzo S. Angelo, 39°33'N, 8°35'E, 850 m, with *Castanea sativa* and *Quercus frainetto*, 23 Aug 1996, *C. Lavorato* (MCVE 18144); CAMPANIA REGION, Avellino, Trevico, 41°03'N, 15°15'E, 850 m, with *Quercus* sp., 22 Aug 1997, *G. Bramini* (MCVE 18216); EMILIA ROMAGNA REGION, Modena, Castelfranco Emilia, Villa Sorra Park, 44°36'45"N, 10°01'15"E, 30 m, on basic soil (pH 8.0) with *Q. robur*, 15 Sep 1985, *F. Fiandri* (MCVE 17352); same loc., with *Q. robur*, 5 Sep 1987, *G. Simonini* (MCVE 17353); same loc., with *Q. robur*, 19 Sep 1987, *G. Simonini* (MCVE 17577); same loc., with *Q. robur*, 19 Sep 1987, *G.*

Simonini (MCVE 27721); same loc., with Q. robur, 05 Oct 1987, F. Fiandri (MCVE 17578); same loc., with Q. robur, 5 Oct 1987, F. Fiandri (MCVE 17579); same loc., with Q. robur, 11 Aug 1990, G. Simonini (MCVE 17581); same loc., with Q. robur, 15 Sep 1985, F. Fiandri (MCVE 17351); Ravenna, 44°25'N, 12°12'E, 4 m, with Quercus sp. and Pinus sp., 23 Aug 1986, A. Zuccherelli (IB 1986/0343); Ravenna, San Vitale, 44°25'N, 12°12'E, 4 m, with Q. cerris, 5 Sep 1996, A. Zuccherelli (IB 1996/0894); Bologna, Parco Cavaioni, 44°27'N, 11°25'E, 250 m, with Q. pubescens (coll. with gasteroid specimens), 20 Aug 1995, G. Consiglio (MCVE 18214-18215); LAZIO REGION, Manziana (Rome), 42°12'34"N, 12°12'20"E, 370 m, on acidic soil with Q. cerris alongside track, 4 Aug 2011, M. Gelardi and V. Migliozzi (MG416a); same loc., with Q. cerris alongside track, 4 Aug 2011, M. Gelardi and V. Migliozzi (MG417a); same loc., with Q. cerris alongside track, 26 Jul 2013, M. Gelardi, B. Picillo and L. Perrone (MG532a); same loc., with Q. cerris, 26 Jul 2013, B. Picillo, M. Gelardi and L. Perrone (MG533a); Ladispoli (Rome), Palo Laziale Oasis, 41°94'01"N, 12°10'00"E, 5 m, with Q. ilex, Arbutus unedo, Pistacia lentiscus, Q. petraea and Pinus pinea, 8 Nov 2013, M. Gelardi, V. Migliozzi and L. Nicoletti (no voucher material available); SARDINIA, Oristano, 39°54'N, 8°35'E, Nov 1985, V. Carcò and E. Mendolia (Rebau-dengo's pers. herb., s.n.); Olbia-Tempio, Aggius, 40°56'N, 9°04'E, with Quercus sp., 16 Oct 1986, G. Littini (Rebaudengo's pers. herb., s.n.); Olbia-Tempio, Tempio Pausania, 40°57/N, 9°05'E, 450 m, with *Quercus* sp., 15 Oct 1987, F. Fiandri (MCVE 17580); same loc., 600 m, with Q. suber, 2 Oct 1996, M. Contu (MCVE 18217); Cagliari, Sinnai, Mount Cresia, 39°18'19"N, 9°12'16"E, 650 m, with Q. ilex, Arbutus unedo and Cistus spp., 17 Sep 2009, A. Mua and M. Casula (AMB 12757).

Habit, ecology, phenology, distribution: Solitary to gregarious, more often caespitose or branched, with specimens rising from the stipe of adjacent basidiomes or with two stipes merging into a single pileus, in warm Mediterranean regions, growing in association with *Quercus* spp. (*Q. ilex, Q. suber, Q. coccifera, Q. cerris, Q. robur, Q. pubescens, Q. petraea, Q. pyrenaica, Q. frainetto*), also with *Cistus* spp., rarely with *Castanea sativa*, on dry soil, ubiquitous, summer to early autumn. Reported from southern Europe, rare.

Commentary: Pulchroboletus roseoalbidus was described (in *Xerocomus*) in early 1987 from collections made by M. Sarnari and G. Littini, in central Italy and V. Carcò and E. Mendolia in Sardinia (Alessio 1987, 1990). A few months later the same taxon was published under the name *Pulveroboletus albopruinosus* Cetto & Zuccherelli (Cetto 1987). Since then, this species was reported from insular and peninsular Italy (Simonini and Fiandri 1989; Littini 1992; Foiera et al. 1993; Zuccherelli 1993 as *P. albopruinosus*; Gennari 1994, 2005; Redeuilh and Simonini 1995; Galli 1998, 2007, 2013; Contu 1999; Lavorato and Rotella 1999; Consiglio and Papetti 2001; Cazzoli 2002, 2006; Migliozzi and Camboni 2002; Ladurner and Simonini 2003; Venturella 2004; Tentori 2006; Brotzu and Colomo 2009) and from other European countries, such as France (Corsica) (Anonymous 1989, Alessio 1991, Roth 1994, Lannoy and Estadès 2001, Eyssartier and Roux 2011), Spain, including the Balearic Islands (Moreno et al. 1995, Ruiz Fernández 1997 as "*X. calañiensis*", Pardo et al. 2004, Calzada Domínguez 2007, Muñoz et al. 2008), Hungary (Károly 2006), Bulgaria (Assyov and Denchev 2009, Denchev and Assyov 2010) and Greece, including the Cyclades Islands (Constantinidis 2009 as "*Rubinoboletus roseoalbidus*", Polemis et al. 2012).

Pulchroboletus roseoalbidus is circumscribed by its medium-small size, pale pastel pink, creampink to whitish with pinkish hues or rarely evenly blood red pileus, yellow to olive hymenophore, tapered and deeply rooting yellow stipe covered with reddish punctuations, especially in the lower half, whitish basal mycelium, a yellowish context that is bright pinkish lilac in the peripheral zones of the pileus (at times pinkish overall in the pileus) and tissues that quickly bruise blue on injury. The stipe of *P. roseoalbidus* is generally devoid of reticulum, although a narrow ring-like pattern of coarse granules is nearly always visible, but sometimes it is ornamented by a rough net or ribs arranged longitudinally (Foiera et al.1993, Lannoy and Estadès 2001, Consiglio and Papetti 2001, Cazzoli 2002, Migliozzi and Camboni 2002, Ladurner and Simonini 2003, Cazzoli 2006, Calzada Domínguez 2007, Galli 2007). Basidiospores are uniformly broad (~7 µm ave.) and, as was reported for *A. ichnusanus*, oversize spores were observed for for *P. roseoalbidus*, up to 25 µm long and 9 µm wide (Alessio 1987, 1990; Migliozzi and Camboni 2002; present study). Some authors (Cetto 1987, Moreno et al. 1995, Contu 1999) report a blue-violet amyloid reaction of the stipe trama with Melzer's solution. However, such a reaction was not observed in our material and we suggest it might be occasional or inconsistent.

In the original diagnosis of *P. albopruinosus* the only major discrepancy was the spore size, which is dramatically smaller than that of *P. roseoalbidus* (Cetto 1987). This divergence, however, was later shown to be measurement error (Alessio 1990). The taxonomic attribution to the genus *Pulevoroboletus* is also questionable because it was based on the presumed solubility of the yellow pigment of the hymenium, a character not included in the delimitation of the genus (Singer 1986) and moreover not detected on *P. albopruinosus* specimens (Simonini and Fiandri 1989).

<u>Simonini and Fiandri (1989)</u> stressed that *Boletus rigelliae* Velen. (<u>Velenovský 1922</u>) might represent an older name for *P. roseoalbidus*, but as already pointed out by <u>Alessio (1985, 1991</u>) the former is most likely conspecific with *Aureoboletus gentilis* (Quél.) Pouzar.

Despite its distinctive features, *P. roseoalbidus* is morphologically similar to the eastern North American species *Boletus patrioticus* Baroni, A.E. Bessette & Roody, but differs from the latter by the dark red to brownish olivaceous red pileus, slight blueing in the context above the tubes and only erratically elsewhere, different pileipellis arrangement and smaller spores; in addition, *B. patrioticus* lacks the ring-like zone on the stipe and its growth is never caespitose (Baroni et al. 1998, Bessette et al. 2000).

Among European species, *Xerocomus bubalinus* (Oolbekkink & Duin) Redeuilh (? = *X. erubescens* Cadiñanos & Muñoz) shares with *P. roseoalbidus* the noticeably pinkish tint beneath the cuticle but is discriminated by the dark reddish brown pileus, gradually fading to ochraceous buff, context turning light blue just above the attachment with the tubes and in the pileus-stipe connection zone, distinctly smaller spores, different pileipellis stucture, non-caespitose growth and association mainly with *Populus* spp. and *Tilia* spp. in wet habitats (<u>Oolbekkink 1991</u>, <u>Muñoz et al. 2008</u>, <u>Gelardi 2009</u>, <u>Assyov and Stoykov 2011a</u>, <u>Knudsen and Taylor 2012</u>).

Xerocomus rubellus Quél. is a more vividly colored species characterized by bright red pileus and stipe, light blue oxidation on external surfaces, uniformly yellow context with carrot orange punctuation at the stipe base and discoloration to light blue only above the tubes, smaller spores, different pileipellis stucture and a growth habit that is never caespitose (Engel et al. 1996, Lannoy and Estadès 2001, Watling and Hills 2005, Klofac 2007, Muñoz et al. 2008, Šutara et al. 2011).

As with *A. ichnusanus*, *P. roseoalbidus* was included in many European bolete field guides and regional taxonomic treatments (Engel et al. 1996, Taylor et al. 2002, Estadès and Lannoy 2004, Horak 2005, Muñoz 2005, Klofac 2007, Eyssartier and Roux 2011, Rödig 2012).

Previous SectionNext Section

Discussion

Alessioporus *and* Pulchroboletus *phylogeny and intergeneric placement:* Although some molecular investigations were published over the past two decades (Binder and Fischer 1997; Binder 1999; Taylor et al. 2001, 2006, 2007; Peintner et al. 2003; Binder and Hibbett 2006; Dentinger et al. 2010; Gelardi et al. 2013; Nuhn et al. 2013; Vizzini et al. 2014), a comprehensive taxonomic classification *Boletus* s.l. and *Xerocomus* s.l. supported by molecular phylogenetic analysis is still lacking. In an analysis based only on ITS sequences, *X. roseoalbidus* was recovered as sister to a clade formed by *Hemileccinum* and *Xerocomus* s.s. (Gelardi et al. 2013) and *X. ichnusanus* as sister to *Boletus* phylogenetus s.l.

Based on molecular analysis (Fig. 1), the phylogenetic relationship among *Alessioporus*, *Pulchroboletus* and *Hemileccinum* in the Hypoboletus group (Nuhn et al. 2013) can be inferred.

Compared with *Alessioporus* and *Pulchroboletus*, the genus *Hemileccinum* is morphologically distinguished by its medium-large to relatively large size, the pileus surface staining violet with NH4OH, its small, roundish pores, the stipe covered by a scabrous, concolorous ornamentation at least in early developmental stages, unstaining tissues, an iodine-like odor at the stipe base, the lateral stipe stratum of the "leccinoid type" (i.e. a $150-400[640] \mu m$ thick layer at the stipe apex consisting of divergent, anticlinally arranged, nongelatinous fascicles of hyphae breaking up to generate scabrosities), gymnocarpic devolopment and non-caespitose growth (<u>Šutara 1989, 2005</u>, 2008; <u>Šutara et al. 2009</u>).

The closely related *Xerocomus* s.s. (= *X. subtomentosus* complex) is distinguished from the two new genera by having a more or less pronounced blue-green staining reaction on the pileus surface with NH4OH, unchanged to faintly and erratically bluing tissues on injury, its bacillate spores under SEM, an hymenophoral trama of the "*Phylloporus*-type", a non-gelatinized lateral stipe stratum, gymnocarpic devolopment and non-caespitose growth (Oolbekkink 1991; Holec 1994; Ladurner and Simonini 2003; Šutara 2005, 2008; Šutara et al. 2009).

Alessioporus *and* Pulchroboletus *taxonomy and biogeography:* This is the first study to investigate evolutionary phylogenetic relationships of the boletoid Mediterranean taxa *Alessioporus ichnusanus* and *Pulchroboletus roseoalbidus*. The two species are ecologically and ontogenetically similar, with their affinities being implemented and strongly corroborated by molecular inference, yet distinct at the generic level from morphological and phylogenetic perspectives (Table II, Fig. 1). Molecular phylogenetic inference confirmed their kinship and showed that these two species represent well supported but distinct lineages within the Hypoboletus group, justifying their formal description.

Alessioporus ichnusanus	Pulchroboletus roseoalbidus
Pileus ochraceous brown, dark olive-brown to copper- brown with brownish black fibrils	Pileus pastel pink, cream-pinkish to whitish pink or occasionally blood red
Pileus margin wavy-lobed in youth	Pileus margin regular to faintly wavy
Stipe surface reticulate to coarsely ribbed	Stipe surface usually smooth to densely punctuate
Stipe exhibiting a narrow pseudo-annulus in the middle/lower part of the stipe	Stipe exhibiting a narrow pseudo-annulus in the middle/upper part of the stipe
Context whitish to yellowish	Pileus context lilac-pinkish
Basidiospores (12.0–)13.3 ± 0.8(–14.0) × (5.5–)5.7 ± 0.3(–6.5) µm, Q = (2.19–)2.36 ± 0.12(–2.59)	Basidiospores (13.5–)14.7 \pm 0.8(–16.0) × (6.5–)6.8 \pm 0.3(–7.5) µm, Q = (1.97–)2.17 \pm 0.11(–2.30)
Pleurocystidia (38–)45–70(–80) \times 8–13 μm	Pleurocystidia (40–)59–76(–80) × (8–)12–18(20) μm

Comparison of the main morphological differences between A. ichnusanus and P. roseoalbidus

The unusual synapomorphic traits of *A. ichnusanus* and *P. roseoalbidus* are: (i) yellow-olive hymenophore, (ii) narrow pseudo-annulus on the stipe, (iii) rooting stipe with whitish basal mycelium, (iv) overall dark indigo staining reaction on rubbing or exposure to air, (v) mild flavor, (vi) olive-brown spore print, (vii) smooth spores with both light microscopy and SEM, (viii) pileipellis a trichoderm of interwoven, filamentous hyphae, (ix) hymenophoral trama of the "*Boletus*-type", (x) fertile stipe surface and "boletoid" lateral stipe stratum, (xi) unusual growth, with both species always occurring gregariously and very often caespitose or irregularly branched with various basidiomes clustered at the base, usually in groups of 5–8 connate but occasionally as many as 12 (Alessio 1985, Galli 2007). The two taxa can be morphologically distinguished following the characters (Table II).

The distinctive set of microscopic characters of *Alessioporus* and *Pulchroboletus* do not fit well with either *Xerocomus* s.s., *Xerocomellus* Šutara or *Hemileccinum* Šutara, according to the remarkable anatomical analyses of <u>Šutara (2005, 2008</u>). They seems to be more congruent with the very broad concept of *Boletus* s.l. defined by <u>Šutara (2008)</u>, underlying the controversial taxonomic position of the two species presently examined (<u>Oolbekkink 1991</u>).

Ontogenetic development of basidiomes appears to be taxonomically relevant and may be treated as one of the most reliable and phylogenetically informative morphological features, suggesting a close affinity of the two species. According to the different ontogenetic patterns proposed by Reijnders (1948, 1963) and subsequently by Singer (1986), carpogenesis of A. ichnusanus and P. roseolabidus is doubtless secondary angiocarpic (Simonini and Fiandri 1988, Roth 1994, Ladurner and Simonini 2003, Cazzoli 2006, Klofac 2007, Assyov and Denchev 2009). This feature appears to be unique to Alessioporus and Pulchroboletus within the European Boletaceae. As noted above. remnants of the connection between stipe and pileus often are observed as a narrow, granulose "ring-zone" in the middle-lower part of the stipe of A. ichnusanus (Fig. 2G, H) and in the middleupper part of the stipe of P. roseoalbidus (Fig. 4E). Singer (1986) accepts various types of basidiome development in Xerocomus s.l. In most species it is gymnocarpic but also paravelangiocarpic; however there is no mention of secondary angiocarpy. The development of many boletes is characterized by an early stage in which the pileus margin develops by curving inward until it touches and pushes onto the stipe surface. In gymnocarpic boletes this development happens without any disturbance of the stipe surface structure, where hyphae remain parallel (see Reijnders [1963: 32, Pl. 2] for *Boletus subtomentosus* L.). In boletes with a secondary angiocarpy, this phenomenon causes an alteration of the stipe arrangement; at the stipe apex, in the region affected by adhesion with pileus margin, the hyphae of the lateral stratum protrude outward and contribute to veil formation, merging with the hyphae coming from the pileus surface (mixangiocarpy). According to Reijnders (1963), This type of development pattern occurs, for example, in Suillus aeruginascens (Opat.) Snell (= S. viscidus [L.] Roussel) and other Suillus species (e.g. S. luteus [L.] Roussel). It is therefore evident that the hyphae of the stipe take part in the formation of the veil (Reijnders 1963). A similar phenomenon is discernible in A. ichnusanus and P. roseoalbidus, where the structures of pileus and stipe projections are nearly the same as in Suillus aeruginescens (compare Fig. 4F with Pl. 3, Figs. 3-4 in Reijnders 1963). The structural alteration of the stipe layer, generated by connection with the pileus margin, occurs in Alessioporus and Pulchroboletus in a similar way as in the Suillus species mentioned by Reijnders. In A. ichnusanus and P. roseoalbidus, the veil is filamentous and ephemeral, and appears in some primordia to be generated by a "swiping" of the pileus margin onto the stipe surface, which soon disappears. In some cases, the connection between the pileus and stipe does not seem to occur, leaving no trace of the ring-like pattern. In other cases however the adhesion between pileus and stipe is more pronounced and the veil appears persistent and disrupts only at maturity to allow

spores discharge, or exceptionally it may be permanent and does not open at all, simulating a truly angiocarpic behavior with a tendency toward gasteromycetization (Fig. 4G, H).

Both genera share noticeable ecological requirements because they are heliophilous and occur in xero-thermophilic forest ecosystems, mostly associated with pure or mixed evergreen sclerophyllous and deciduous oak communities with which they show a certain degree of specificity (*Quercus cerris*, *Q. ilex* and *Q. suber* are the preferential ectomycorrhizal partners). They also may be found however with other fagaceous hosts such as *Castanea sativa* (Ladurner and Simonini 2003, Muñoz 2008), rockroses (*Cistus* spp., Cistaceae) (Foiera et al. 1993, Lavorato 1991 as "*B. siculus*", Littini 1992, Lannoy and Estadès 2001, Ladurner and Simonini 2003, Brotzu and Colomo 2009) and doubtfully with introduced *Eucalyptus* species (Myrtaceae) (Ladurner and Simonini 2003). Their growth is typically on arid, bare soil, along coastal regions and shoreline during the driest period of the year.

These boletes are rarely encountered even in their preferred habitat and gradually decrease in frequency toward the north. Their distribution patterns appear to be somewhat restricted and predominantly meridional, and they are reported only from southern European countries, extending eastward into Balcans and as far north as Austria and Hungary. Their presence all over the Mediterranean basin, including lowland coastal regions of northern Africa and western Middle East with similar environmental conditions cannot be excluded. *A. ichnusanus* and *P. roseoalbidus* are presently considered rare and potentially threatened taxa, inasmuch as they are documented only from a few locations across their known ranges.

As stated above, the type collection of *A. ichnusanus* was discovered in R. Galli's personal herbarium, but no holotype material is available for *P. roseoalbidus*. Authentic collections of both taxa identified by C.L. Alessio from Sardinia in the 1980s were found in E. Rebaudengo's personal herbarium; however there is no way to establish with certainty whether they come from the type localities. Furthermore the dried samples were preserved in poor condition and repeated attemps to extract DNA were unsuccessful. Accordingly, we decided to anchor the names *A. ichnusanus* and *P. roseoalbidus* by epitypification with recent Sardinian samples from which we were able to obtain good ITS, nrLSU and *tef-1* α sequences and that might prove adequate for future investigations.

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Footnotes

- <u>4</u>1 Corresponding author. <u>alfredo.vizzini@unito.it</u>
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