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Molecular confirmation of *Gyroporus lacteus* and typification of *Boletus cyanescens*

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Abstract

Gyroporus lacteus is fully described based on recent collections from sandy areas in Italian littoral woods consisting of *Pinus pinea* and *Quercus ilex*. Léveillé's plate 9 (1–2) (in Annls Sci. Nat., Bot., 1848) is selected as a lectotype and a recent sequenced collection as an epitype. Its independent position from *Gyroporus cyanescens* is supported by phylogenetic analyses of the nuclear ribosomal internal transcribed spacer (ITS) and the nuclear ribosomal large subunit (LSU) regions. In addition, *Gyroporus cyanescens* is typified by selecting Bulliard's Plate 369 (in Herbier de la France 8, 1788) as a lectotype and a sequenced collection as an epitype.

Key words: *Basidiomycota*, *Boletales*, *Gyroporaceae*, rDNA, *Sclerodermatineae*, Taxonomy

Introduction

The *Sclerodermatineae* Binder & Bresinsky, also called the sclerodermatoid fungi (Watling 2006), is a suborder within the large, and ecologically important order *Boletales* E.-J. Gilbert (Binder & Bresinsky 2002), which includes the boletoid genera *Boletinellus* Murrill, *Phlebopus* (R. Heim) Singer, and *Gyroporus* Quél. and some gasteroid genera such as *Scleroderma* Pers., *Astraeus* Morgan, *Calostoma* Desv., *Pisolithus* Alb. & Schwein., *Tremellogaster* E. Fisch., and *Diplocystis* Berk. & M.A. Curtis (Louzan *et al*. 2007; Wilson *et al*. 2011, 2012). The sclerodermatoid fungi are commercially important as ectomycorrhizal partners of many forest trees, and some, especially *Pisolithus* and *Scleroderma*, have been utilized for inocula in forestry practice (Turjaman *et al*. 2005; Watling 2006, 2008; Sebastiana *et al*. 2013).

In particular, the genus *Gyroporus*, typified by *Boletus cyanescens* Bull., is circumscribed by fleshy small to medium-sized epigeal basidiomes, minutely velvety to floccose-scaly pileus often becoming shaggy, firm and leathery, villose-pruinose to velvety stipe often with horizontal fissures or cracks, sometimes with a ring-like zone and internally with large and distinct chambers or totally hollow, white then straw-color, free tubes, minute, simple pores, white then lemon-yellow, white or pallid context, unchanging or strongly turning light blue to violaceus on handling due to the presence of gyrocyanins (Besl *et al*. 1973; Gill & Steglich 1987), mild taste, lemon-yellow spore-print, shortly ellipsoid adaxially applanate, smooth spores, whitish to pale straw-yellow, cyanophilic, without iodine reactions and rarely exceeding 11 µm in length, usually 4-spored basidia, pileipellis a cutis of repent to erect hyphae, cheilocystidia and pleurocystidia present, clamp-connections usually present; ontogenetic development gymnocarpic (hymenophore naked), metavelangiocarpic (development of the hymenophore enclosed in hyphal growth from all tissues of the basidiomes) or pileoangiocarpic (hymenophore at first enclosed in outgrowths of the pileus) (Reijnders 1963; Singer 1986; Watling 2008). The development of the stipe tissue is very unusual, since instead of being composed of hyphae running lengthwise, the hyphae are arranged transversely, enveloping a stuffed, floccose area that gradually disintegrates, ultimately producing a central cavity. The growth is terrestrial, in ectomycorrhizal symbiosis with deciduous trees (*Fagaceae*) and conifers (*Pinaceae*) (Agerer 1999; Raidl *et al*. 2006; Watling 2006, 2008; Wilson *et al*. 2012). *Gyroporus* is a small genus probably encompassing no more than 20 species—Index Fungorum (http://www.indexfungorum.org/, accessed 22 May 2015) lists 68 validly published names—scattered throughout temperate and

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tropical areas (Chiu 1948; Heinemann 1954; Corner 1972; Heinemann & Rammeloo 1983; Imazeki *et al*. 1988; Both 1993; Watling & Li 1999; Bessette *et al*. 2000; Lannoy & Estadès 2001; Nagasawa 2001; Li *et al*. 2003; Muñoz 2005; Watling & Hills 2005; Zang 2006; Watling 2008; Šutara *et al*. 2009; Horak 2011; Wilson *et al*. 2011, 2012). The genus is sister (with weak support) to a clade consisting of *Astraeus*, *Pisolithus* and *Scleroderma* species (Wilson *et al*. 2011, 2012).

In Europe three species are currently reported (Muñoz 2005): *G. cyanescens* (Bull.) Quèl., *G. castaneus* (Bull.) Quèl., and *G. ammophilus* (M.L. Castro & L. Freire) M.L. Castro & L. Freire. The first two are widespread North temperate species that are also found in Asia, Australia, North America, and Africa (*G. castaneus*); the third seems so far circumscribed to Europe (Castro & Freire 1995).

Preliminary molecular analyses suggested that some individual species of *Gyroporus* appear to represent multiple cryptic taxa (Wilson *et al*. 2011, 2012); similarly Watling (2006) suggested investigating those species such as *G. cyanescens* and *G. castaneus* which presently are thought to have a wide distribution and variability range in their classical morphological characters.

The aim of the present paper was to morphologically and molecularly (nrITS and nrLSU sequence analysis) study recent collections attributable to *Gyroporus lacteus* Quèl., a taxon usually considered merely a white-capped form of *G. cyanescens* (Watling & Hills 2005), and to assess its status in *Gyroporus*.

Materials and Methods

Morphological studies

The macro-morphological descriptions follow the detailed field notes taken on fresh material for each collection. The micro-morphological descriptions are based on study of herbarium material rehydrated in 5% KOH and stained in ammoniacal Congo red, Cresyl blue and Melzer's reagent. Basidiospore size (in Melzer's) is expressed both as a range and a mean value. The width of basidia was measured at the widest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. The notation [n/m/p] indicates that measurements were made on "n" randomly selected spores from "m" basidiomes of "p" collections. The following abbreviations are used: Q = the quotient of length and width of the spores in side view; Qm = average quotient. Author citations follow the Index Fungorum Authors of Fungal Names (http://www.indexfungorum.org/authorsoffungalnames.htm).

Herbarium abbreviations are according to Thiers (2015). All material examined is housed at MCVE (Herbarium del Museo Civico di Storia Naturale, Venezia, Italy).

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from dry specimens (MCVE 28580 and MCVE 28582) blending a portion of the pileus context (about 20 mg) with the aid of a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min. at 65ºC. A similar volume of chloroform: isoamyl alcohol (24:1) was added and carefully mixed with the samples until emulsified. It was then centrifuged for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70%, centrifuged again for 2 min and dried. It was finally re-suspended in 200 µL ddH_2O. PCR amplification was performed with the primers ITS1F and ITS4 (White *et al*. 1990; Gardes & Bruns 1993) for the nrITS region, while LR0R and LR5 (Vilgalys & Hester 1990) were used to amplify the nrLSU region. PCR reactions were performed under a program consisting of a hot start at 95 ºC for 5 min, followed by 35 cycles at 94 ºC, 54 ºC and 72 ºC (45, 30 and 45 s, respectively) and a final 72 ºC step 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with primer ITS4. Chromatograms were checked searching for putative reading errors, and these were corrected. The sequences are deposited in GenBank and their accession numbers are reported in Figs. 1–2.

Sequence alignment, data set assembly and phylogenetic analysis

The sequences obtained in this study were checked and assembled using Geneious v.5.3 (Drummond *et al*. 2010). Based on BLASTn results, preliminary phylogenetic analysis and outcomes of recent molecular studies on the *Sclerodermatineae* (Wilson *et al*. 2011, 2012), sequences were retrieved from GenBank and UNITE (unite.ut.ee/) databases for comparative analysis. Two separate analyses of ITS and LSU sequences were carried out. Alignments were generated for each ITS and LSU data set using MAFFT (Katoh *et al*. 2002) with default conditions for gap

openings and gap extension penalties. Alignments were then imported into MEGA 6.0 (Tamura *et al*. 2013) 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 2.0. (Darriba *et al*. 2012) to provide a substitution model for the alignment. GTR + Γ model was chosen for both the alignments. Based on the results of Wilson *et al*. (2011, 2012), *Scleroderma* and *Pisolithus* species were chosen as outgroup taxa for both data set.

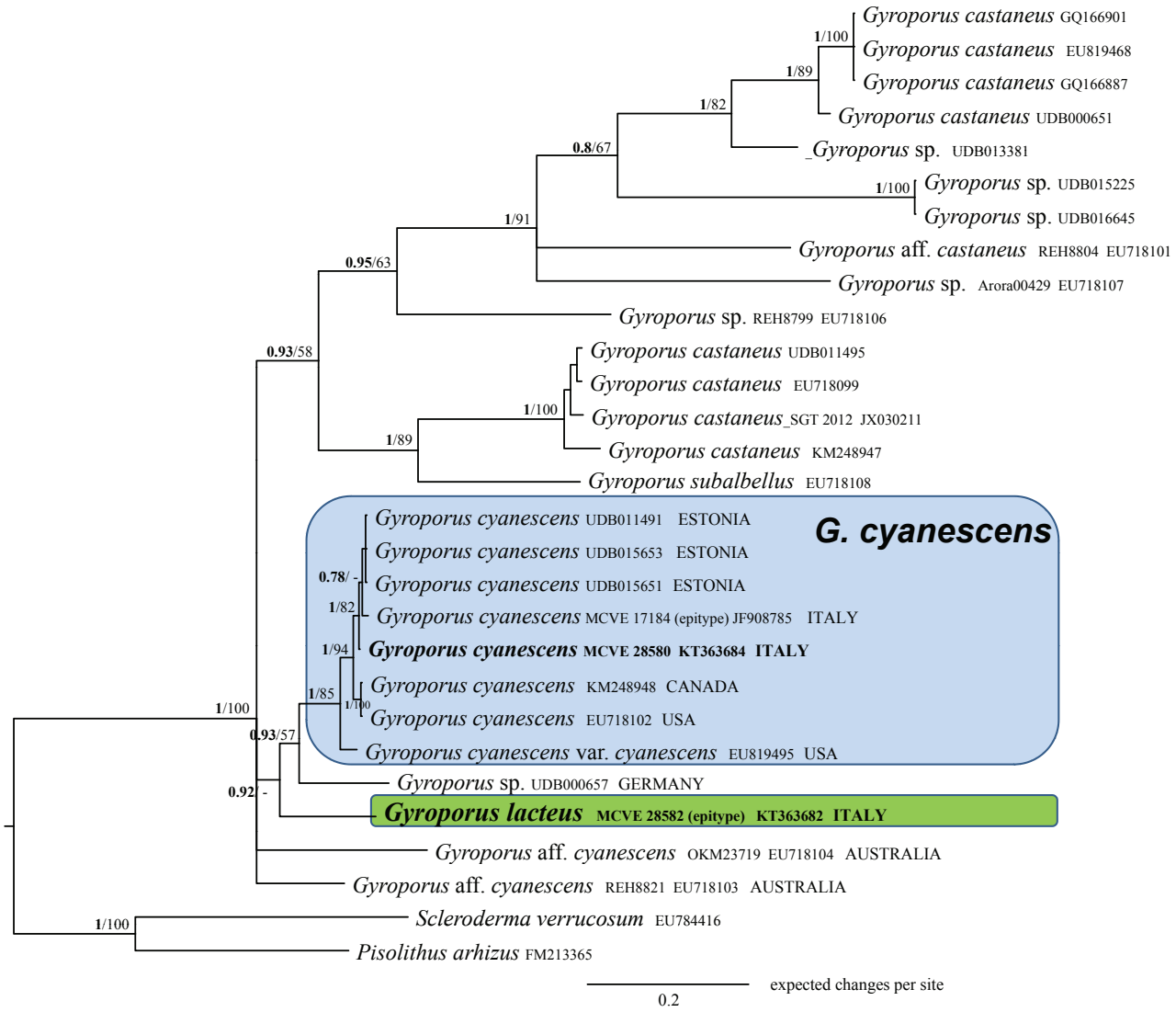

FIGURE 1. Bayesian phylogenetic analysis based on the ITS sequences of *Gyroporus* spp., with *Pisolithus arhizus* and *Scleroderma verrucosum* as outgroup taxa. BPP values (in bold) ≥ 0.70 and MLB values ≥ 50 % are shown on the branches. For each sequence taxon name and Genbank/UNITE accession number are given. Newly sequenced collections are in bold.

Phylogenetic hypotheses were constructed using Bayesian inference (BI) and maximum likelihood (ML) criteria. The BI was performed with MrBayes 3.2 (Ronquist *et al*. 2012) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run for 10 million generations, under the selected evolutionary model. Trees were sampled every 1,000 generations, resulting in overall sampling of 10,001 trees; the first 2,500 trees were discarded as "burn-in" (25 %). 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 v.7.3.2 (Stamatakis 2006) with 1,000 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.

Only MLB values over 50 % and BPP values over 0.75 are reported in the resulting trees (Figs. 1–2). Branch lengths were estimated as mean values over the sampled trees. Pairwise % identity values of ITS sequences were calculated using MEGA 6.0 (Tamura *et al*. 2013).

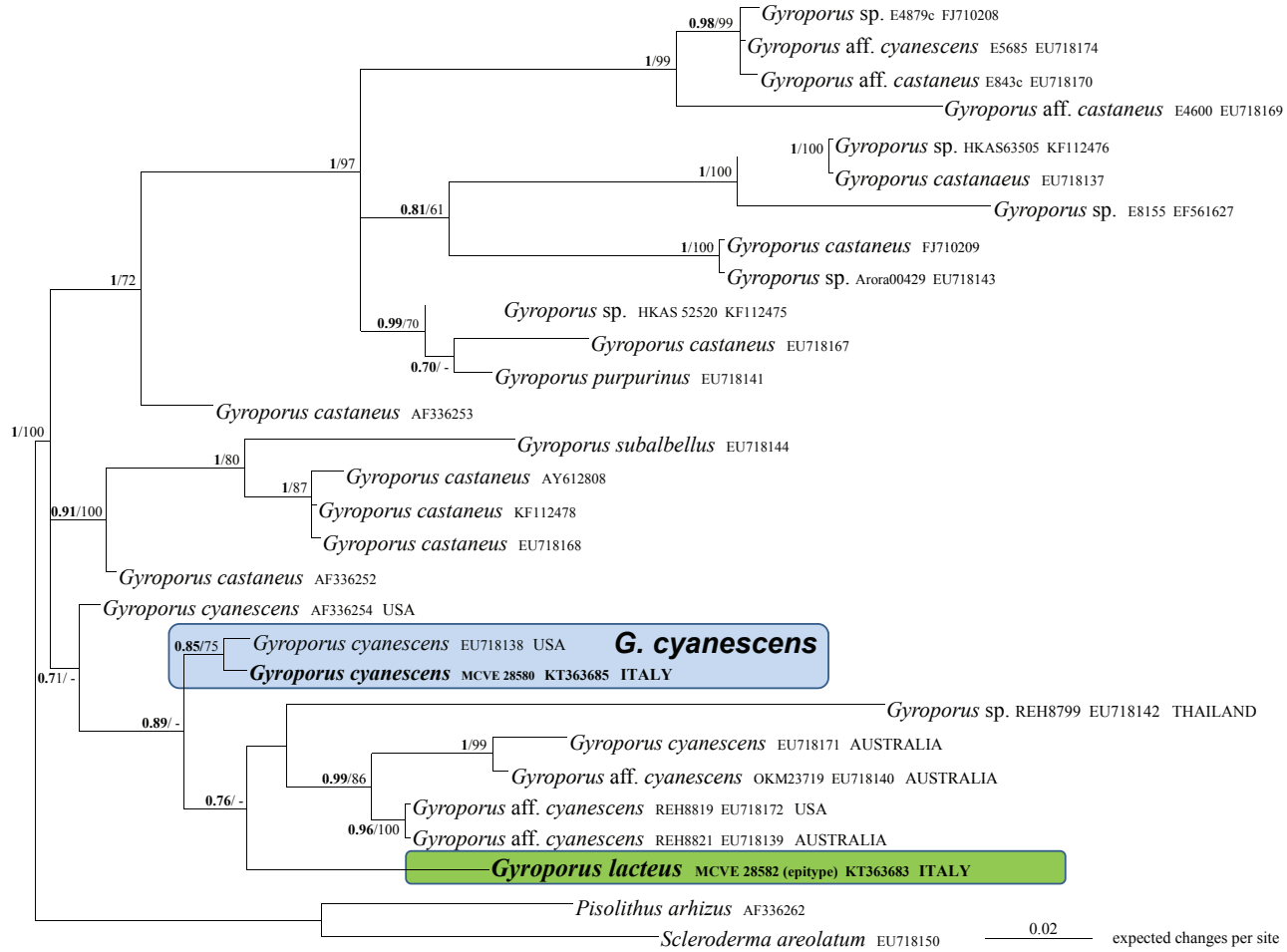

FIGURE 2. Bayesian phylogenetic analysis based on the LSU sequences of *Gyroporus* spp., with *Pisolithus arhizus* and *Scleroderma areolatum* as outgroup taxa. BPP values (in bold) ≥ 0.70 and MLB values ≥ 50 % are shown on the branches. For each sequence taxon name and Genbank accession number are given. Newly sequenced collections are in bold.

Results

Molecular results

Both Bayesian and maximum likelihood analyses produced the same topology; therefore, only the BI trees with both BPP and MLB values are shown (Figs. 1–2). The ITS data matrix comprised a total of 29 sequences (including 18 from GenBank and 9 from UNITE databases); the alignment comprised 799 characters, and contains 448 (56.1%) variable sites. The LSU matrix consisted of 29 sequences (including 27 from GenBank); the alignment comprised 956 characters, and contains 251 (26.3%) variable sites.

In the ITS analysis (Fig.1), our collection of *G. cyanescens* (MCVE 28580) forms, together with collections from Italy, Estonia, USA and Canada, a well-supported clade (BPP = 1, MLB = 85%). The sequences of the *G. cyanescens* clade show a pairwise % identity value of 98.5. *Gyroporus lacteus* (MCVE 28582) occupies a sister position to the clade consisting of *Gyroporus* sp. (UDB000657) from Germany and the *G. cyanescens* clade. *Gyroporus lacteus* shares with the *G. cyanescens* complex a pairwise % identity value of 90.2. In the LSU analysis (Fig. 2), our Italian collection of *G. cyanescens* clusters (BPP = 85, MLB = 75) with an American collection (EU718138) of *G. cyanescens*, whereas *G. lacteus* falls into a weak supported clade formed by collections named as *G. cyanescens*, *G.* aff. *cyanescens* and *Gyroporus* sp. from Australia, USA and Thailand.

Taxonomy

Gyroporus cyanescens (Bull.) Quél. *Enchir. fung.*(Paris): 161 (1886) Fig. 3

Basionym: *Boletus cyanescens* Bull., *Herb. Fr.* 8: tab. 369 (1788)

≡ *Suillus cyanescens* (Bull.) P. Karst., *Bidr. Känn. Finl. Nat. Folk* 37: 1 (1882)

≡ *Leucoconius cyanescens* (Bull.) Beck, *Z. Pilzk.* 2: 142 (1923)

= *Boletus constrictus* Pers., *Syn. meth. fung.* (Göttingen) 2: 508 (1801)

≡ *Leccinum constrictum* (Pers.) Gray, *Nat. Arr. Brit. Pl.* (London) 1: 647 (1821)

Lectotype (iconotype) (designated here): Bulliard 1788, Herbier de la France 8, Tab. 369 (MBT202278) (Fig. 3a).

Epitype (designated here): MCVE 17184 (MBT202279), Italy, Trentino Alto Adige, Baselga di Piné (TN), Laghestèl, under *Pinus sylvestris*, 24 September 1997, *G. Simonini.*

Collections examined:—ITALY, Veneto: Posina (VI), under *Fagus sylvatica*, 650 m a.s.l., 3 October 2014, *C. Feltrin*, *MCVE 28580* (MCVE!). Piemonte: Colle del Lys (TO), under *F. sylvatica*, 1300 m a.s.l., 22 August 1996, *A. Vizzini*, *TO AV220896g* (TO!). Trentino Alto Adige, Baselga di Piné (TN), Laghestèl, under *Pinus sylvestris*, 940 m a.s.l., 24 September 1997, *G. Simonini*, *MCVE 17184* (epitype, MCVE!).

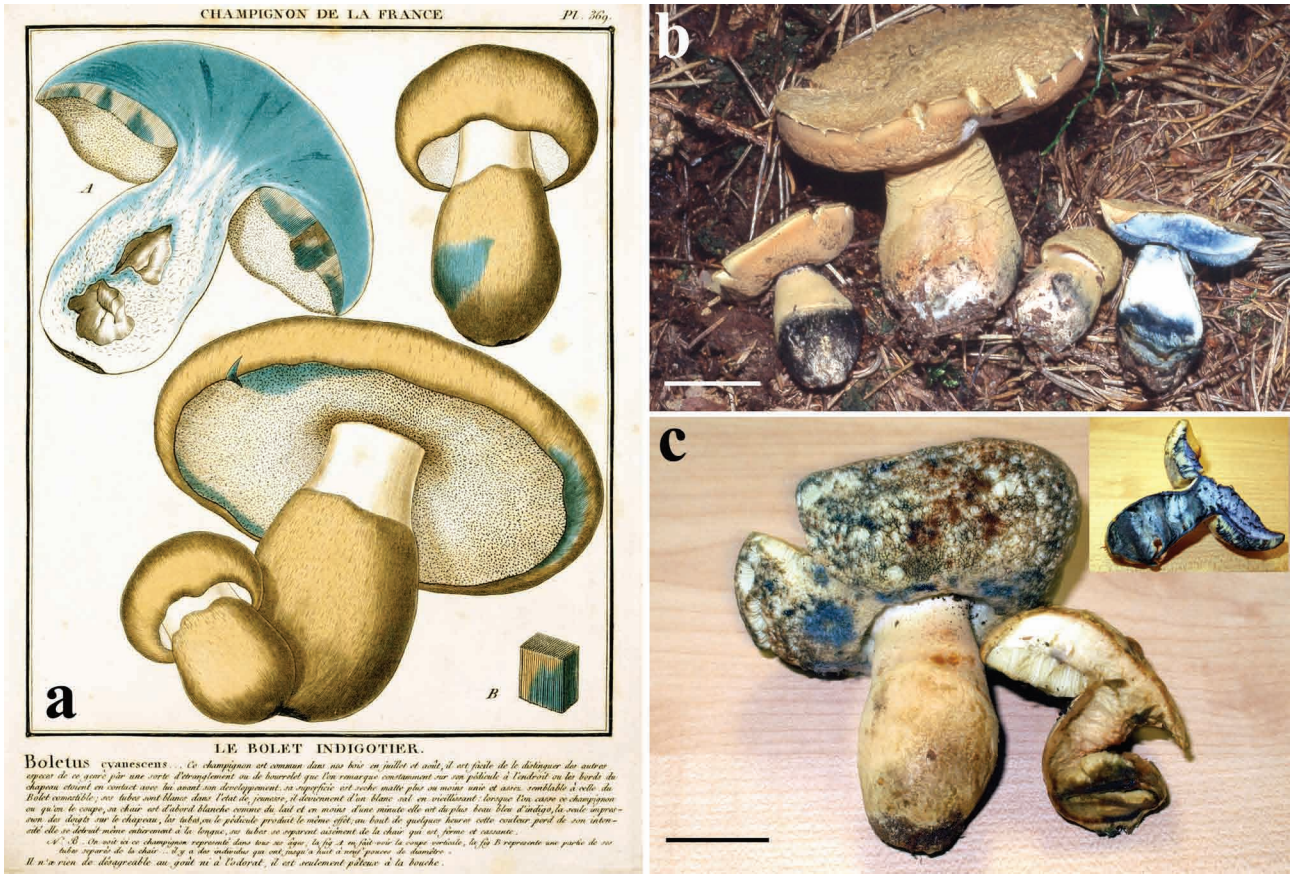

FIGURE 3. *Gyroporus cyanescens.* Basidiomata. **a**. Bulliard's plate 369 (lectotype, iconotype). **b**. Collection MCVE 17184 (epitype, photo by G. Simonini). **c**. Collection MCVE 28580 (photo by P. Di Piazza). Scale bars: 2 cm.

Gyroporus lacteus Quél., *Enchir. fung.* (Paris): 161 (1886) Figs. 4–6

Basionym: *Boletus lacteus* Lév., *Annls Sci. Nat.*, Bot., sér. 3, 9: 124 (1848), nom. illegit. (Art. 53), non *Boletus lacteus* Batsch, *Elench. fung.* (Halle): 103 (1783)

≡ *Gyroporus cyanescens* var. *lacteus* (Quél.) Quél., *Fl. mycol. France* (Paris): 425 (1888)

Lectotype (iconotype) (designated here): Léveillé 1848, Fragments mycologiques. Annales des Sciences Naturelles Botanique, Tab. 9 (1–2) (MBT202280) (Fig. 4a).

Epitype (designated here): MCVE 28582 (MBT202281), Italy, Veneto, Bosco Nordio, Chioggia (VE), sandy soil, with *Pinus pinea* and *Quercus ilex*, 04 October 2014, *C. Angelini.*

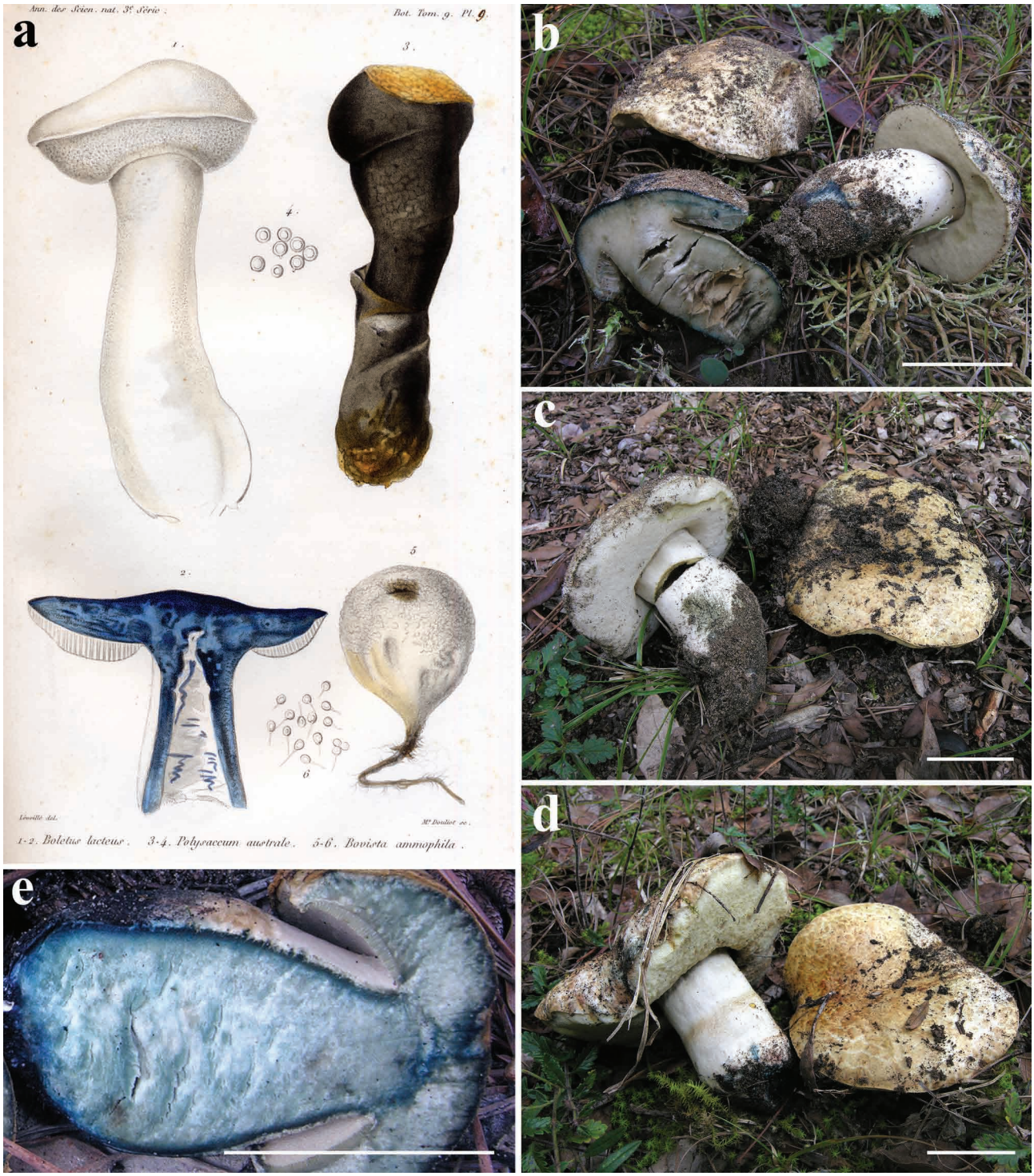

FIGURE 4. *Gyroporus lacteus*. Basidiomata. **a**. Léveillé' s plate 9 (1–2) (lectotype, iconotype). **b–e**. Collection MCVE 28582 (epitype, photos by C. Angelini). Scale bars: 5 cm.

Pileus fleshy, 9–15(–17) cm diam., at first hemispheric, then convex, finally fully expanded, applanate and pulvinate; surface matt and dry, cuticle slightly exceeding the pileus margin, at first unbroken and velutinous, but when expanding breaking up into more or less large and irregular scales, consisting of bunches of coalescing hairs letting the whitish context shine through, entirely and strongly covered with sand which is difficult to wipe off, dull, whitish as long as the specimens remain buried, ochraceous-cream when they come out from the substrate, or typically orange-brown, like "bread crust", in prematurely emerged specimens; immediately and strongly staining deep indigo blue or ink blue if handled or bruised. *Pores* irregular, straight-flattened as long as the pileus is unexpanded or semi-expanded, then with a swollen appearance like "beer foam", small, snow white and more or less round when young, cream, greenish-cream, larger and sometimes slightly angular with age, bluish-grey when touched. *Tubes* free, thin, rather short when young,

medium long in expanded specimens, concolorous with the pores, staining faded-watery blue when exposed. *Stipe* up to 14 × 7 cm, thick-set and proportional to the pileus, sometimes longer than the pileus diameter, other times shorter or equal, almost always buried for most of its length, mostly cylindrical, rarely slightly enlarged downwards, usually with a rounded base, only occasionally shortly pointed, rarely subrooting and then curved at the lowermost part of the base; surface almost smooth or slightly fibrillose-floccose, without horizontal cracks, snow white in the part under the ground, but cream, orange-brownish, like the pileus surface but less deep in tones in the part above the ground, entirely covered with sand which is difficult to wipe off, typically confined to the part not touching the hymenophore when at the primordial stage, instantly and strongly staining ink blue where touched; corticated in section, with a spongy context tending to crack horizontally, then cavernous, with large rhomboid cavities in fully-grown specimens, whitish, when cut immediately indigo blue, but the colour change is less strong than that of the outer surfaces of the pileus and above all of the stipe; almost odourless when young, then the smell becomes more definite, aromatic, in any case unpleasant, in fully-grown specimens, especially at stipe base. *Spore print* yellowish.

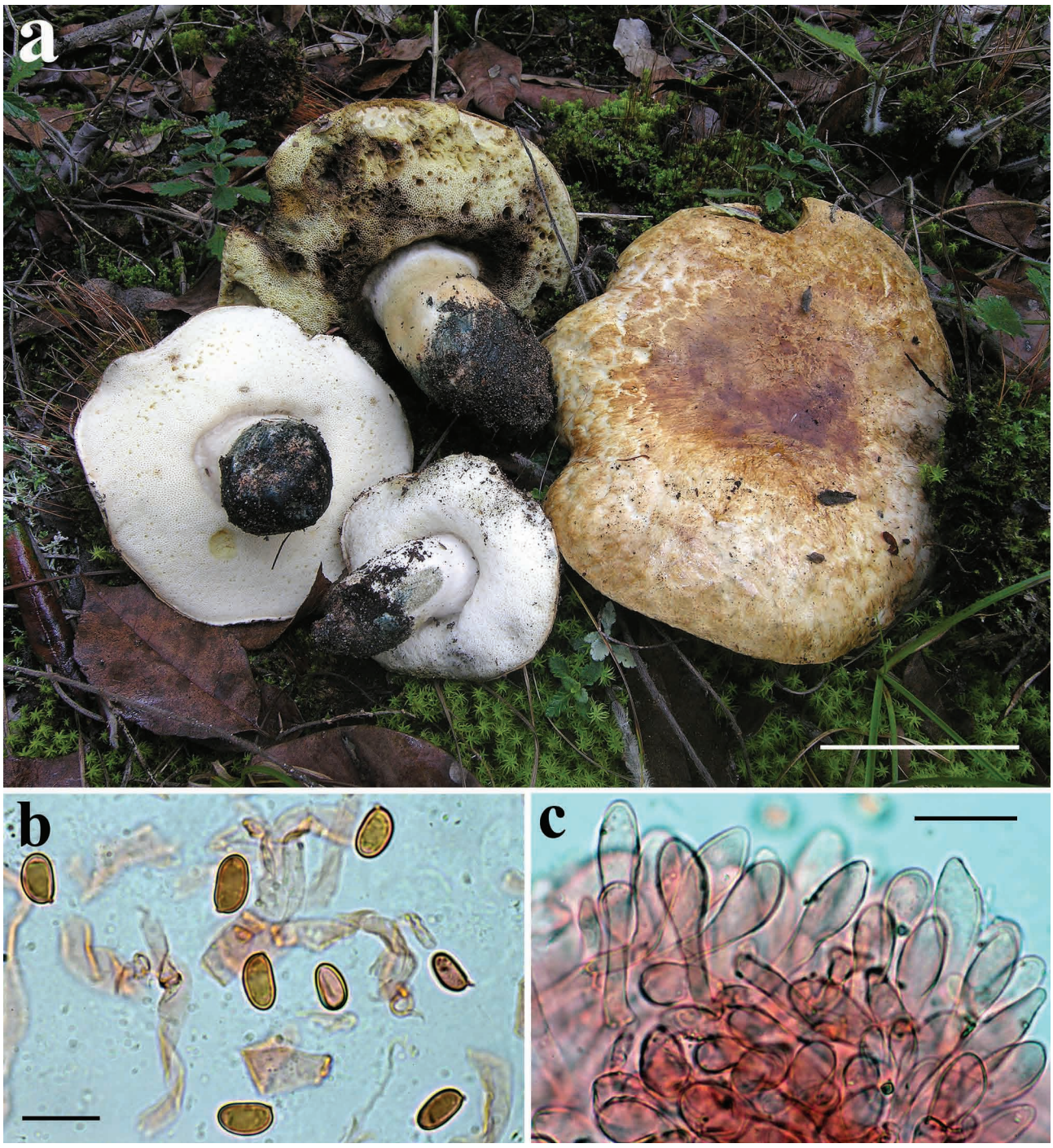

FIGURE 5. *Gyroporus lacteus*. **a**. Basidiomata. **b**. Basidiospores (in ammoniacal Congo red). **c**. Cheilocystidia (in ammoniacal Congo red). All from MCVE 28582 (epitype, photos by C. Angelini). Scale bars: **a** = 5 cm; **b** = 10 µm; **c** = 20 µm.

FIGURE 6. *Gyroporus lacteus*. **a**. Habitat. **b–c**. Habitat and habit. Photos by C. Angelini.

Basidiospores [40/4/2] (8.0–) 8.2–10.5 (–11.0) × (4.2–) 5.0–6.0 (–6.2) µm, on average 9.47 × 5.54 µm, Q = (1.45) 1.47 × 2.1 (2.3), Qm = 1.67, smooth, elliptic in face view, subelliptic or slightly reniform-phaseoliform (dorsal line

nearly straight) in profile, rarely subcylindrical, light yellow-greenish in water. *Basidia* clavate, usually 4-spored, rarely 2-spored, 30–40 × 10–15 µm, with sterigmata up to 7 µm long. *Cheilocystidia* abundant, up to 50 × 10 µm, very variable, fusiform, lageniform, cylindrical to clavate, rarely septate and sometimes minutely encrusted at apex. *Pleurocystidia* similar to cheilocystidia but infrequent. *Caulocystidia* not seen. *Pileipellis* as a cutis consisting of thin-walled radially arranged hyphae, with some repent terminal elements, up to 15 µm wide. *Pigment* yellowish-ochre, both intracellular and parietal, sometimes also minutely encrusting. *Stipitipellis* with hyphae similar to those of the pileipellis. *Clamp connections* present in all tissues.

Habit, habitat and distribution:—autumnal (early October to mid November), growing gregariously and deeply buried (long semihypogean) in sandy soil, at the edge, or more frequently in paths or in clearings, of Mediterranean woods with pines and holm oaks (*Pinus pinea* and *Quercus ilex*) close to the sea. So far known from France, Italy and Spain.

Collections examined:—ITALY, Veneto: Bosco Nordio, Chioggia (VE), sandy soil, with *Pinus pinea* and *Quercus ilex*, 02 November 2013, *C. Angelini*, *MCVE 28581* (MCVE!); ibidem 04 October 2014, *C. Angelini*, *MCVE 28582* (epitype, MCVE!).

Discussion

Variability, typification and cryptic species in Gyroporus cyanescens

Gyroporus cyanescens is easily distinguished on the basis of the metavelangiocarpic development of the basidiome (Reijnders 1963), the context rapidly turning blue on cutting, the small to medium-sized, velutinous-tomentose, usually straw-yellow, buff to ivory-colored pileus, the stipe typically narrowed upwards, with apical pseudo-annular zone and horizontal fissures (Fig. 3a), whitish to pale lemon and pruinose at apex but gradually concolorous with the pileus and fibrillose-tomentose below and spores shorter than 10 µm on average. It typically grows in hill and montane forests closely associated to fagaceous trees (family *Fagaceae*, *Fagus*, *Quercus* and *Castanea*) and conifers (usually *Pinus sylvestris*) (Alessio 1985; Breitenbach & Kränzlin 1991; Lannoy & Estadès 2001: Muñoz 2005; Watling & Hills 2005; Šutara *et al*. 2009; Knudsen & Taylor 2012).

Our molecular analyses demonstrated its occurrence outside Europe, in North America (Canada and United States, Figs. 1–2), as previously reported by Both (1993) and Bessette *et al*. (2000). The species seems to display a wide morphological range in its classical characters, and many infraspecific taxa or even putative species were recognized based on differences in color changes of the context when exposed, colors of the pileus surface or whether the outer surfaces green as the basidiomes dry: var. *lacteus* (Quél.) Quél., f. *immutabilis* J. Charb., C. Lej. & M. Xavier, var. *sulphureus* (Kalamees) Kalamees, var. *vinosovirescens* G. Riousset, Riousset & Bertéa and var. *violaceotinctus* Watling. As there are no herbarium specimens for the type of *Boletus cyanescens* Bull., we decided to select a lectotype and an epitype to preserve current usage of the name. Therefore, Bulliard's Plate 369 (in Herbier de la France 8, 1788) is here selected as lectotype (iconotype, see Fig. 3a) and a recent sequenced collection (MCVE 17184) is defined as epitype; this collection was chosen as epitype because it agrees well with the brief original diagnosis, the iconotype as well as with descriptions by several authoritative authors (e.g.: Alessio 1985; Lannoy & Estadès 2001; Muñoz 2005; Watling & Hills 2005; Knudsen & Taylor 2012).

Molecular analyses by Wilson *et al*. (2011, 2012) and our studies (Fig. 1–2) suggest that in *G. cyanescens* cryptic speciation events have taken place and that *G. cyanescens*, similarly to other species in *Sclerodermatineae* thought to have a wide distribution (e.g. *Pisolithus arhizus*, *Astraeus hygrometricus*, Moyersoen *et al*. 2003; Phosri *et al*. 2013), appears to represent multiple cryptic taxa (complex of species). In particular, *G. cyanescens* var. *violaceotinctus* Watl. from North America is a real independent species (Vilgalys, personal communication, unpubl. data in Watling 2006), as well as *Gyroporus lacteus* (Figs. 1–2) and collections named *G*. aff. *cyanescens* from Australia and USA (Figs. 1–2 and Wilson *et al*. 2011, 2012).

Status and differential characters of Gyroporus lacteus

Boletus lacteus was established by Léveillé (1848, nom. inval., preoccupied by *Boletus lacteus* Batsch 1783) for a taxon growing in sandy areas (Tète de Buch, with *Pinus pinaster*, Bordeaux, France) close to *G. cyanescens* but differing mainly in having a large pileus at first white, a thorough whitish stipe without the apical constriction miming a pseudo-annular, ring-like zone. Quélét (1886) first included *B. lacteus* in his new genus *Gyroporus* as a species, then (1888) reduced it to a variety. This taxon was treated so far only as a white-capped form of *G. cyanescens* by most modern

authors (Alessio 1985; Lannoy & Estadès 2001; Watling & Hills 2005; Knudsen & Taylor 2012). Muñoz (2005) based on Spanish collections highlighted that var. *lacteus* shows pilei up to 20 cm diam, pileus and stipe surfaces strongly turning ink-blue on handling, stipe without the pseudo-annular zone and growth in sandy soils with *Pinus pinaster* and *Cystus* spp. Microscopically it perfectly matches the type. The taxon has also been found in Italy, from littoral sandy zones in Tuscany, in association with *Quercus ilex*, *Pinus pinea* and *Cistus salvifolius* (Bertolini 2014) and Latium (M. Gelardi, pers. comm.). Our collections, from Venice, with *Pinus pinea* and *Q. ilex*, are in full agreement with the observations by Muñoz; we also would like to point out that i) most basidiomes displayed a semi-hypogeous growth, growing deeply rooted in the sandy soil; ii) pileus color is at first ice-white, soon evolving to buff-ochre in basidiomes exposed to air and then squamulose and *Agaricus semotus*-like; iii) stipe is always without the apical ring-like constriction and whitish throughout even though in the basidiomes soon exposed to air they may present a ochre coloration; it lacks the horizontal fissures or cracking so typical of *G. cyanescens*.

ITS and LSU analyses (Fig. 1–2) indicate that *G. lacteus* is an independent species. Its ITS sequence shows a pairwise % value of only 90.2% with those of *G. cyanescens*.

As there is no original material, Léveillé's plate 9 (1–2) is here selected as lectotype (see Fig. 4a), and the collection MCVE 28582 as epitype. Plate 9 (1–2) (showing a white pileus and a long stipe) probably illustrates a young specimen growing in semi-hypogeous conditions.

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