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# ***Hygrophorus roseodiscoideus* Bon & Chevassut: epitypification and updated biogeography of a poorly known but widespread thermophilous species**

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**KEY WORDS :** Hygrophoraceae, oak, *Quercus*, Mediterranean Sea, woodwax, distribution, epitypification.

## **ABSTRACT**

*Hygrophorus roseodiscoideus* Bon & Chevassut is a poorly known species so far reported from Mediterranean *Quercus* ecosystems of western Europe. The lack of reference sequences for this species hampers its reliable identification by mycologists and fungal ecologists in the DNA era. We here fix this issue by epitypifying *H. roseodiscoideus* with a sequenced collection from the Aix-en-Provence area, where the species has been described from. We also report several sequenced collections from central European countries and Lebanon, that considerably extend the species' biogeographical distribution. Based on our findings, *H. roseodiscoideus* can be characterized as a morphologically distinct thermophilous species, associated with oaks on calcareous soils, distributed along the northern coasts of the Mediterranean, from Spain to the Levant, but also colonizing the warmest *Quercus* woodlands of central Europe.

## RÉSUMÉ

*Hygrophorus roseodiscoideus* Bon & Chevassut : épitypification et mise à jour biogéographique d'une espèce thermophile méconnue mais répandue. *Hygrophorus roseodiscoideus* Bon & Chevassut est une espèce méconnue jusqu'ici supposée restreinte aux chênaies méditerranéennes d'Europe occidentale. À l'heure où les sciences naturalistes sont entrées dans l'ère phylogénétique, l'absence de séquence de référence pour cette espèce prive les mycologues, ainsi que les experts en écologie fongique, d'un outil d'identification fiable de ce taxon. Nous solutionnons ici ce problème en épitypifiant *H. roseodiscoideus* avec une récolte séquencée issue des environs d'Aix-en-Provence, d'où l'espèce a été initialement décrite. Nous présentons aussi ici plusieurs récoltes séquencées d'Europe centrale et du Liban, qui étendent considérablement la distribution biogéographique de l'espèce. Sur la base de ce travail, *H. roseodiscoideus* se présente comme une espèce morphologiquement bien typée, thermophile, associée aux chênes sur sol calcaire, distribuée tout au long de la côte nord de la Méditerranée, depuis l'Espagne jusqu'au Levant, mais colonisant aussi les chênaies les plus chaudes d'Europe centrale.

## INTRODUCTION

*Hygrophorus roseodiscoideus* Bon & Chevassut (Basidiomycota, Hygrophoraceae) is a remarkable species originally described from southern France (Bon & Chevassut 1985). The authors characterized it as resembling *Hygrophorus discoideus* (Pers.) Fr., but with a stouter habit, vividly coloured basidiomata with rose tinges on the pileus, lamellae and stipe, and larger spores (up to 9-10  $\mu\text{m}$  versus 7-8  $\mu\text{m}$  in *H. discoideus*). The species was originally found under *Quercus ilex* L. and *Q. pubescens* Willd. on calcareous bedrock, and only in years with abundant rainfall with relatively warm late autumn temperatures. These conditions are infrequent in southern France, so *H. roseodiscoideus* cannot be considered as a common species, even if locally it fruits abundantly in years when favourable conditions occur. For these reasons, it remains poorly known to many field mycologists and it is often misidentified as *H. arbustivus* var. *quercetorum* Bon, or *H. leucophaeo-ilicis* Bon & Chevassut, that display only remote resemblance with *H. roseodiscoideus*, but co-occur in the same Mediterranean *Quercus* L. woodlands (and were described simultaneously by Bon & Chevassut 1985).

Later on, the knowledge of distribution range of the species has been extended to Italy (Candusso 1997; Campo 2015) and Spain (Ortega Díaz 1992), including the Balearic Islands (Galli 2012), still with *Quercus* (*Q. cerris* L., *Q. robur* L.). However, because no reference sequence of *H. roseodiscoi-*

*deus* is currently available in public databases, all these collections have been identified purely on morpho-anatomical and ecological grounds. So far, there is no published record of *H. roseodiscoideus* outside the Mediterranean. However, we found *Hygrophorus* Fr. basidiomata that fit the description of this species in several countries of central Europe. DNA sequencing revealed that all this material belongs to the same lineage, together with several French collections phenotypically considered as typical of *H. roseodiscoideus*.

We here report on these sequenced and other well-documented collections to update the biogeography of *H. roseodiscoideus* as well as its morphological, anatomical and ecological variability. In order to fix usage of the name and yield a reference sequence for this species, we moreover epitypify *H. roseodiscoideus* with a sequenced collection from southern France.

## MATERIAL AND METHODS

### FIELD WORK, MORPHOLOGY, DOCUMENTATION

Data on occurrence of *H. roseodiscoideus* are based on our collections or records kindly provided by our colleagues. They represent examples of well-documented records for individual countries, not complete occurrence data, especially for southern Europe, where the species is widely distributed and well documented (see Introduction). Description of morphological

TABLE 1. — Basidiospore size in selected collections across the distribution range of *Hygrophorus roseodiscoideus* Bon & Chevassut. Abbreviations: **L**, length; **L10**, main lower length (10 % of shortest spores subtracted); **L90**, main upper length (10 % of largest spores subtracted); **W**, width; **W10**, **W90**, same as for L; **Q**, quotient of length/width; **av**, average; **min**, minimal; **max**, maximal.

Collection	Lmin	L10	L90	Lmax	Wmin	W10	W90	Wmax	Qmin	Qav	Qmax
AT: WU 0007470	7.5	7.7	10.2	11.3	4.8	5.1	6.7	7.6	1.30	1.59	2.00
CZ: PRM 955166	8.0	8.0	10.0	10.0	5.5	5.5	6.5	7.0	1.33	1.50	1.67
CZ: PRM 954665	7.5	8.0	10.0	10.0	5.3	5.5	6.0	6.3	1.33	1.52	1.67
CZ: BRNU 677820	8.0	9.0	10.0	10.5	5.5	5.5	6.0	6.0	1.45	1.60	1.82
FR: LIP 0402283	7.1	7.4	8.6	10.3	4.6	5.4	6.5	7.5	1.20	1.40	1.70
FR: DiBo041202	7.5	7.5	9.0	10.0	4.5	5.0	6.0	6.5	1.23	1.55	2.0
HR: MCVE 28439	8.5	9.0	11.4	12.3	5.8	6.0	7.5	7.7	1.26	1.44	1.68
HU: DB 2020-11-12-2	7.4	8.2	11.2	12.4	4.8	5.4	6.8	7.2	1.30	1.54	2.00
HU: AL 280/15	7.8	8.5	10.8	11.8	5.2	5.6	6.5	7.5	1.30	1.48	1.92
IT: WU 0025200	7.5	8.1	9.9	10.3	4.8	5.2	6.2	6.5	1.30	1.53	1.80
IT: EC16102825	8.0	8.5	10.5	11.5	5.2	5.5	6.7	7.0	1.33	1.56	1.85
LB: T2-11	8.0	8.0	9.55	10.0	4.5	5.0	6.0	6.0	1.33	1.62	2.00

characters is based on collections listed in Table 1. Descriptive terminology follows Vellinga (1988). Micromorphological characters were studied in 5 % KOH using dried material. Spore size in Table 1 is presented as the main data range (10–90 percentile values), flanked by limit values in parentheses, of all spores measured (20 measurements per collection, 35 for the epitype). “Q” refers to the quotient of length/width for each spore measured, Qav average value per collection. Herbarium codes follow Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>). Voucher specimens are kept in herbaria BP, BRNU, LIP, MCVE, PRM, TUR, WU-Myc, Eötvös Loránd University (ELTE), Associazione Micologica Bresadola-Gruppo di Muggia e del Carso (AMB-Muggia) and personal herbaria of L. Albert (AL), D. Borgarino (DiBo), E. Campo (EC), P. Finy (FP), and the Center of Evolution and Functional Ecology of Montpellier (CEFE).

#### DNA STUDY

Newly generated sequences (MZ576433–MZ576442) were obtained by several authors of the present study. Methods used for DNA extraction, amplification and sequencing are given as references to their previously published works carried out with the same methods: J.-M. Bellanger (Liimatainen *et al.* 2017), B. Dima (Papp & Dima 2018), I. Krisai-Greilhuber (Voitk *et al.* 2020), P. Zehnález (Holec & Zehnález 2020). Phylogenetic analyses were conducted online at [www.phylogeny.fr](http://www.phylogeny.fr) (Dereeper *et al.* 2008). Multiple sequence alignments were performed with MUSCLE v.3.7 (Edgar 2004). Maximum likelihood (ML) phylogenetic analysis was achieved with PhyML v.3.0 (Guindon *et al.* 2010), using the GTR + I +  $\Gamma$  model of evolution and the Shimodaira Hasegawa version of the approximate likelihood-ratio test (SH-aLRT) of branch support (Anisimova *et al.* 2011). Bayesian inference (BI) of phylogeny was performed with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003), after 1 000 000 generations, tree and parameter sampling every 1000 generations and an initial burn-in of 25 % (250 trees). Branch support was assessed by Bayesian posterior probabilities (BPP). Phylograms were built using TreeDyn 198.3 (Chevenet *et al.* 2006) and edited with Inkscape 0.91 (<https://inkscape.org/fr>).

## RESULTS

### Family HYGROPHORACEAE Lotsy Genus *Hygrophorus* Fr.

#### *Hygrophorus roseodiscoideus* Bon & Chevassut

*Documents mycologiques* 15 (59): 42 (Bon & Chevassut 1985).

ORIGINAL DIAGNOSIS. — Pileus 5–12 cm, convexus vel late mammosus, carnosus, margine involuta, cuticula viscosa, coloribus versus discum fulvobrunneis vel rubrocastaneis, versus marginem paulatim pallidioribus, aurantio-ochraceis vel cremeo-roseis. Lamellae arcuatae, decurrentes, distantes, pallide cremeo roseae, vel ab imo subincarnatae. Stipes 6–10(13) × 1–2.5 cm, deorsum attenuato, sursum albido-punctuato, zona viscosa subannuliformi, fugaci atque versus basim nonnullis fasciis +/- ochraceis. Caro pallide incarnata, odore saporeque subnullo vel fungoso. Sporae 9–10 × 5.5–6.5  $\mu$ m, ellipticae. Trama bilateralis; basidia 45–65 × 7–10  $\mu$ m, tetraspora. Cuticula pilei hyphis × 3–5  $\mu$ m, gelatinosis, pigmento mixto vel membranari interdum verrucoso. Caulocutis sursum pilis +/- refringentibus, deorsum hyphis elongatis in micaceo muco coerctis. In Querceto ilicis vel pubescentis lectus, serotinus. Holotypus no. 84 391 in herbario MB – Cotypus GC 3326.

HOLOTYPE. — **France**. Gard, close to Quissac, *Quercus ilex* and *Q. pubescens* on calcareous bedrock, 12.XII.1984, leg. G. Chevassut, det. M. Bon, *Bon 84391* (holo-, LIP[LIP 0102284]); iso-, in herb. *G. Chevassut 3326* (current location of this herbarium is unknown) (Fig. 1).

EPITYPE. — **France**. Bouches-du-Rhône, Simiane-Collongue, calcareous bedrock, under *Q. ilex* and *Pinus halepensis* Mill., 31.X.2000, leg. & det. P.-A. Moreau, *PAM00103101* (epi-, designated here in support of the holotype cited above, LIP[LIP 0402283]), GenBank ITS: MZ576440, MycoBank: MBT 10003653 (Fig. 2).

ADDITIONAL MATERIAL EXAMINED. — **Austria**. Burgenland, district Oberpullendorf, community Horitschon, Ragerwald, 47°34'30"N, 16°32'30"E, 290 m a.s.l., bedrock, Inner Alpine Neogene Basin, clay, marl, marlstone, sand, gravel, limestone, in thermophilous oak forest, near *Quercus cerris*, *Carpinus betulus* L., and *Acer* L., c. 10 basidiomata, 29.IX.1988, leg. W. Klofac, det. A. Hausknecht (WU[WU-Myc 007470]).

**Croatia**. Istria Region, Novigrad, Tar, sea coast, Mediterranean area, under *Quercus ilex* and *Arbutus unedo* L., 6.XII.2003, leg. & det.

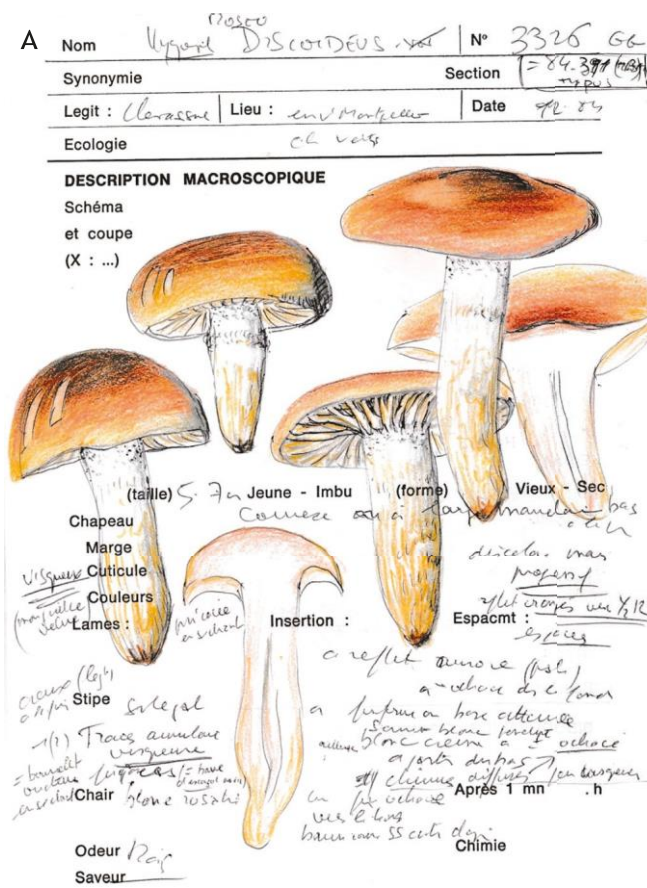


FIG. 1. — Original material of *Hygrophorus roseodiscoideus* Bon & Chevassut: **A**, hand-written description sheet and color drawing of specimens of the holotype of *H. roseodiscoideus* by M. Bon; **B**, photograph of the dried basidiomata (holo-, Bon 84391 kept in LIP).

E. Campo (TUR[TUR-A 162859]); under *Quercus pubescens*, on calcareous soil, 6.XII.2014, leg. & det. E. Campo (MCVE[MCVE 28439]); Kvarner Region, Krk, Malinska, in Mediterranean area, on calcareous soil under *Q. pubescens* and *Q. cerris*, 17.XI.2018, leg. & det. E. Campo (specimens not kept).

**Czech Republic.** Central Bohemia, near the village of Mceľ, Čtvrtě National Nature Reserve, 50°17'54"N, 15°4'11.579"E, 225 m a.s.l., under *Carpinus* L., *Tilia* L., *Corylus* L. and *Quercus robur* L. on calcareous bedrock (arenaceous marl), 10.IX.2010, leg. Jan Borovička, voucher lost; 50°17'39.96"N, 15°3'9.84"E, 250 m a.s.l., thermophilous oak forest, calcareous bedrock, under *Quercus robur* L. on calcareous bedrock (arenaceous marl), 10.IX.2010, leg. Jan Borovička, voucher lost; 50°17'39.96"N, 15°3'9.84"E, 250 m a.s.l., in warm broadleaved forest under *Carpinus* and *Quercus*, 1.XI.2020, leg. & det. J. Borovička, 3192 (PRM[PRM 954665]) (This site is 1.4 km NEE of the previous one and very close [about 50-100 m] to the first record from Čtvrtě in September 2010 [see above].); South Moravia, near Mokrý-Horákov, Sivický les, 49°13'16.2"N, 16°45'46.439"E (accuracy several hundred meters), 350-400 m a.s.l., thermophilous oak forest, calcareous bedrock, under *Quercus petraea* (Matt.) Liebl., *Carpinus* and *Sorbus torminalis* (L.) Crantz, 4.XI.2007, leg. & det. D. Dvořák, DD 501/07 (BRNU[BRNU 677820]; Fig. 4B).

**France.** Bouches-du-Rhône, Simiane-Collongue, calcareous bedrock, under *Q. ilex* and *Pinus halepensis*, 31.X.2000, leg. & det. P.-A. Moreau, PAM00103101 (epi-, LIP[LIP 0402283]; Fig. 2); Hérault, Montpellier, campus du CEFÉ-CNRS, calcareous bedrock, under *Q. ilex* and *Pinus halepensis*, 5.XII.2010, leg. & det. F. Richard, FR0512001 (CEFE); Hérault, Puechabon, site expérimental du CEFÉ-CNRS, 43°44'29"N, 03°35'45"E, 270 m a.s.l., calcareous bedrock, soil under *Q. ilex*, 28.I.2009, leg. O. Shahin, det. F. Richard (environmental sample VAC25b-09), GenBank: JF506763 (Richard *et al.* 2011); Vaucluse, Cadenet, La Royère, calcareous bedrock, under *Q. ilex*, *Q. pubescens* and *P. halepensis*, 7.XII.2004, leg. & det. D. Borgarino, DiBo041144; Vaucluse, Cucuron, La Capagière, calcareous bedrock, under *Q. ilex*, *Q. pubescens* and *P. halepensis*, 10.XII.2004, leg. & det. D. Borgarino, DiBo041202 (Fig. 4C).

**Hungary.** Budai-hegység Mts., Budapest, Mt. Széchenyi-hegy, 47°29'16.56"N, 18°58'56.279"E, 400 m a.s.l., thermophilous oak forest (*Ceraso mahaleb-Quercetum pubescentis*), calcareous bedrock, under *Quercus pubescens*, *Quercus cerris*, *Fraxinus ornus* L., *Tilia cordata* Mill., *Prunus mahaleb* L., *Cornus mas* L., 8.XI.2015, leg. & det. L. Albert, AL 280/15; 12.XI.2020, leg. & det. L. Albert & B. Dima, AL 342/20, DB 2020-11-12-2 (ELTE) (Fig. 4D); Budai-hegység Mts., Budapest, Mt. János-hegy, 47°31'18"N, 18°56'59.7"E, thermophilous oak forest, calcareous bedrock, under *Quercus pubescens*, *Quercus cerris*, *Fraxinus ornus*, 10.X.2014, leg. & det. B. Dima, DB5586 (ELTE); Budai-hegység Mts., Budakeszi, Wildlife Park, in deciduous forest, calcareous bedrock, under *Quercus petraea*, *Carpinus betulus*, 21.X.1976, leg. G. Bohus & M. Babos (as *Hygrophorus fagi* G. Becker & Bon), det. B. Dima (BP[BP58973]), GenBank: MK088109 (Zajta *et al.* 2019); Bükk Mts., Borsod-Abaúj-Zemplén County, Miskolc, Csanyik-völgy, in deciduous forest, calcareous bedrock, under *Carpinus betulus*, *Fagus sylvatica* L., *Quercus petraea*, 16.X.2007, leg. & det. L. Albert & B. Dima, AL 07/90; Vértes Mts., Fejér County, Csákvár, thermophilous oak forest, calcareous bedrock, under *Quercus cerris*, 1.XI.2015, leg. P. Finy, det. B. Dima, FP 2015-11-01.

**Italy.** Friuli Venezia Giulia, Trieste, Parco di Miramare, 45°42'16.18"N, 13°42'52.37"E, 10 m a.s.l., on calcareous soil under *Q. ilex*, 28.XII.2012, leg. & det. P. Picciola (AMB-Muggia[AMB-Muggia 5824]); 21.XII.2014 (AMB-Muggia[AMB-Muggia 5657]); 28.XI.2015 (AMB-Muggia[AMB-Muggia 5846]); Friuli Venezia Giulia, Trieste, Muggia, Santa Barbara, 45°35'14.89"N, 13°47'4.50"E, 200 m a.s.l., on calcareous soil in mixed wood with *Q. pubescens* and *P. nigra* J.F. Arnold, 11.X.2017, leg. & det. P. Picciola (AMB-Muggia[AMB-Muggia 7196]); Friuli Venezia Giulia, Trieste, Basovizza, Bosco Igouza, 45°39'14.47"N, 13°52'24.48"E, 400 m a.s.l., on calcareous soil in mixed wood with *P. nigra*, *Quercus* sp. and

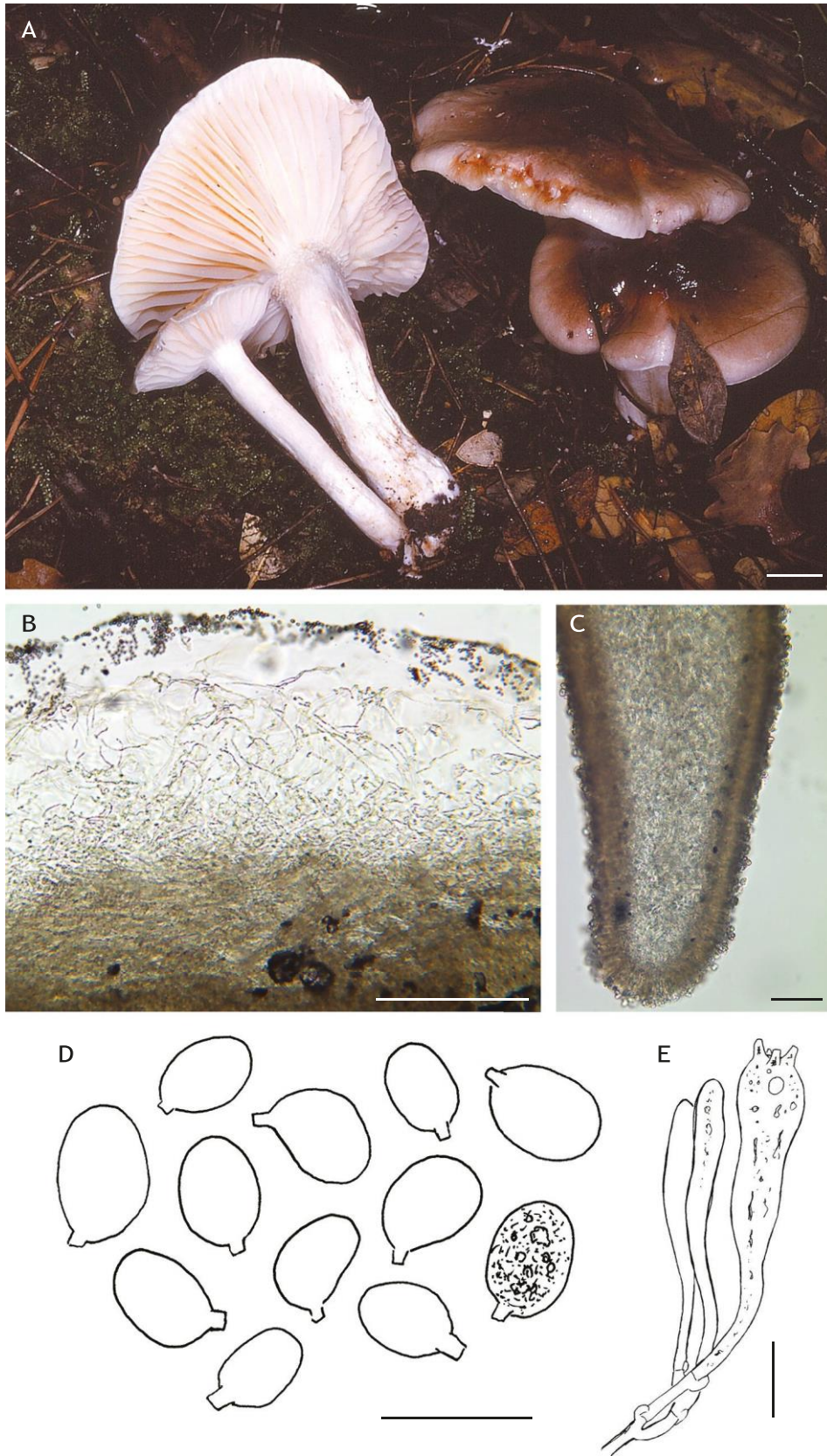


FIG. 2. — Macro- and microcharacters of the epitype of *Hygrophorus roseodiscoideus* Bon & Chevassut. In situ photograph, violet hues are exaggerated as the photo has been taken with a flash. **A**, photo by P.-A. Moreau; **B**, pileipellis structure; **C**, lamellar trama structure; **D**, spore drawing; **E**, basidia drawing of PAM00103101 (LIP[LIP 0402283]). Scale bars: A, 1 cm; B, C, 100 µm; D, E, 10 µm.

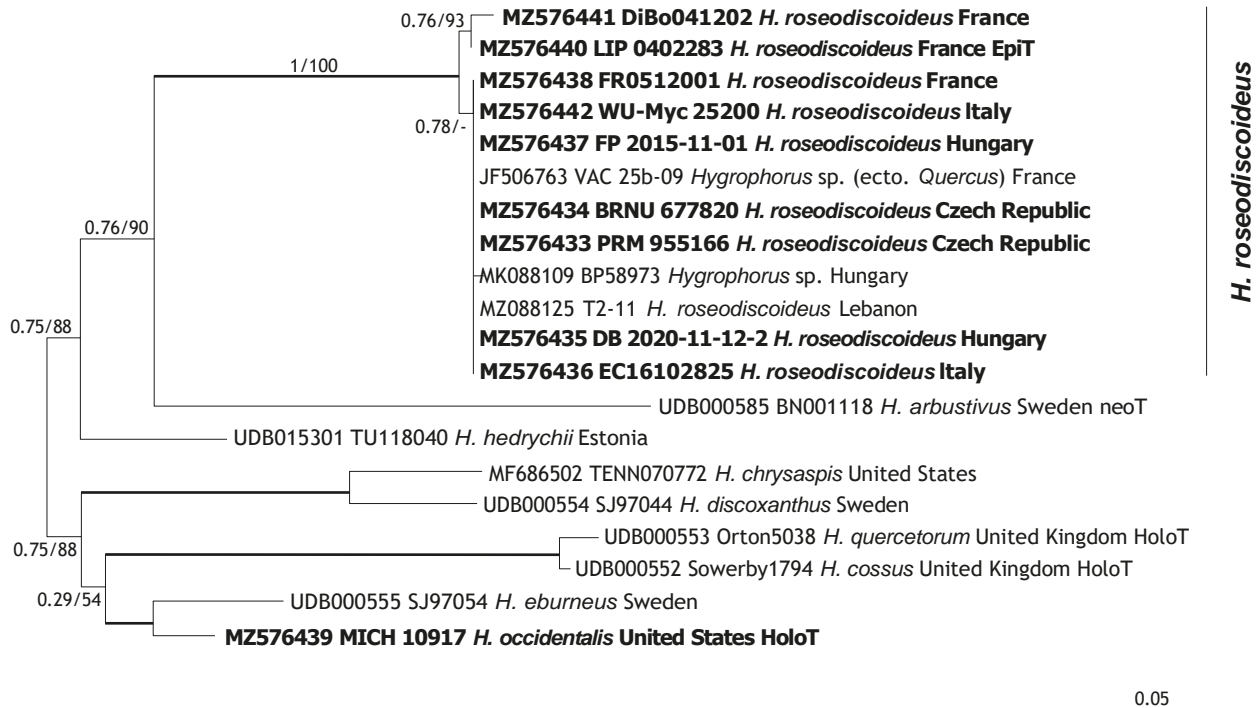


FIG. 3. — ITS phylogeny of *Hygrophorus* Fr. *pro parte*. Maximum likelihood phylogenetic tree of 12 ITS sequences of *H. roseodiscoideus* Bon & Chevassut, including that of the epitype designated in the present work. Thick branches are supported by SH-aLRT > 0.81 and BPP ≥ 95 %, others display support values as SH-aLRT/% BPP. Sequences printed in **bold** were obtained during this study.

other deciduous trees, 15.X.2017, leg. & det. P. Picciola (AMB-Muggia[AMB-Muggia 7175]); Toscana, Siena, Castellina in Chianti, Cipresseta S. Agnese, on calcareous soil under *Quercus pubescens* and *Q. ilex*, 28.X.2016, leg. & det. E. Campo, EC16102825 (Fig. 4E); Umbria, Carpegna, Passo Cantoniera, deciduous forest with *Quercus cerris*, 13.X.2004, leg. & det. A. Hausknecht (WU[WU-Myc 25200]; Fig. 4F).

**Lebanon.** North Lebanon, Akkar district, near the village of Teshah, 34°29'24.048"N, 36°11'34.343"E, 1354 m a.s.l., mixed limestone, sandstone and volcanic soil in *Quercus calliprinos* Webb forest with understory of *Styrax officinalis* L. and *Juniperus oxycedrus* L., 15.XII.2018, leg. & det. S. Sleiman (CEFE), GenBank: MZ088125 (Sleiman *et al.* 2021).

**ECOLOGY AND DISTRIBUTION.** — **Austria.** The only known locality (Burgenland, Ragerwald) is a thermophilous oak forest with *Quercus cerris* on calcareous bedrock. Altogether, some 500 species of fungi are known from this forest, among them rare in northern localities thermophilic species like *Amanita caesarea* (Scop.) Pers., *Neoboletus xanthopus* (Klofac & A.Urb.) Klofac & A.Urb., *Rubroboletus legaliae* (Pilát & Dermek) Della Maggiora & Trassinelli, *R. rhodoxanthus* (Krombh.) Kuan Zhao & Zhu L. Yang, *R. satanas* (Lenz) Kuan Zhao & Zhu L. Yang, *Russula pannonica* Pidlich-Aigner, as well as *Omphalotus olearius* (DC.) Singer, *Desarmillaria tabescens* (Scop.) R.A.Koch & Aime, *Butyriboletus regius* (Krombh.) D.Arora & J.L.Frank, and *Astraeus hygrometricus* (Pers.) Morgan.

**Croatia.** The only records known to us are from the coastal Mediterranean areas of North Croatia (Istria, Krk), on calcareous soils, under *Quercus ilex*, *Q. pubescens* or *Q. cerris*.

**Czech Republic.** *Hygrophorus roseodiscoideus* is known only from two localities. Both of them are thermophilous oak forests on calcareous bedrock (arenaceous marl, limestone). In Čtvrtě (Central Bohemia), the forest is mainly composed by *Quercus robur* but also other trees like *Q. pubescens*, *Carpinus betulus*, *Cornus mas*, *Sorbus torminalis*, or *Tilia*. Interestingly, *H. roseodiscoideus* was not found at the hottest

sites typical by occurrence of *Q. pubescens*, but all three collections were under *Quercus robur*. In Sivický les (Southern Moravia), the host tree was most likely *Q. petraea*. Čtvrtě site is characteristic by a typical community of thermophilous ectomycorrhizal fungi (Šebek 1990; Zíbarová 2014; Holec 2020) like *Amanita solitaria* (Paulet ex Vittad.) Bertill., *A. franchetii* (Boud.) Fayod, *Butyriboletus appendiculatus* (Schaeff.) D.Arora & J.L.Frank, *B. fechtneri* (Velen.) D.Arora & J.L.Frank, *Entoloma sinuatum* (Pers.) P.Kumm., *Hemileccinum impolatum* (Fr.) Šutara, *Hygrophorus penarioides* Jacobson & E.Larss., *Inocybe godeyi* Gillet, *Lactarius acerrimus* Britzelm., *L. evosmus* Kühner & Romagn., *L. pterosporus* Romagn., *Leccinellum crocipodium* (Letellier) Della Maggiora & Trassinelli, *L. pseudoscabrum* (Kallenb.) Šutara, *Russula decipiens* (Singer) Svrček, *R. luteotacta* Rea and numerous *Cortinarius* (Pers.) Gray (*Phlegmacium* (Fr.) Wünsche) species. The locality is protected as a national nature reserve and about 500 species of fungi are recorded there. Sivický les hosts a rich community of mainly ectomycorrhizal thermophilous and calciphilous species (Antonín *et al.* 2007), e.g. *Amanita solitaria*, *Aureoboletus gentilis* (Quél.) Pouzar, *Butyriboletus appendiculatus*, *Cortinarius cotoneus* Fr., *C. dibaphus* Fr., *C. sodagnitus* Rob.Henry and other cortinari, *Hemileccinum depilatum* (Redeuilh) Šutara, *H. impolatum*, *Hygrophorus arbustivus*, *H. russula* (Schaeff. ex Fr.) Kauffman, *Lactarius zonarius* (Bull.) Fr., *Lyophyllum rhopalopodium* Cléménçon, *Rubroboletus rhodoxanthus*, *R. satanas*, *Suillellus queletii* (Schulzer) Vizzini, Simonini & Gelardi, *Tricholoma filamentosum* (Alessio) Alessio and many others. The site is currently declared as Special Area of Conservation (Natura 2000 site) with occurrence of Galio-Carpinetum oak-hornbeam forests (habitat 9170) and Euro-Siberian steppic woods with *Quercus* spp. (habitat 9110). A small part of the area is protected as the Nature Monument Na Líchách.

**France.** All molecularly confirmed collections of *H. roseodiscoideus* come from thermophilous, calciphilous *Quercus* (*Q. ilex*, *Q. pubescens*) woodlands of the Mediterranean area (cf. above). However, the species is reported from several localities in the western part of the country, under the influence of the Atlantic Ocean and always



FIG. 4. — Basidiomata of *Hygrophorus roseodiscoideus* Bon & Chevassut: **A**, Czech Republic, Čtvrtě, photo by J. Holec (PRM[PRM 955166]); **B**, Czech Republic, Sivický les, photo by D. Dvořák (BRNU[BRNU 677820]); **C**, France, Cucuron, photo by D. Borgarino (DiBo041202); **D**, Hungary, Mt. Széchenyi-hegy, photo by B. Dima (DB 2020-11-12-2); **E**, Italy, Cipresseta S. Agnese, photo by E. Campo (EC16102825); **F**, Italy, Passo Cantoniera, photo by A. Hausknecht (WU[WU-Myc 25200]). All collections were sequenced. For full collection data, see section Additional material examined.

in the vicinity of *Quercus* trees, apparently following the geographical range of *Q. ilex* (cf. [www.mycocharentes.fr](http://www.mycocharentes.fr) and [www.societe-mycologique-poitou.org](http://www.societe-mycologique-poitou.org)). Although the identity of these collections remains to be confirmed, published photographs and descriptions are indeed compatible with *H. roseodiscoideus* and this species might be present there as well. Bon & Chevassut (1985) reported collections from acidic soils, but they appear to be less abundant than those from calcareous ground.

**Hungary.** *Hygrophorus roseodiscoideus* is known from five localities, of which three are situated around the forests of the capital, Budapest. Two older collections were determined as *Hygrophorus fagi* (Babos

1989) and *H. leporinus* Fr. s. Moser (Albert 2007). All localities are thermophilous calcareous deciduous forests dominated by *Quercus* species. Two of the localities (Mt. Széchenyi-hegy and Mt. János-hegy) are dominated by *Q. pubescens* with additional tree and shrub species such as *Q. cerris*, *Fraxinus ornus*, *Tilia cordata*, *Cornus mas*, etc. These habitats are hot-spots for many rare and thermophilous/Mediterranean macrofungi such as *Cortinarius atrovirens* subsp. *ionochlorus* (Maire) Vizzini & Gasparini, *C. quercilicis* (Chevassut & Rob. Henry) Melot, *C. violaceipes* Bidaud & Consiglio, *C. flavoaurantians* Boccardo, Clericuzio & Vizzini, *C. diffractosuaavis* Chevassut & Rob. Henry, *C. ochrolamellatus* Ballarà *et al.*, *C. chevassutii* Rob. Henry,



*C. prodigiosus* B. Bušek *et al.*, *Hygrophorus russula*, *Tricholoma basirubens* (Bon) A. Riva & Bon, *Boletus aereus* Bull. The three other localities are not so thermophilic, they are dominated by *Q. cerris* or *Q. petraea* with admixture of *Carpinus betulus*.

**Italy.** Documented collections are from northern and central Italy (see section Additional material examined). In all cases, *H. roseodiscoideus* was collected on calcareous soil, mostly under *Quercus ilex*, but also under *Q. pubescens* or *Q. cerris*, sometimes in mixed tree communities with *Pinus nigra*. In addition, E. Campo observed the species in the regions of Veneto (commune Chioggia), Liguria (Portofino), Emilia Romagna (Bettola), Toscana (Castellina in Chianti, Lucca, Capalbio), Marche (Ancona), Lazio (Tolfa), Sardegna (Isili, Nurallao) and Abruzzo (Loreto Aprutino). In these regions the occurrence was linked to *Quercus* (*Q. ilex*, *Q. pubescens*, *Q. cerris*) on calcareous soils. It is very curious that the species is not reported from southern Italy (G. Schirizzi, pers. comm.).

**Lebanon.** The first Asian record of *H. roseodiscoideus* is from northern Lebanon, in *Quercus calliprinos* forest with *Styrax officinalis* and *Juniperus oxycedrus* understory. Other species found at this site the same day were *Tricholoma argyraceum* (Bull.) Gillet, *Cortinarius caligatus* Malençon, *Cortinarius subbulliardii* Rob. Henry, *Cortinarius violaceipes*, *Cortinarius puellaris* Brandrud *et al.*, *Cortinarius luteocingulatus* Bidaud & Fillion and *Inocybe tenebrosa* Qué. All of them are new records for Lebanon and notably, this ecosystem appears to be the richest in fungal diversity compared to *Abies cilicica* (Antoine & Kotschy) Carrière and *Quercus cerris* ecosystems in the region (Sleiman *et al.* 2021).

## NOTES

In the protologue, Bon & Chevassut (1985) cited a number of collections from the Montpellier area and from Roussillon, in the current Occitanie Region, as well as from *Quercus* woodlands near Aix-en-Provence and further east along the Côte d'Azur in the Provence-Alpes-Côte d'Azur Region. In its original concept, *H. roseodiscoideus* is thus a species fruiting along the whole French Mediterranean coast and in nomenclatural terms, all this cited material can be considered as paratyptic. Unfortunately, the holotype could not be successfully sequenced (neither by the "classical" Sanger method nor the Illumina Miseq method), and none of the paratypes cited by Bon & Chevassut (1985: 44) could be located in Bon's collections at LIP. Georges Chevassut's herbarium has been lost after his death and no collection from his herbarium could be located either. To fix usage of the name, we thus epitypify it here using a sequenced collection from Simiane-Collongue, a locality 15 km south from Aix-en-Provence. This collection perfectly fits the description of *H. roseodiscoideus*, both macro- and micro-anatomically (Fig. 2), and its ITS sequence can thus be used as a barcode for the species in future studies.

## REVISED DESCRIPTION

Basidiomata medium-sized to large, rather stout and fleshy, growing individually or in small groups (Figs 1; 2; 4). Pileus 40–120 mm, hemispherical, broadly conical to campanulate when young, with involute margin, sometimes also truncately broadly conical, then convex to plano-convex with a low broad umbo and inflexed margin, covered with thick layer of slime when wet, greasy lustrous when dry, at centre rusty to orange brown with a dark grey tinge, paler in outer half, of greyish beige or milk coffee colour with a pink to pale lilac tinge, whole pileus pallescent with age and sometimes covered with yellow to

rusty-yellow patches when old; when expanding, pileus cuticle sometimes breaking to form small adpressed scales. Lamellae distant, L = 40–60, l = 1–3, sinuate to sometimes subdecurrent, thick, ceraceous, whitish at first, then cream-coloured to pale yellow, with pink tinge. Stipe 60–150 × 8–20(25) mm, cylindrical, conically attenuated towards base, whitish to pale creamy-yellowish, sometimes with a pinkish tinge, slightly slimy when young or wet, otherwise dry, tomentose-floccose, especially in upper part, with yellow-rusty spots when old or bruised. Context whitish to light ochre-orange, somewhat yellowing. Smell not specific. Taste mild, neutral. Basidiospores (7.1)7.4–11.2(12.4) × (4.6)5.1–6.8(7.6) μm, Q = 1.2–2.0, Q<sub>av</sub> = 1.4–1.6 (Table 1), smooth, hyaline, ellipsoid, less frequently obovoid or ovoid, slightly lacrymoid when immature, hilar appendix very prominent, with granular content or several to one oil droplets. For other micromorphological characters see e.g. Bon (1990) and Candusso (1997).

## PHYLOGENY

Nine ITS sequences newly generated from collections morphologically identified as *H. roseodiscoideus* form a well-supported clade in both ML and BI analyses (Fig. 3), together with three sequences previously published by us in GenBank as *Hygrophorus* sp. (JF506763; MK088109) or *H. roseodiscoideus* (MZ088125). Sequences are slightly polymorphic and nest as two sister clades distant to each other by one substitution and three indels. However, because of null or weak statistical support and lack of associated morphological, ecological or biogeographical signal for these clades, we consider the inclusive lineage as representative of the species. Based on available ITS sequences in the genus, the closest relatives to *H. roseodiscoideus* are *H. arbutivus* Fr. and *H. hedrychii* (Velen.) K. Kult, but the support for these relationships is weak (Fig. 3).

## DISCUSSION

The present study settles the longstanding problems concerning the genetic profile of *H. roseodiscoideus* through epitypification of this species and contributes to better circumscribe its morpho-anatomical features and precise its ecological preferences and distribution range.

The diagnostic characters of the species are the relatively large and fleshy basidiomata, slimy pileus with rusty to orange brown centre (sometimes having dark grey tinge) and much paler margin coloured greyish beige, milk coffee or yellowish-whitish, always with typical, more or less obvious pink to pale lilac tinges. The pink hue can be observed also on lamellae which are basically cream to pale yellow. In some collections, pileus cuticle disrupts into small punctulate scales which is not known in similar species. The species is also characteristic by its occurrence in thermophilous oak-dominated stands on calcareous soils. The slimy ring zone on young stipe mentioned by some authors (Bon & Chevassut 1985; Galli 2012) is an inconsistent and often missing character, unsuitable to diagnose the species in our opinion. Bon (1990) and Candusso (1997) mention the pink tinge even in context.

Our data show that the spore size range of *H. roseodiscoideus* is also greater than reported in literature (Bon & Chevassut 1985; Bon 1990; Candusso 1997; Galli 2012). Typical size is  $7.4\text{--}11.4 \times 5.0\text{--}7.5 \mu\text{m}$  with extreme range  $7.1\text{--}12.4 \times 4.5\text{--}7.7 \mu\text{m}$ .

*Hygrophorus roseodiscoideus* is found in association with various species of oak, both evergreen (*Quercus ilex*, *Q. calliprinos*) and deciduous ones (*Q. cerris*, *Q. pubescens*, *Q. robur*, probably also *Q. petraea*). Its habitats are primarily thermophilous Mediterranean oak-dominated stands (often the coastal ones) mixed with trees and shrubs like *Pinus halepensis*, *P. nigra*, *Styrax officinalis*, *Arbutus unedo* or *Juniperus oxycedrus*. Other putative host trees are given by Candusso (1997). In central Europe (Czech Republic, Austria, Hungary), *H. roseodiscoideus* occurs in distinctly warm areas (Central Bohemia, Southern Moravia, Burgenland, vicinity of Budapest, slopes of Bükk Mts.) populated by oak-dominated thermophilous forests (deciduous ones, see above) with admixed *Carpinus betulus*, *Sorbus torminalis*, *Fraxinus ornus*, *Tilia*, *Cornus mas*, *Prunus mahaleb* or *Corylus* (only some of these species are present in particular localities). The central European sites are located in hilly areas, not in lowlands, mostly up to 400 m a.s.l. There are also montane sites of occurrence, namely in Italy (Passo Cantoniera, about 1000 m) and Lebanon (Tesheh, 1354 m) (see also Candusso 1997). The species is also known from the Atlantic coast of western France, although these collections need to be molecularly confirmed.

The substrate preferences of *H. roseodiscoideus* include almost exclusively calcareous soils on bedrock of limestone, clay, or marl, sometimes mixed with sandstone or volcanic rocks. The phenology slightly differs across the species range, Mediterranean and Atlantic populations fruiting from October to January (mostly December), versus September to November for central European populations.

In the same habitats, remotely similar species to *H. roseodiscoideus* are: 1) *H. leucophaeo-ilicis*, which displays ochre-brown pileus centre and whitish-cream margin without pink tinge throughout; 2) *H. pseudodiscoideus* (Maire) Malençon & Bertault, having slender basidiomata with yellow to ochre-brown pileus centre and whitish margin, also without pink colour; and 3) *H. arbustivus*, especially its var. *quercetorum*, with ochre to more or less brown, distinctly radially innately fibrillose pileus and smaller spores ( $7\text{--}9 \times 4.5\text{--}5.5 \mu\text{m}$ ). Fruitbodies of *H. leucophaeus s.l.* are more slender, without greyish tinges on pileus and form mycorrhiza with *Carpinus*, *Corylus* and *Fagus L.*

Judging from some well-documented sites (see Ecology above), the mycobiota is very rich at localities where *H. roseodiscoideus* occurs, and includes primarily thermophilous

ectomycorrhizal species belonging to boletoid genera, *Russula* Pers., *Lactarius* Pers., *Amanita* Pers., *Cortinarius*, *Hygrophorus* and *Tricholoma* Fr.

In central Europe, we currently know only two sites in the Czech Republic, one in Austria, and five in Hungary in which *H. roseodiscoideus* occurs. Recent records from this area can be attributed to an improved understanding of the morphological profile of *H. roseodiscoideus* among mycologists,

rather than ongoing climatic change (warming). The reason is that there are collections already from the seventies (Hungary) and eighties (Austria) of the twentieth century (see section Additional material examined), i.e., from a period predating climate warming. In contrast, the species is widely distributed in the European part of the Mediterranean, even if uncommon and fruiting only in seasons when favourable climatic conditions occur. Given its known distribution range and ecological preferences, we would expect it to occur in other countries with thermophilous oak forests on calcareous soils, e.g. in Portugal, Slovakia, Slovenia, Balkan countries, and maybe also as far east as Turkey. Its occurrence in suitable Mediterranean habitats of North Africa is also to be expected.

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