A first lichenicolous *Corticium* species (Corticiaceae, Corticiales), described from *Thamnolia* in Switzerland

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Abstract. The new lichenicolous fungus, *Corticium silviae* Diederich, E.Zimm. & Lawrey is described from Switzerland, where it grows on *Thamnolia*. Phylogenetic results also suggest that *Limonomyces* should best be regarded as a synonym of *Laetisaria*, a genus that has a sister position to *Marchandiomyces*. The following new combinations are proposed: *Laetisaria buckii* (Diederich & Lawrey) Diederich, Lawrey & Ghobad-Nejhad (= *Marchandiomyces buckii*), *L. culmigena* (R.K.Webster & D.A.Reid) Diederich, Lawrey & Ghobad-Nejhad (= *Exobasidiellum culmigenum*), *L. marsonii* (Diederich & Lawrey) Diederich, Lawrey & Ghobad-Nejhad (= *M. marsonii*), *L. nothofagicola* (Diederich & Lawrey) Diederich, Lawrey & Ghobad-Nejhad (= *M. nothofagicola*), *L. roseipellis* (Stalpers & Loer.) Diederich, Lawrey & Ghobad-Nejhad (= *Limonomyces roseipellis*).

1. Introduction

Most lichenicolous fungi belong to the ascomycetes, while only five percent of them are basidiomycetes. These include species from different orders and classes, the most diverse being the Tremellales, Cantharellales and Corticiales (Diederich et al. 2018). A total of four lichenicolous species were known from the Corticiaceae, belonging to *Erythricium* J.Erikss. & Hjortstam (= *Marchandiobasidium* Diederich & Schultheis), *Laetisaria* Burds. and *Marchandiomyces* Diederich & D.Hawksw. Two of these are known only from the asexual bulbil-forming stage, one only from the sexual stage, and one from both stages.

Here we report the discovery of a further lichenicolous sexual species of Corticiaceae, discovered on *Thamnolia* in Switzerland. Phylogenetic analyses based on nuLSU and ITS sequences suggest that the new species belongs to the genus *Corticium* and that the circumscription of the genera *Laetisaria* and *Marchandiomyces* should be revised.

2. Material and Methods

2.1. Morphological examination

Type material of the new species is deposited in G and in the private collection of P. Diederich. External morphology was examined using a Leica MZ 7.5 dissecting microscope. Macroscopic photographs were taken using a Canon 40D camera with a Canon MP-E 65 macro lens, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Microscopical structures were studied using hand-cut sections stained with a mixture of phloxine, Congo red and KOH (5%). Microscopic photographs were prepared using a Leica DMLB microscope with DIC, using a Leica EC3 camera and Helicon Focus. Basidiospore measurements are indicated as (min.–) X-σ – X+σ (–max.), followed by the number of measurements (n).

2.2. Molecular data

Tissue scraped from the specimen was washed in 70% ethanol and then genomic DNA was extracted using the Fast DNA Spin Kit from MP Biomedicals (Santa Ana, CA) according to the manufacturer's protocol. About 10 ng of extracted DNA were subjected to a standard PCR in a 20 µL reaction volume using Taq Gold polymerase (Applied Biosystems, Foster City, CA), also according to manufacturer's protocols, with the objective of amplifying the ITS and nuclear large subunit (nuLSU) rDNA. The products were purified with Ampure magnetic beads (Agencourt Bioscience, Beverly, MA) and the purified PCR products were used in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). The sequencing reactions were purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, MO), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run on an ABI3130-xl capillary sequencer (Applied Biosystems). The data collected were analyzed using ABI Sequencing Analysis v5.4 software, and the sequences were then assembled together with the Sequencher version 5.0.1 (Gene Codes, Ann Arbor, MI) software for manual corrections in base calling and to make contiguous alignments of overlapping fragments. The primers used were LR0R, LR3R, LR5, LR7, LR16, ITS4 and ITS5 (http://www.biology.duke.edu/fungi/mycolab/primers.htm).

2.3. Phylogenetic analysis

In addition to our newly generated sequence (GenBank accession: *Corticium* sp. ITS and nuLSU: MH520061), we included sequences used in earlier analyses of the Corticiales (Sikaroodi et al. 2001; DePriest et al. 2005; Lawrey et al. 2007, 2008; Ghobad-Nejhad & Hallenberg 2011; Ghobad-Nejhad et al. 2010) and those from a broad range of taxa representing recognized clades in the *Corticium* clade (Langer 2002; Larson 2004; Binder et al. 2005; Hibbett et al. 2007, 2014; Larson et al. 2007; Diederich et al. 2011). We had difficulty aligning ITS sequences available in GenBank, so we trimmed our sequence to include only the nuLSU and used only published nuLSU sequences from GenBank in our analyses. The final data set (all GenBank accession numbers included in Fig. 1) contained 35 ingroup terminals, and sequences from Gloeophyllales [*Veluticeps abietina* (Pers.) Hjortstam & Tellería, *Heliocybe sulcata* (Berk.) Redhead & Ginns, *Gloeophyllum sepiarium* (Wulfen) P.Karst., and *G. abietinum* (Bull.) P.Karst.] were used as outgroups.

The newly generated nuLSU sequences were edited in Geneious v.8.1.6 (http://www.geneious.com/) and automatically aligned with MAFFT using the --auto option (Katoh & Toh 2005). The alignments were trimmed and subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn et al. 2010a,b); regions aligned with low confidence (below 0.93) were removed. The final nuLSU data set had an alignment length of 862 bases, 231 of which were variable. The GUIDANCE score for the nuLSU alignment was 0.9622. Maximum likelihood (ML) searches were done using RAxML 7.2.6 (Stamatakis 2006; Stamatakis et al. 2005) with non-parametric bootstrapping of 1000 replicates under the universal GTRGAMMA model. A Bayesian analysis was also performed for the same data sets using Markov chain Monte Carlo sampling (Larget & Simon 1999) in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). A substitution model was selected in jModelTest 0.1.1 (Posada 2008), which employs PhyML 3.0 (Guindon & Gascuel 2003) to estimate the likelihood of the data under 24 models of evolution using a fixed topology. The AICc values under each model were compared and the model with the lowest AICc value (GTR+I+G) was selected. Two parallel analyses were then run in MrBayes for 10,000,000 generations, with 4 chains each, sampling every 100 generations. Burn-in trees (initial 25%) were discarded for each run and posterior probabilities (PP) of the nuLSU matrix were determined by calculating a majority-rule consensus tree generated from the post-burnin trees by the MCMCMC runs using the sumt

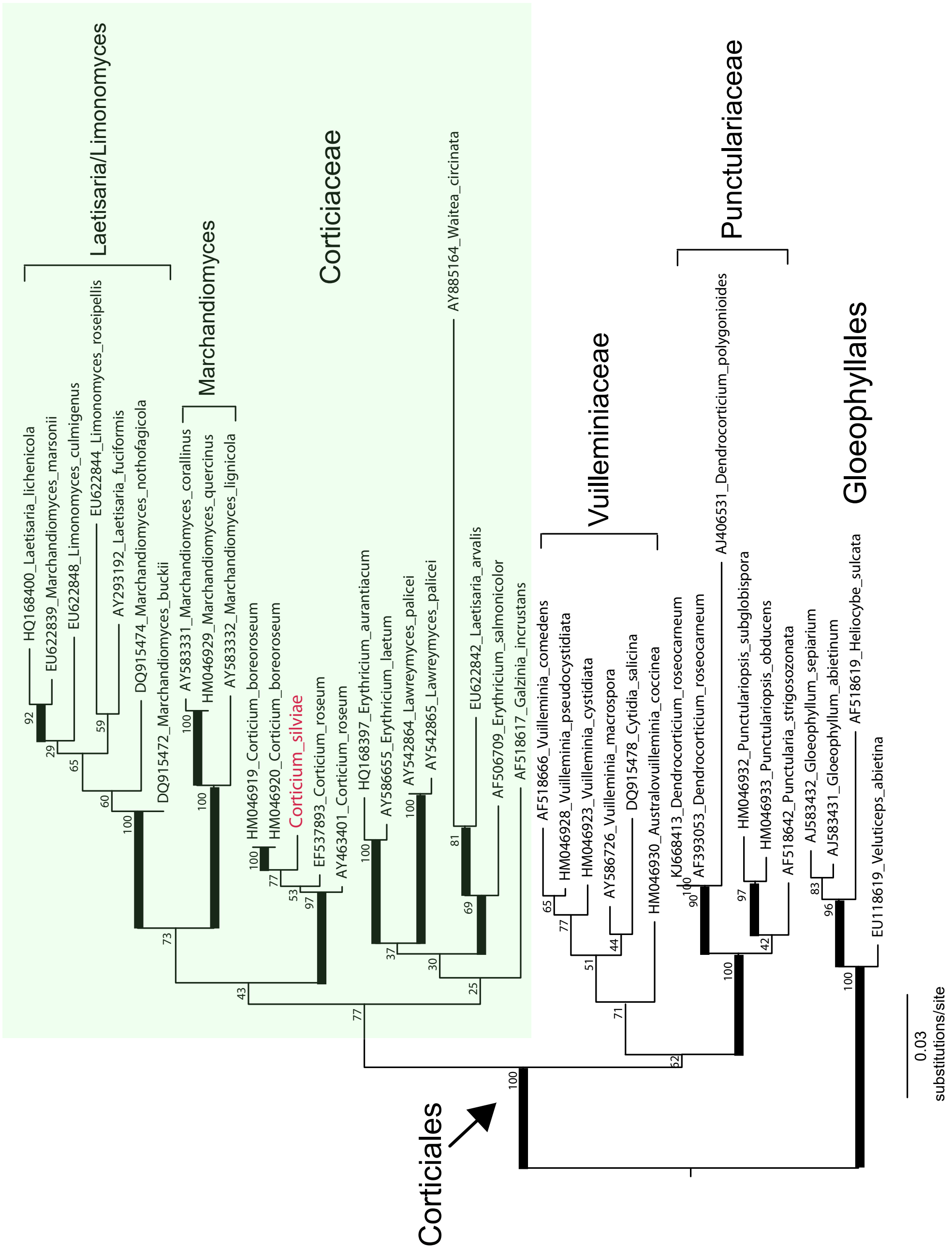

Fig. 1. Best-scoring nuLSU RAxML phylogram of species used in the analysis, showing the placement of *Corticium silviae*. Internal branches in boldface indicate posterior probabilities ≥0.95 and numbers are ML-BS values ≥70.

option of MrBayes. RAxML and MrBayes analyses were performed using the CIPRES Web Portal 3.1 (Miller et al. 2010) and the University of Oslo Bioportal (http://www.bioportal.uio.no). The most likely tree was then produced (–lnL=4550.4209). Relationships were considered supported if they had ML-BS values of 70 or greater and Bayesian posterior probabilities (PP) of 0.95 or greater. Phylogenetic trees were visualized using FigTree v. 1.4.2 (Rambaut 2012).

3. Results

3.1. Phylogenetic placement of new sequences in the Corticiales

ML and Bayesian analyses of the nuLSU alignment recovered trees with the same topology for the strongly supported branches, so only the ML tree is shown (Fig. 1), with the branches having ML bootstrap values ≥70 % in bold and with the posterior probabilities of the Bayesian analysis added above the internal branches. The phylogeny consistently resolved three clades in the Corticiales currently recognized by Ghobad-Nejhad et al. (2010) at the family level as Punctulariaceae, Vuilleminiaceae and Corticiaceae. The unknown was recovered in a well-supported clade containing sequences of *Corticium roseum* Pers. and *C. boreoroseum* Boidin & Lanq., sister to a clade containing two well-supported clades, one formed by sequences of *Marchandiomyces* species and the other with *Laetisaria*/*Limonomyces* species. Each of these clades contains lichenicolous species, with our unknown representing the first known lichenicolous member of *Corticium* (described below). It is also apparent from Fig. 1 that some species originally described in the asexual genus *Marchandiomyces* do not belong to the clade containing the generic type *M. corallinus* (Roberge) Diederich & D.Hawksw., but instead are members of the clade containing *Laetisaria* and the generic type of *Limonomyces*, a result that requires several nomenclatural changes discussed below.

3.2. Taxonomy

Corticium silviae Diederich, E.Zimm. & Lawrey, sp. nov. (Figs 2–3)

Characterized by pale pink basidiomata, relatively small basidia [35–60(–80) × 4–9 µm], relatively small basidiospores [(8–)8.4–10.5(–14) × (5.7–)6.2–7.7(–8.7) µm], the presence of clamp connections, the lack of cystidia, and the lichenicolous habitat.

Type: Switzerland, Valais, Arolla, La Follisses, 46°00’07” N, 7°29’27” E, alt. 2126 m, on *Thamnolia*, 5 June 2017, S. Feusi (G – holotype; herb. Diederich – isotype).

MycoBank MB828093

Basidiomata pale pink, effused, thin, adnate, floccose, margin poorly distinct, up to 0.1 mm thick, reaching 1 cm diam. *Basal hyphae* hyaline, thin-walled, smooth, straight, 2–3 mm wide, septa with clamps. *Subhymenial* hyphae hyaline, thin-walled, smooth, 2–4 µm thick, septa with clamps. *Hymenium* comprising one layer of basidia on vertically branching, thin-walled hyphae, without crystals. *Dendrohyphidia* common in hymenium, with few rather short side branches, 2–2.5(–3) µm diam., hyaline. *Cystidia* and other sterile hymenial elements lacking. *Probasidia* ellipsoid, 13–16 × 6–7 µm, with a basal clamp. *Basidia* initially elongate, cylindrical, ca. 38–60 × 4–6 µm, sometimes with a basal lateral, ellipsoid probasidial bladder; when mature, clavate to suburniform, 30–45(–55) µm long, up to 8 µm in the upper part, exceptionally with a thin, transverse septum in the upper third; wall c. 0.5 µm thick; sterigmata 3–4 per basidium, to 4 µm long and 2 µm wide at the base, curved. *Basidiospores* hyaline, smooth, aseptate, not repetitive, pyriform or lacriform, one side frequently flattened or slightly concave, with a prominent truncate apiculus of 1–2 µm, (8–)8.4–10.5(–14) × (5.7–)6.2–7.7(–8.7) µm, ratio length/breadth (1.1–)1.2–1.5(–1.6) (n=37); wall c. 0.5 µm thick; germ tubes 1.5–2 µm thick. *Conidial and bulbilliferous morphs* unknown.

Duhem & Michel (2006) published a worldwide key to 18 *Corticium* s.l. species. These species all differ from our new lichenicolous taxon by larger or smaller basidiospores, longer or broader basidia, the absence of clamp connections, or the presence of capitate cystidia.

This is the first lichenicolous species of *Corticium*. The similar *Laetisaria lichenicola* Diederich, Lawrey & Van den Broeck is distinguished by pink to coral basidiomata, shorter basidia, c. 30–35 × 9.5–12 µm when mature, 2 sterigmata per basidium, larger basidiospores, 14.5–18.5(–20) × (8–)10.5–12.5 µm, and a different host selection (*Physcia adscendens* and *P. tenella*) (Diederich et al. 2011), and *Erythricium aurantiacum* (Lasch) D.Hawksw. & A.Henrici by orange basidiomata, the absence of clamp connections, shorter and broader basidia, 25–40 × 12–15 µm, larger basidiospores, 13–17.5(–18.5) × 8–11.5(–13.5) µm, and a different ecology (on corticolous *Physcia*, *Xanthoria*, etc.) (Diederich et al. 2003).

Distribution and host. The new species is currently known only from the type locality in Switzerland on the thallus of *Thamnolia*. The host thalli are not or slightly damaged (swollen and bent) by the lichenicolous fungus. During a recent visit of the type locality by the second author and Mrs Silvia Feusi, two populations of *Corticium silviae* have been observed and photographed (Fig. 1).

Etymology. The new species is dedicated to the collector of the type specimen, Silvia Feusi (Switzerland).

Our phylogenetic tree (Fig. 1) strongly suggests that the genus *Marchandiomyces* Diederich & D.Hawksw. should just include three species, the generic type *M. corallinus* (Roberge) Diederich & D.Hawksw., *M. lignicola* Lawrey & Diederich and *M. quercinus* (J.Eriksson & Ryvarden) D.Hawksw. & A.Henrici. Three other species described in *Marchandiomyces* belong to the *Laetisaria* clade, which also includes the type of *Limonomyces*. *Laetisaria* and *Limonomyces* must consequently be regarded as synonyms, and the following nomenclatural changes become necessary. An amended description of the genus *Laetisaria* is also given. One of us (M. G.) recently obtained an ITS sequence from a further species, *Laetisaria agaves* Burds. & Gilb., and a preliminary analysis (results not shown here) suggests that it belongs to the *Laetisaria* clade as well.

Laetisaria Burds., Trans. Brit. Mycol. Soc. 72: 420 (1979); type: *L. fuciformis* (McAlpine) Burds.

Syn. nov.: *Limonomyces* Stalpers & Loer., Canad. J. Bot. 60: 533 (1982); type: *L. roseipellis* Stalpers & Loer.

Asexual stage, when present, consisting of small pink to pale red bulbils. Teleomorph stage, when present, as thin, pink to pale red, resupinate basidiomata. Basidiomata annual, corticioid, pink, crustose to membranaceous, smooth. Hyphal system monomitic, hyphae with or without clamps. Cystidia absent, hyphidia occasionally present. Basidia first bladder-like, then subcylindrical to clavate, with 2-4 sterigmata. Basidiospores large ovoid, thin to moderately thick-walled, not reacting in Melzer's reagent. Species mostly parasitic on leaves of monocotyledons (Poaceae, *Agave*, *Pandanus*), on lichen thalli or lignicolous.

Laetisaria buckii (Diederich & Lawrey) Diederich, Lawrey & Ghobad-Nejhad, comb. nov.; basionym: *Marchandiomyces buckii* Diederich & Lawrey, Mycol. Progr. 6: 70 (2007); MycoBank MB828094.

Laetisaria culmigena (R.K.Webster & D.A.Reid) Diederich, Lawrey & Ghobad-Nejhad, comb. nov.; basionym: *Exobasidiellum culmigenum* R.K.Webster & D.A.Reid, Trans. Brit. Mycol. Soc. 52: 20 (1969); MycoBank MB828095.

Laetisaria marsonii (Diederich & Lawrey) Diederich, Lawrey & Ghobad-Nejhad, comb. nov.; basionym: *Marchandiomyces marsonii* Diederich & Lawrey, Amer. J. Bot. 95: 820 (2008); MycoBank MB828096.

Laetisaria nothofagicola (Diederich & Lawrey) Diederich, Lawrey & Ghobad-Nejhad, comb. nov.; basionym: *Marchandiomyces nothofagicola* Diederich & Lawrey, Mycol. Progr. 6: 71 (2007); MycoBank MB82097.

Laetisaria roseipellis (Stalpers & Loer.) Diederich, Lawrey & Ghobad-Nejhad, comb. nov.; basionym: *Limonomyces roseipellis* Stalpers & Loer., Canad. J. Bot. 60: 534 (1982); MycoBank MB828098.

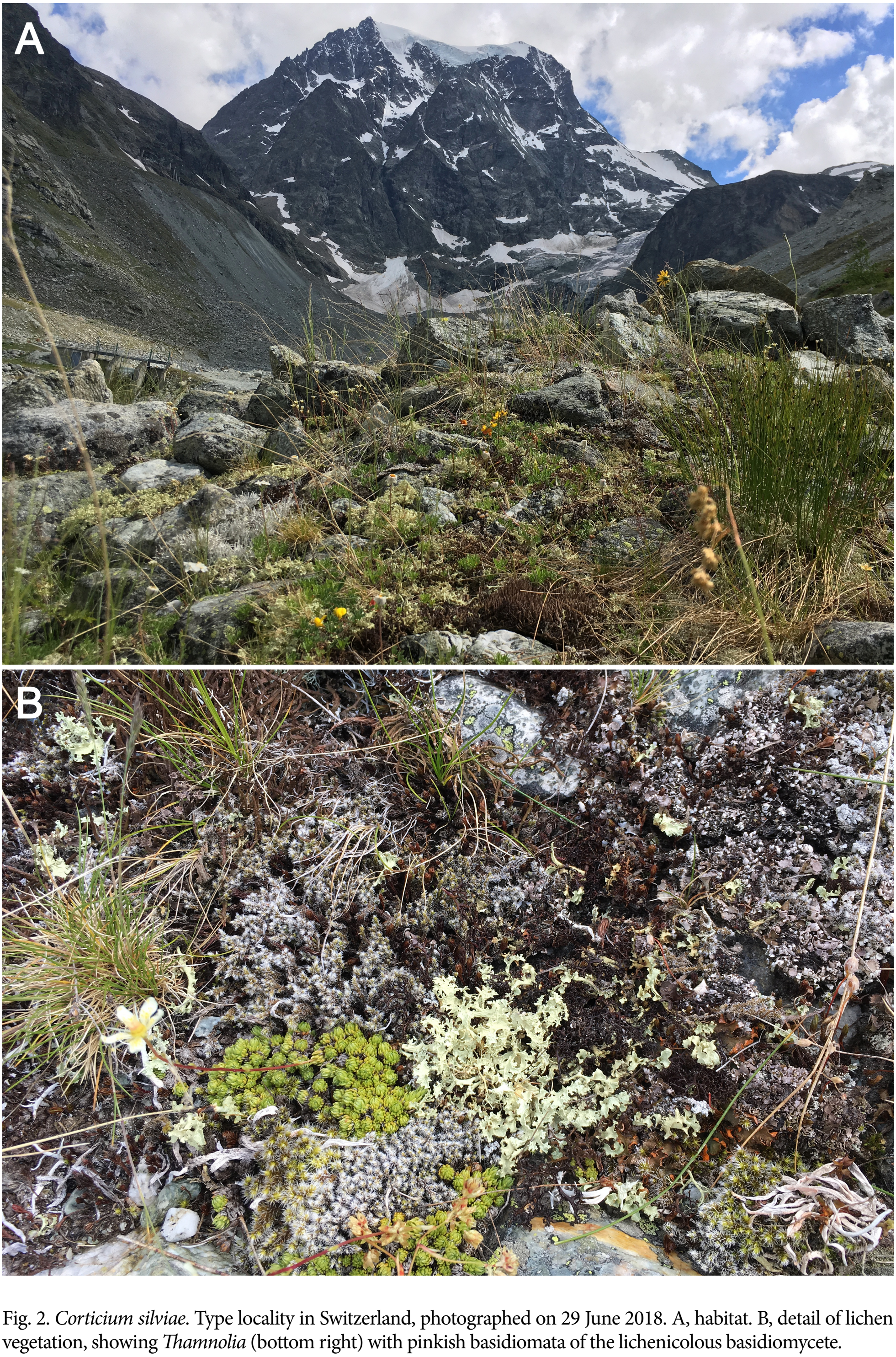

Fig. 2. *Corticium silviae*. Type locality in Switzerland, photographed on 29 June 2018. A, habitat. B, detail of lichen vegetation, showing *Thamnolia* (bottom right) with pinkish basidiomata of the lichenicolous basidiomycete.

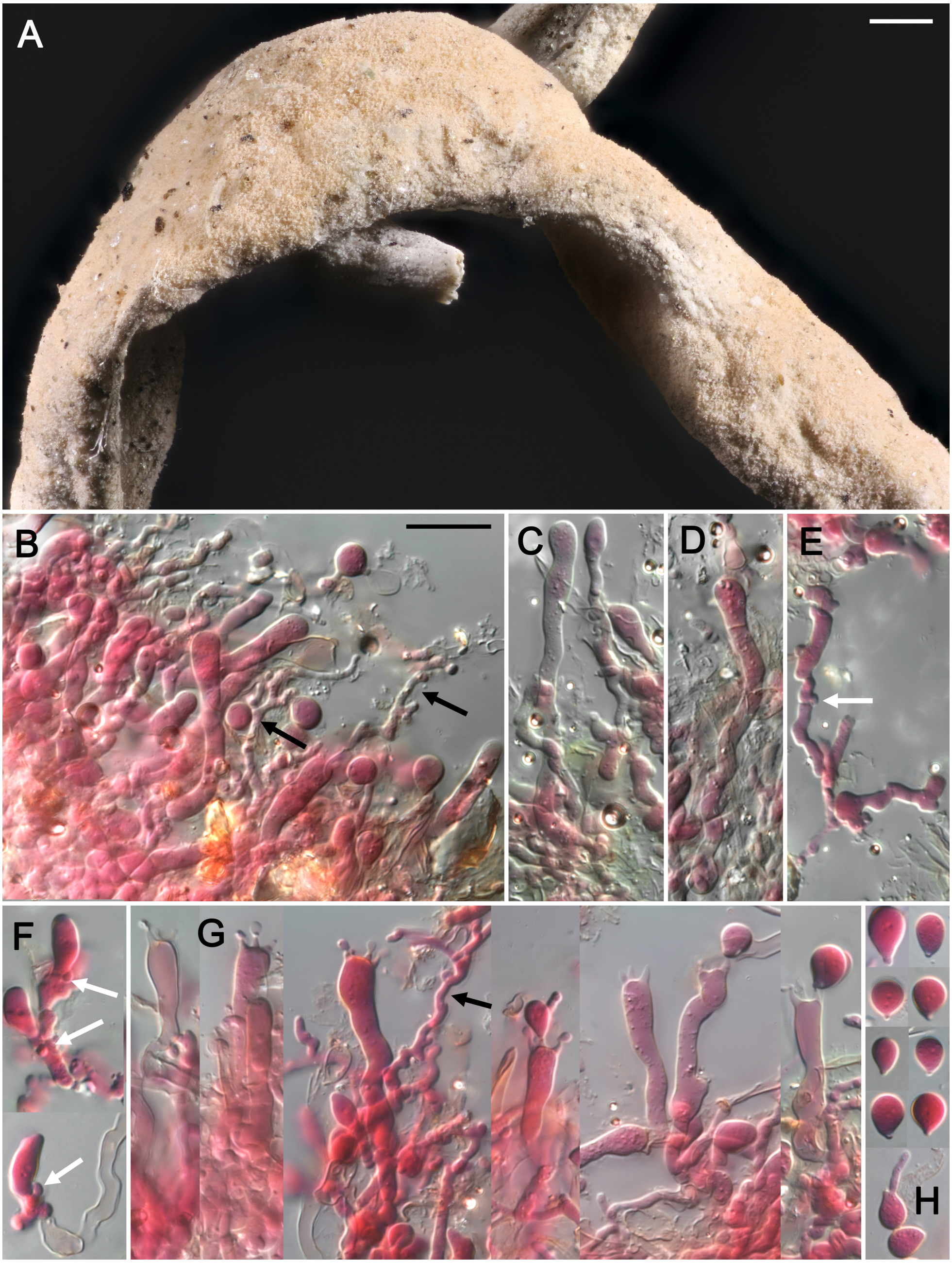

Fig. 3. *Corticium silviae* (G – holotype). A, basidioma over thallus of *Thamnolia*. B, hymenium. C, basidia. D, septate basidium. E, subhymenial hyphae with clamp connections. F, probasidia with a basal clamp. G, mature basidia with 3–4 sterigmata. H, basidiospores, one germinating. Microscopical photos using DIC optics, in a mixture of 5% KOH, phloxine and Congo red. Black arrows: dendrohyphidia; white arrows: clamps. Scale bars: A = 500 µm. B–H (the same bar for all) = 20 µm.

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