

***Candolleomyces eurysporus*, a new *Psathyrellaceae* (*Agaricales*) species from the tropical Cúc Phương National Park, Vietnam**

ENRICO BÜTTNER^{1,*}
ALEXANDER KARICH¹
DO HUU NGHI²
MAXIMILIAN LANGE¹
CHRISTIANE LIERS¹
HARALD KELLNER¹
MARTIN HOFRICHTER¹
RENÉ ULLRICH¹

*E-mail: enrico.buettner@tu-dresden.de

¹TU Dresden - Internationales Hochschulinstitut
Zittau
Markt 23
02763 Zittau, Germany

²Experimental Biology Lab.
Institute of Natural Products Chemistry
Vietnam Academy of Science and Technology
18 Hoang Quoc Viet, Cau Giay,
Hanoi, Vietnam

Accepted 18. December 2020. © Austrian Mycological Society, published online 22. December 2020

BÜTTNER, E., KARICH, A., NGHI, D. H., LANGE, M., LIERS, C., KELLNER, H., HOFRICHTER, M., ULLRICH, R., 2020: *Candolleomyces eurysporus* – A new *Psathyrellaceae* (*Agaricales*) species from the tropical Cúc Phương National Park, Vietnam. – Austrian J. of Mycology 28: 79–92.

Key words: *Basidiomycota*, *Candolleomyces*, *Psathyrellaceae*, sp. nov., taxonomy, wood-rot. – Funga of Vietnam. – 1 new species.

Abstract: Basidiomata of a hitherto undescribed *Candolleomyces* species were collected during a macrofungal foray in North Vietnam. They grew on deciduous deadwood in the Southeastern part of the Cúc Phương National Park (Vietnamese: *Vườn quốc gia Cúc Phương*), Ninh Bình Province. The new species *Candolleomyces eurysporus*, sp. nov., is characterized by broadly ellipsoid to broadly ovoid basidiospores [$5.5\text{--}7.0 \times 4\text{--}5\text{--}(6) \mu\text{m}$] without visible germ pore, utriform to ventricose-clavate cheilocystidia, heteromorphic caulocystidia and the absence of pleurocystidia and pileocystidia. Based on an isolated pure culture, the genome was sequenced and a full ribosomal RNA operon including 18S, internal transcribed spacer 1 (ITS), 5.8S, ITS2 and 28S rRNA gene annotated. Phylogenetic analysis confirmed the taxonomic placement of *C. eurysporus* within the *C. sulcatotuberculosis* species clade.

Zusammenfassung: Während einer Exkursion in der Provinz Ninh Bình (Nordvietnam) wurden im Cúc Phương Nationalpark (vietnamesisch: *Vườn quốc gia Cúc Phương*) Fruchtkörper einer bisher nicht beschriebenen Pilzart gesammelt. Die Basidiomata wuchsen auf abgefallenen Zweigen eines nicht näher bestimmbaren Laubbaumes. *Candolleomyces eurysporus* sp. nov., ist ein neuer Faserling, der durch folgende Merkmalskombination charakterisiert ist: breit ellipsoide bis breit eiförmige Basidiosporen [$5,5\text{--}7 \times 4\text{--}5\text{--}(6) \mu\text{m}$] ohne sichtbaren Keimporus, utriforme bis bauchig-keulige Cheilozystiden, heteromorphe Kaulozystiden sowie das Fehlen von Pleuro- und Pileozystiden. Durch die Sequenzierung einer Reinkultur konnte das Genom des Pilzes ausgewertet und ein sich wiederholendes rRNA Operon, inklusive der Regionen für 18S, 5.8S, 28S rRNA Gene sowie die internal transcribed spacer ITS1 und ITS2, annotiert werden. Phylogenetische Analysen bestätigten die taxonomische Stellung von *C. eurysporus* sp. nov. innerhalb der *C. sulcatotuberculosis*-Klade.

Three new genera *Britzelmayria* D. WÄCHT. & A. MELZER, *Candolleomyces* D. WÄCHT. & A. MELZER, and *Olotia* D. WÄCHT. & A. MELZER have recently been separated from the dark-spored agaric genus *Psathyrella* (FR.) QUÉL. (WÄCHTER & MELZER 2020). Species that were traditionally placed in the genus *Psathyrella* (incl. the new three genera) are ubiquitously distributed across the world (HOASHI 2008, SINGER 1978, VAN WAVEREN 1985) and thus, can be found in arctic or alpine regions as well as in the subtropics and tropics (PEGLER 1983, 1977; SMITH 1972). Although, over 900 species have been described so far (<https://www.mycobank.org/>), their number is steadily increasing (MELZER & al. 2018, SICOLI & al. 2019, YAN & BAU 2018).

Species of the family *Psathyrellaceae* VILGALYS, MONCALVO & REDHEAD, including *Candolleomyces*, are usually following saprotrophic lifestyles and thus, grow on deadwood, plant debris, dung, leaf-litter or humus-rich soil. The type name of the family is *Psathyrella* and refers to the fragile or brittle constitution of fruiting bodies that all members of *Psathyrellaceae* have in common (diminutive of *Psathyra*, from Old Greek ψαθυρος = friable, crumbling).

Recent molecular genetic studies have indicated closer relation of the genus to other psathyrelloid genera, such as *Coprinellus* P. KARST. and *Coprinopsis* P. KARST. (MATHENY & al. 2006, NAGY & al. 2009, ÖRSTADIUS & al. 2015, REDHEAD & al. 2001, WÄCHTER & MELZER 2020). A current state of relationship within *Psathyrellaceae* is given in WÄCHTER & MELZER (2020) and includes the following genera: *Britzelmayria*, *Candolleomyces*, *Coprinellus*, *Coprinopsis*, *Cystoagaricus* SINGER, *Hausknechtia* D. WÄCHT. & A. MELZER, *Homophron* (BRITZELM.) ÖRSTADIUS & E. LARSS., *Kauffmania* ÖRSTADIUS & E. LARSS., *Lacrymaria* PAT., *Narcissea* D. WÄCHT. & A. MELZER, *Olotia*, *Parasola* REDHEAD, VILGALYS & HOPPLE, *Psathyrella*, *Punjabia* D. WÄCHT. & A. MELZER, *Tulosesus* D. WÄCHT. & A. MELZER, and *Typhrasa* ÖRSTADIUS & E. LARSS.. The recently erected genus *Candolleomyces* contains taxa with small to large basidiomata, which grow terrestrially, lignicolously, or rarely fimicolously (WÄCHTER & MELZER 2020). It differs from other closely related genera such as *Typhrasa*, *Olotia*, *Britzelmayria*, *Kauffmania* and *Psathyrella* by the absence of pleurocystidia. The genus' etymology refers to the type species *Candolleomyces candolleanus* (FR.) D. WÄCHT. & A. MELZER, the epithet of which honors the Swiss botanist AUGUSTIN PYRAMUS DE CANDOLLE.

Two basidiomata resembling the recently described species *Psathyrella aberdarensis* A. MELZER, KIMANI & R. ULLRICH, now *Candolleomyces aberdarensis* (A. MELZER, KIMANI & R. ULLRICH) D. WÄCHT. & A. MELZER, were collected during a field trip with focus on macrofungi in the Cúc Phương National Park (Vietnamese: *Vườn quốc gia Cúc Phương*, Ninh Bình Province, North Vietnam) in November 2018. These specimens and the strain isolated turned out to deviate microscopically and genetically from all known *Psathyrella* s.l. species and therefore, are described as new. In addition, the strain was sequenced and its full genome sequence, providing relevant data for further phylogenetic and functional analyses, was deposited at NCBI (National Centre for Biotechnology Information) Bioproject PRJNA647680.

Although, Vietnam is without doubt a hotspot of biodiversity, only little information is currently available on the funga of this country (DE QUEIROZ & al. 2013). The enormous diversity of large uncharted regions in Vietnam (COLLEN & al. 2014) will surely lead to the discovery of more fungal species, as it has recently been reported in a survey on comparable Chinese regions (YAN & BAU 2018).

Tab. 1. Species (65) used for the phylogenetic analyses of the *Candolleomyces* within the *Psathyrella* s.str. main tree including GenBank accession numbers of the ITS regions.

Organism	GenBank no.	Organism	GenBank No.
<i>Agaricus atomatus</i>	DQ389665	<i>Psathyrella cotonea</i>	KC992870
<i>Britzelmayria multipedata</i>	AM712279	<i>Psathyrella duchesnayensis</i>	KC992869
<i>Britzelmayria multipedata</i>	KC992888	<i>Psathyrella effibulata</i>	DQ389672
<i>Britzelmayria supernula</i>	KC992867	<i>Psathyrella fatua</i>	DQ389681
<i>Candolleomyces aberdarensis</i>	MH880928	<i>Psathyrella fatua</i>	KC992879
<i>Candolleomyces badhyzensis</i>	KC992883	<i>Psathyrella gordonii</i>	KC992925
<i>Candolleomyces candolleanus</i>	DQ389720	<i>Psathyrella lacuum</i>	KC992887
<i>Candolleomyces euryosporus</i>	MT651560	<i>Psathyrella longicauda</i>	KC992889
<i>Candolleomyces leucotephrus</i>	KC992885	<i>Psathyrella lutensis</i>	DQ389685
<i>Candolleomyces luteopallidus</i>	KC992884	<i>Psathyrella lutulenta</i>	KC992875
<i>Candolleomyces sulcatotuberculosis</i>	KJ138422	<i>Psathyrella magnispora</i>	KC992863
<i>Candolleomyces trinitatensis</i>	KC992882	<i>Psathyrella magnispora</i>	KC992864
<i>Candolleomyces tuberculatus</i>	KC992886	<i>Psathyrella marquana</i>	MF668178
<i>Candolleomyces tuberculatus</i>	KC992934	<i>Psathyrella microrrhiza</i>	DQ389684
<i>Candolleomyces typhae</i>	DQ389721	<i>Psathyrella obtusata</i>	DQ389711
<i>Coprinellus micaceus</i>	HM240519	<i>Psathyrella obtusata</i>	AM712273
<i>Coprinellus silvaticus</i>	KC992943	<i>Psathyrella kauffmanii</i>	AM712277
<i>Coprinellus xanthothrix</i>	HF543673	<i>Psathyrella piluliformis</i>	DQ389699
<i>Psathyrella</i> aff. <i>casca</i> BRNM 705627	AM712278	<i>Psathyrella pratensis</i>	DQ389678
<i>Psathyrella almerensis</i>	KC992873	<i>Psathyrella prona</i>	DQ389666
<i>Psathyrella almerensis</i>	KC992874	<i>Psathyrella prona</i>	DQ389673
<i>Psathyrella amarescens</i>	KC992852	<i>Psathyrella pseudogracilis</i>	DQ389675
<i>Psathyrella ammophila</i>	KC992871	<i>Psathyrella pseudogracilis</i>	KC992853
<i>Psathyrella ammophila</i>	KC992872	<i>Psathyrella romellii</i>	KC992859
<i>Psathyrella bipellis</i>	DQ389679	<i>Psathyrella</i> sp. 10 EL-2013	KC992877
<i>Psathyrella bipellis</i>	DQ389680	<i>Psathyrella</i> sp. 5 EL-2013	KC992851
<i>Psathyrella bipellis</i>	KC992865	<i>Psathyrella</i> sp. 9 EL-2013	KC992868
<i>Psathyrella calcarea</i>	DQ389671	<i>Psathyrella spadiceogrisea</i>	DQ389682
<i>Psathyrella owyheensis</i>	KC992880	<i>Psathyrella spadiceogrisea</i>	KC992878
<i>Psathyrella</i> cf. <i>longicauda</i> LO382-89	DQ389667	<i>Psathyrella stercoraria</i>	DQ389669
<i>Psathyrella clivensis</i>	DQ389683	<i>Psathyrella thujina</i>	KC992876
<i>Psathyrella corrugis</i>	DQ389674	<i>Psathyrella vinosofulva</i>	KC992861
<i>Psathyrella cotonea</i>	AM712283		

Material and methods

Material:

A twig bearing two fresh basidiomata was collected in Cúc Phương National Park and transferred to Zittau (Germany) for further cultivation and examination. The *in situ* collected twig was placed under saturation vapor at 23 °C in an incubation chamber. After about five weeks, three basidiomata grew on the twig consecutively over a month. An axenic pure culture (fungal strain) was isolated from this material using 2 % malt-agar plates supplemented with antibiotics (50 µg/ml streptomycin, penicillin, chloramphenicol, benomyl and 40 µg/ml nystatin). The strain was deposited at the Vietnam Type Culture Collection (VTCC, Hanoi, Vietnam) under VTCC 930004. The specimen voucher is deposited at the Herbarium Senckenbergianum Görlitz, Germany (GLM-F126263).

Tab. 2. Fungal species (27 records) used for phylogenetic analyses of *Candolleomyces* including GenBank accession numbers of the ITS regions.

Organism (current name)	Organism (GenBank entry) †	GenBank No.
<i>Candolleomyces</i>	<i>Psathyrella</i>	
<i>C. aberdarensis</i> (A. MELZER, KIMANI & R. ULLRICH) D. WÄCHT. & A. MELZER	<i>P. aberdarensis</i>	MK421517
<i>C. badhyzensis</i> (KALAMEES) D. WÄCHT. & A. MELZER	<i>P. badhyzensis</i>	KC992883
<i>C. cacao</i> (DESJARDIN & B. A. PERRY) D. WÄCHT. & A. MELZER	<i>P. cacao</i>	KX017210
<i>C. cacao</i>	<i>P. cacao</i>	NR148106
<i>C. candolleanus</i> (FR.) D. WÄCHT. & A. MELZER	<i>P. candolleana</i>	DQ389720
<i>C. efflorescens</i> (SACC.) D. WÄCHT. & A. MELZER	<i>P. efflorescens</i>	KC992941
<i>C. euryспорus</i> sp. nov.	<i>P. euryспорa</i> ‡	MT651560
<i>C. halophilus</i> (BERK. & BROOME) D. WÄCHT. & A. MELZER	<i>P. halophila</i>	MG825900
<i>C. leucotephrus</i> (BERK. & BROOME) D. WÄCHT. & A. MELZER	<i>P. leucotephra</i>	KC992885
<i>C. luteopallidus</i> (A.H. SM.) D. WÄCHT. & A. MELZER	<i>P. luteopallida</i>	KC992884
<i>C. luteopallidus</i>	<i>P. luteopallida</i>	MG734736
<i>C. secotioides</i> (G. MORENO, HEYKOOP, ESQUEDA & OLARIAGA) D. WÄCHT. & A. MELZER	<i>P. secotioides</i>	KR003281
<i>C. singer</i> (A.H. SM.) D. WÄCHT. & A. MELZER	<i>P. singeri</i>	MG734718
<i>C. subsingeri</i> (T. BAU & J.Q. YAN) D. WÄCHT. & A. MELZER	<i>P. subsingeri</i>	MG734714
<i>C. subsingeri</i>	<i>P. subsingeri</i>	MG734742
<i>C. subsingeri</i>	<i>P. subsingeri</i>	NR160505
<i>C. sulcatotuberculosis</i> (J. FAVRE) D. WÄCHT. & A. MELZER	<i>P. typhae</i> var. <i>sulcatotuberculosa</i>	KJ138423
<i>C. sulcatotuberculosis</i>	<i>P. typhae</i> var. <i>sulcatotuberculosa</i>	KJ138422
<i>C. trinitatensis</i> (R. E. D. BAKER & W. T. DALE) D. WÄCHT. & A. MELZER	<i>P. trinitatensis</i>	KC992882
<i>C. tuberculatus</i> (PAT.) D. WÄCHT. & A. MELZER	<i>P. tuberculata</i>	KC992886
<i>C. tuberculatus</i>	<i>P. tuberculata</i>	KC992934
<i>C. typhae</i> (KALCHBR.) D. WÄCHT. & A. MELZER	<i>P. typhae</i>	JX077004
<i>C. typhae</i>	<i>P. typhae</i>	DQ389721
<i>Coprinellus</i>	<i>Coprinellus</i>	
<i>C. micaceus</i> (BULL.) VILGALYS, HOPPLE & JACQ. JOHNSON	<i>C. micaceus</i>	HM240519
<i>C. silvaticus</i> (PECK) GMINDER	<i>C. silvaticus</i>	KC992943
<i>C. xanthothrix</i> (ROMAGN.) VILGALYS, HOPPLE & JACQ. JOHNSON	<i>C. xanthothrix</i>	HF543673
<i>Psathyrella</i>	<i>Psathyrella</i>	
<i>Psathyrella lacuum</i> HUIJSMAN	<i>P. lacuum</i>	KC992887

† queried 14.12.2020

‡ initially submitted as *P. euryспорa***Morphology:**

All macroscopic characteristics were recorded based on fresh material. Photographs of the cultivated basidiomata were taken and colour codes are based on the RGB color model (POYNTONU 2003). Microscopic characteristics were analyzed based on hand sections of fresh and revived material (Zeiss, Axio Scope A1, Oberkochen, Germany; Canon EOS 60 D, Tokyo, Japan). The spore size was measured in water using spores from a spore print at the stipe apex. Color of spores was assessed in water, ammonia solution (10 %) and potassium hydroxide solution (5 % w/v KOH). Cystidia and other microscopic structures were studied in 5 % KOH.

DNA extraction, genome sequencing and ribosomal RNA gene retrieval:

Biomass of a culture plate of *Candolleomyces euryспорus* was scraped off to extract genomic DNA by a standard cetyltrimethylammonium bromide (CTAB)-based method. Genomic DNA was sonographically sheared with a S2 ultrasonicator (Covaris, Woburn, MA, USA) to subsequently construct a 400bp library using the Ion Plus Fragment Library Kit (Thermo Fisher, Darmstadt, Germany). The library was sequenced on an Ion GeneStudio™ S5 System using the Ion 530 Chip Kit. The resulting 11.3 million reads were filtered to include lengths between 300 and 625 bp and were assembled using MIRA 4.0

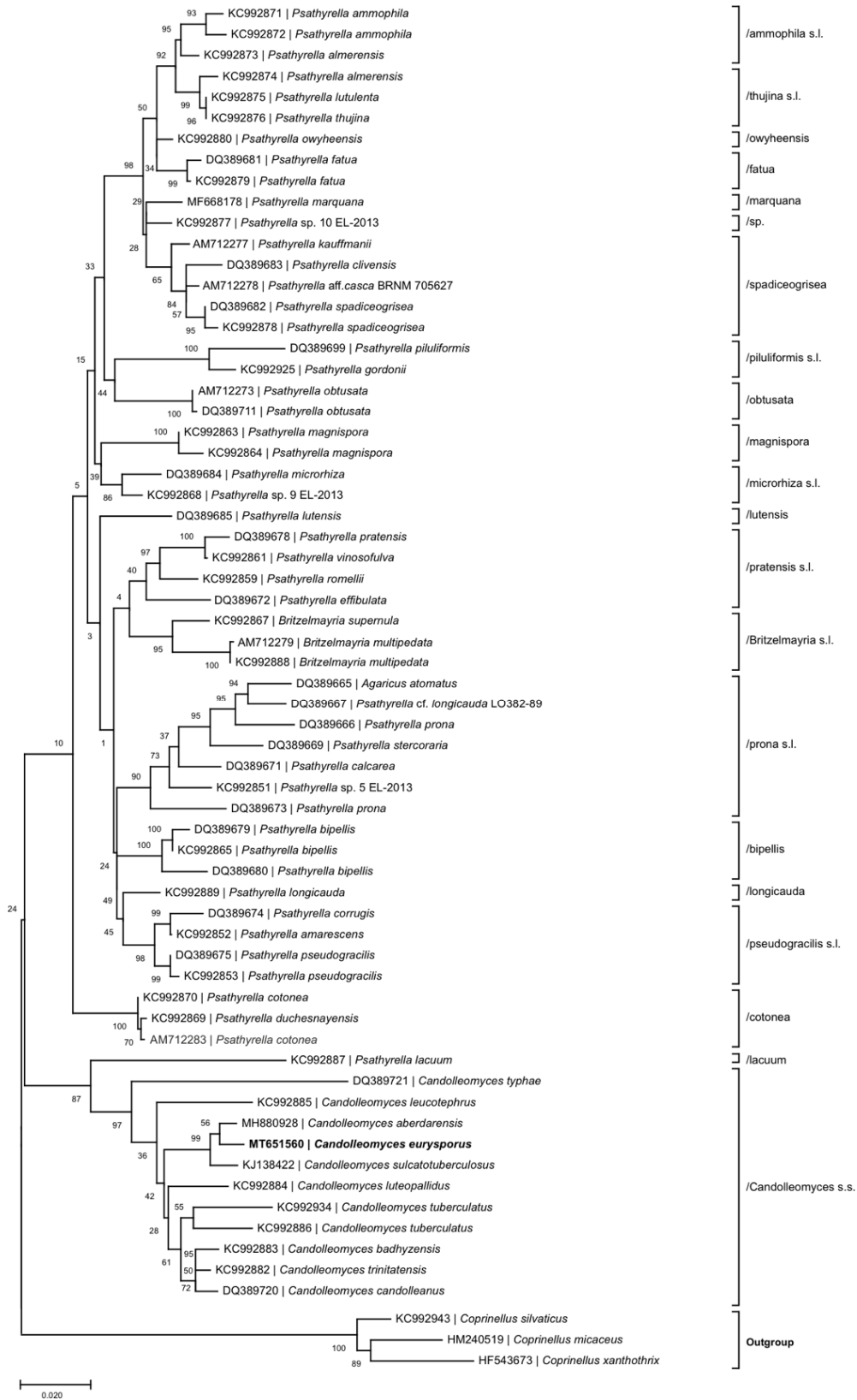


Fig. 1. Maximum Likelihood tree inferred from an alignment including ITS and 28s rRNA gene sequences of *C. eurysporus* (bold) and 64 reference sequences. Support of branches were calculated using a maximum likelihood bootstrap (MCL) approach (1,000 replicates, values >80 below branches; KUMAR al. 2018, NEI & KUMAR 2000). Gamma distribution was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 0.1676). Shown branch values refer to percentage of trees in which the associated taxa clustered together. The tree is rooted using *Coprinellus* spp. as outgroup.

(minimum reads per contig = 100, mode = accurate; CHEVREUX & AL. 1999). Overlapping contigs were joined and duplicate contigs were filtered using, in a second step, the Geneious assembler R11.4.1 (parameter: highest sensitivity/slow; KEARSE & al. 2012). For complete annotation of the full ribosomal RNA gene operon including 18S, internal transcribed spacer 1 (ITS), 5.8S, ITS2 and 28S rRNA gene regions and spacer, the contig was manually identified comparing references obtained from NCBI (nr-database) to the full assembly including all contigs. The full ribosomal operon (MT651560) including SSU, LSU and 5.8S rRNA gene regions and additional genes β -tubulin (MW369460) and translation elongation factor (Tef)1 α (MW369459) are available at GenBank NCBI.

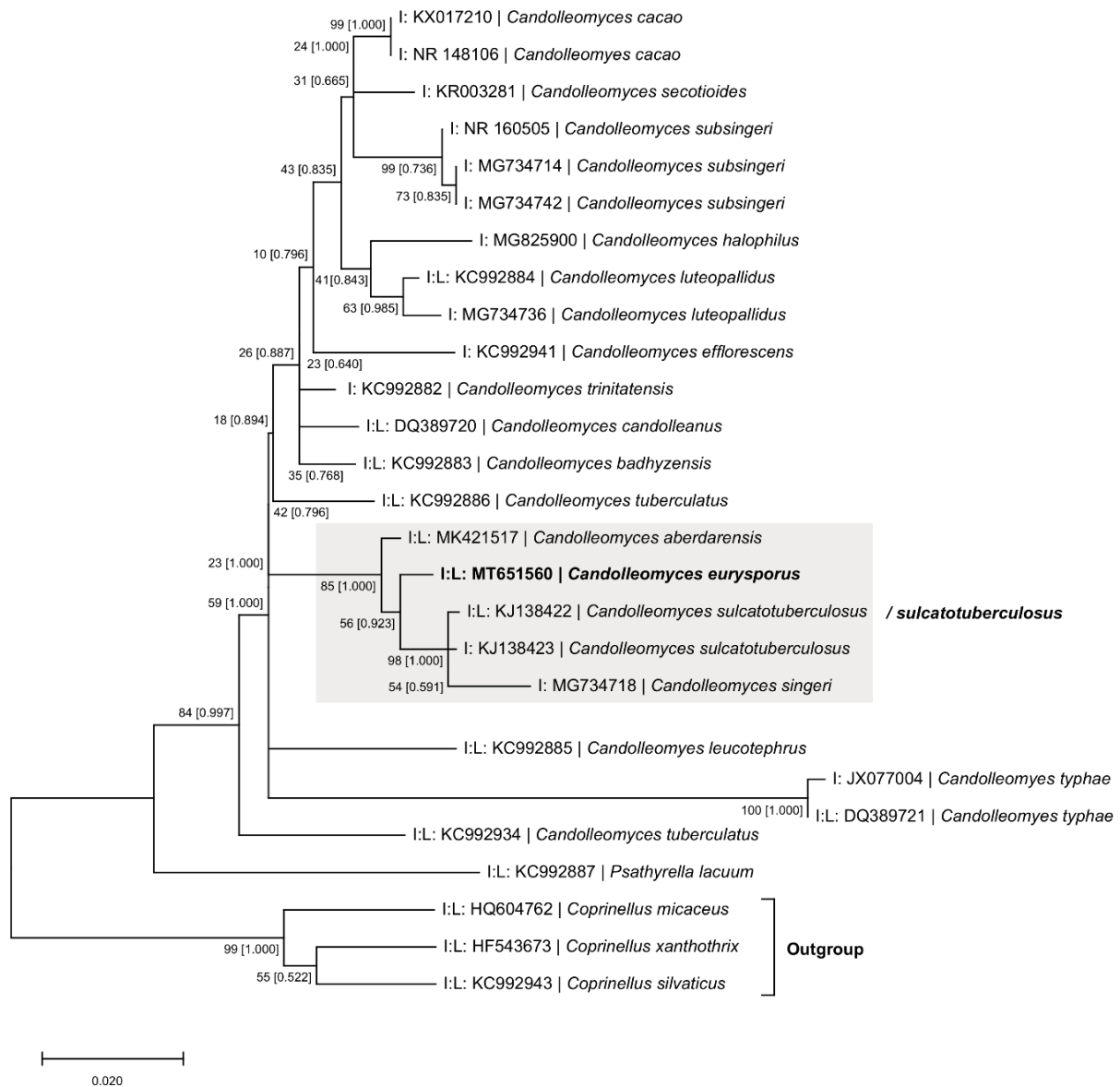


Fig. 2. Maximum Likelihood tree inferred from ITS (I) and LSU (L) sequences of *C. euryспорus* (bold) and 26 references estimated using the Maximum Composite Likelihood (MCL) approach (FELSENSTEIN 1985, KUMAR & al. 2018) of *Candolleomyces* and *Psathyrella lacuum*. Gamma distribution with invariant sites was used to model evolutionary rate differences among sites (5 categories; +G, parameter = 0.6561) with Kimura (G+I) 2-paramater model (KIMURA 1980). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Shown branch values refer to percentage of trees in which the associated taxa clustered together. Sampling from the ML tree of the existing molecular data using the reconstruction method MCMCMC (GEYER 1991) using MrBayes, values are contained in square brackets. The tree is rooted using *Coprinellus* spp. as outgroup.

Alignment and phylogenetic analysis:

In a first step, the identified *C. eurysporus* ITS and 28S rRNA (D1-D3) gene regions were used to find similar sequences within the genus *Psathyrella* and specifically *Candolleomyces* in NCBI GenBank using a blastn search (nr/nt database). This first result concerning the phylogeny is given in Fig. 1, species are listed in Tab. 1. Furthermore, we compiled a set of sequence data including ITS region and the 28S rRNA gene region (as far as available) of the genus *Candolleomyces* (Tab. 2). Alignments were calculated using MUSCLE 3.8.425 (EDGAR 2004) with default settings integrated in Geneious R11. Phylogenies were constructed using MEGA 10.0.5 (KUMAR & al. 2018). For both sets, a maximum likelihood approach after a model selection was used (GUINDON & GASCUEL 2003). The substitution model with the lowest Bayesian Information Criterion (BIC) score was chosen (K2+G+I, KIMURA 1980). Branch support was estimated by using a bootstrap approach using 1,000 replicates. Furthermore, a Bayesian approach using MrBayes 3.2.6 (HUELSENBECK & RONQUIST 2001, RONQUIST & al. 2012) integrated in Geneious was used (substitution model: general time reversible (GTR) with gamma distributed rate variation among sites (+G); chain length: 1,100,000; burn-in: 100,000; chains: 4; sample frequency: 200).

Results

Phylogeny

The draft genome of *Candolleomyces eurysporus* is represented by 1,966 assembled contigs, a size of 70.0 Mb and a G+C content of 50.0 %. Altogether 16,680 genes were predicted using AUGUSTUS (STANKE & al. 2004). A query of the ITS region by known representatives of the genus *Psathyrella* s.l. provided a clear picture. Regions of ITS1 (272bp), 5.8S rRNA gene (157bp) and ITS2 (237bp) were strictly annotated. Further regions (SSU, LSU) for phylogenetic studies were adapted from known sequences. In comparison, ITS/28S rRNA gene sequence showed the highest similarity (98.7 %) to the recently described species *C. aberdarensis* from Kenya. Furthermore, sequences of β -tubulin and tef1 α protein of both species were to 99.77 % and 98.91 %, respectively, identical. The ML (maximum likelihood) phylogeny using ITS/28S rRNA genes placed the new species in the well-supported genus *Candolleomyces*, more distinctly in a clade together with *C. sulcatotuberculosis* (J. FAVRE) D. WÄCHT. & A. MELZER. and *C. aberdarensis* (Fig. 1). A detailed analysis of the genus *Candolleomyces* was performed including ITS and 28S rRNA gene regions (Fig. 2) showing that *C. sulcatotuberculosis* and *C. aberdarensis*, the new Vietnamese specimen and the sequence of a specimen that is regarded as *Candolleomyces singeri* (A.H. SM.) D. WÄCHT. & A. MELZER form a well-supported *sulcatotuberculosis* clade (n=1,000 bootstrap).

Taxonomy

Candolleomyces eurysporus A. KARICH, E. BÜTTNER & R. ULLRICH, spec. nov.
(Figs. 2–4)

MycoBank no.: MB836196

GenBank acc. no.: MT651560

Etymology: The name refers to the rather broad spores – eurýs, ancient greek εὐρύς = broad, wide.



Fig. 3. Basidiomata of *Candolleomyces eurysporus*. Bars: 1 cm. Phot. A. KARICH.

Latin diagnosis:

Pileus usque ad 12 mm latus, conicus vel hemisphaericus, deinde applanatus, cremeus vel pallide brunneus, umide usque ad 2/3 pellucide striatus. Velum album, floccosum, usque ad medium pilei. Lamellae c. 30, mediis intervallis distantes, adnexe vel adnatae, primum pallide brunnea, acie albae, non deliquescens. Stipes usque ad 20 × 1 mm, cylindraceus, albus vel cremeus, apice pruinosis, basi tomentosus. Basidia 12–19,5 × 6,5–9 μm, clavata, 4-sporigera. Sporae (5–)5,5–7,0(–7,5) × 4–5(–6) μm, lato-ellipsoideae vel ovoideae, pallide brunneae, poro germinativo nullo. Cheilocystidia 18–25 × 9–11,5 μm, utriformia vel clavata. Pleurocystidia nulla. Caulocystidia lageniformia, subcylindrica vel utriformia, cellulis sphaeropedunculatis et clavatis immixtis. Cellulae veli cylindraceae et subhyalinae vel pallide brunneae. Fibulae praesentes. Basidiomata solitaria ad lignum emortuum.

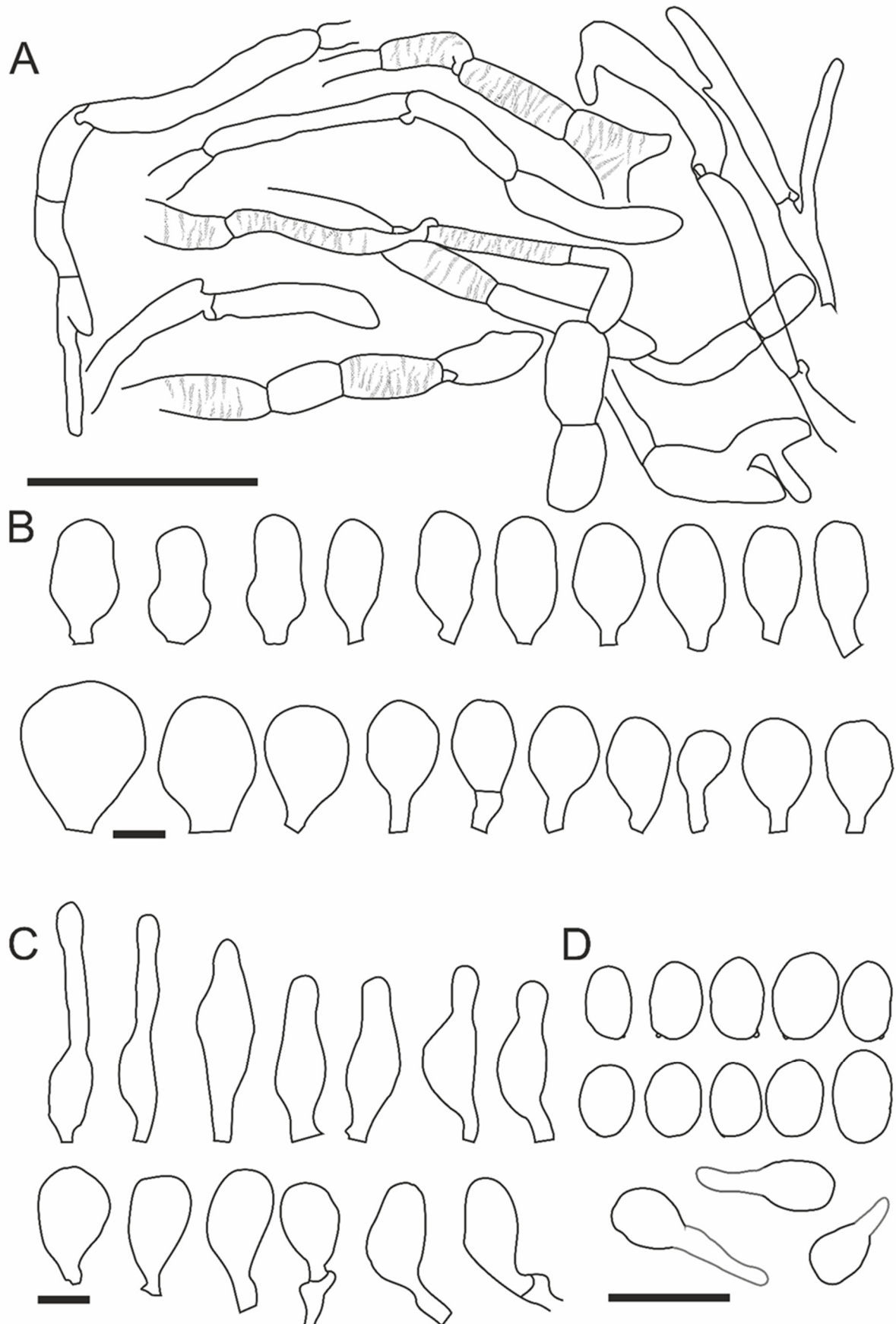


Fig. 4. Microcharacters of *Candolleomyces eurysporus*; *A* veil elements, *B* cheilo- and paracystidia, *C* caulocystidia, *D* spores incl. three germinating spores. Bars: *A* 50 µm, *B*–*D* 10 µm. Drawing by A. KARICH.

Holotypus: Cultivated in Zittau, Germany, December 13, 2018; holotype deposited as basidiomata at the Herbarium Senckenbergianum Görlitz, Germany (GLM-F126263), ex-type culture deposited at the Vietnam Type Culture Collection Hanoi under VTCC 930004. Origin: Vietnam: Ninh Binh Province: Cúc Phương National Park, 20.293500 N, 105.667528 E; approx. 267 m s.m.; November 21st 2018; leg. E. BÜTTNER.

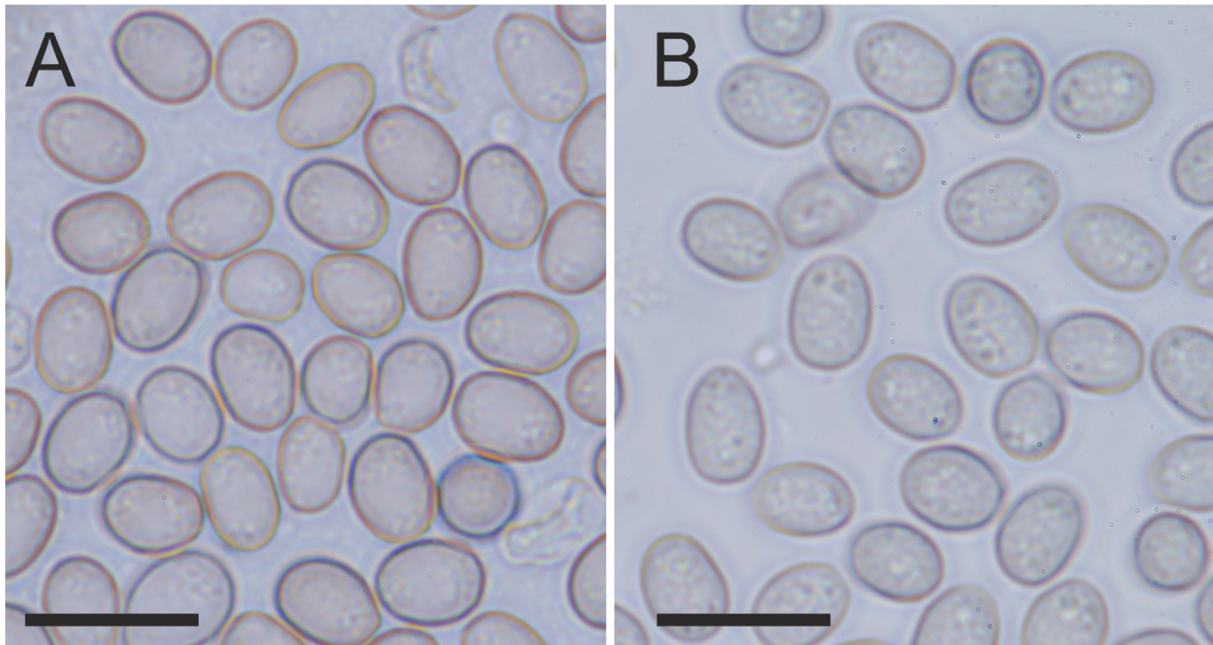


Fig. 5. Basidiospores of *Candolleomyces eurysporus* in water (A) and in 5% KOH (B), bars: 10 μm . Phot. A. KARICH.

English description:

Pileus: 6–12 mm wide, at first conical to campanulate, later semiglobate to broad conical to flattening, old with a wavy margin, at first whitish-cream (R:190 G:190 B:140) later becoming more brownish (R:180 G:180 B:120), finally darker brown with olive tinge (R:100 G:90 B:40) centre remaining brighter, translucently striate up to 2/3rd when fresh, hygrophanous, universal veil as loosely adhering but quite persistent floccules on the pileus (Fig. 3).

Lamellae: medium spaced to crowded, L=28–30, interspersed with 1–3 lamellulae, adnexed to adnate, dark-cream to brownish, finally becoming brown at age, lamellar edge white, pruinose (lens).

Stipe: up 15–20 mm \times 1 mm, cylindrical, white in the upper part, downwards somewhat brownish-creamy, tip pruinose from caulocystidia, base white tomentose.

Basidiospores: (5–)5.5–7.0(–7.5) \times 4–5(–6) μm , average 6.2 \times 4.4 μm , Q=1.2–1.6(–1.7), Q_{av}=1.4, in front view broadly ellipsoid to ovoid, in side view broadly ellipsoid and sometimes adaxially very slightly flattened, apiculus tiny, germ pore not visible; in water and ammonia solution pale brown, in KOH nearly hyaline (Figs. 4, 5); a small portion of spores (approx. 5%) in the spore print (i.e. at the stem of basidioma) germinating, with an up to 10 μm long hyphae growing of the apical part of the spore.

Basidia: 12–19.5 \times 6.5–9 μm , 4-spored, clavate.

Cheilocystidia: 18–25 × 9–11.5 µm, broadly utriform to clavate, very rare and hard to distinguish from sphaeropedunculate to clavate-pyriform cells (paracystidia), 15.8–23 × 11–16.6 µm; all marginal cells thin-walled and colourless.

Pleurocystidia: absent.

Caulocystidia: 18–43 × 8–12 µm, heteromorphic, broadly to narrowly utriform, subfusiform, lageniform, at the tip of the stem only, numerous, intermixed with sphaeropedunculate and clavate-pyriform elements, 18.5–33 × 11–16 µm.

Veil: consisting of 20–75 × 4–11 µm, cylindrical, slightly diverticulate, subhyaline to pale brown hyphae, some hyphae finely to distinctly banded incrustated.

Pileipellis and epithelium: consist of up to 40 µm wide, globose to subglobose hyaline to pale brown elements.

Clamp connections: present in veil, hymenium and mycelium.

Habit and habitat: Solitary on deadwood (fallen twigs) of broad-leaved trees. Humid and near-ground habitat.

Discussion

Candolleomyces eurysporus is characterized by its small basidiomata, its flocculose yet relatively persistent veil consisting of rather thin-walled and moderately encrusted, cylindrical hyphae as well as by its small, broad (QAV. <1.5) and pale spores without a visible germ pore.

According to DNA sequence analysis, *C. eurysporus* is closely related to *C. aberdarensis* and *C. sulcatotuberculosis*. The latter, a seemingly rare species, had been listed as a variety of *Candolleomyces typhae* (KALCHBR.) D. WÄCHT. & A. MELZER for a long time (BATTISTIN & al. 2014, MATHENY & al. 2006). Both species, however, can bear lageniform cheilocystidia and seem to be restricted to wet habitats, which is in contrast to *C. eurysporus* (BATTISTIN & al. 2014, LUDWIG, 2007).

Another closely related species is *Candolleomyces singeri* (A. H. SM.) D. WÄCHT. & A. MELZER (WÄCHTER & MELZER 2020), which was originally described as a twig-dwelling fungus from Florida (SMITH 1972). SMITH did not mention any veil and described the lamellae as crowded. The ITS sequence of a specimen collected in China and determined as *P. singeri* (*C. singeri*) was deposited in GenBank (see Tab.1) but differs from sequences of *C. eurysporus*. It is unclear, however, whether this specimen in fact represents *P. singeri* (MELZER & al. 2018). Nevertheless, all mentioned species have rather pale spores and share the same clade */sulcatotuberculosis* within *Candolleomyces* (see Fig. 4). On the other hand, *C. eurysporus* differs from other species within */sulcatotuberculosis* by smaller and more rotund spores. Obviously, there are two different veil types within */sulcatotuberculosis*. The veil of *C. aberdarensis* consists of two types of cells, i.e. (i) diverticulate, often thick-walled and brownish pigmented cells and (ii) globose elements (MELZERN & al. 2018). In contrast, the veil elements of *C. eurysporus* consist of cylindrical, subhyaline to pale brownish and - in some parts - slightly encrusted hyphae, and thus resemble the veil hyphae of *C. sulcatotuberculosis* (BATTISTIN & al. 2014).

Other species with more or less pale spores, lacking or indistinct germ pore and without pleurocystidia were already discussed in MELZER & al. (2018) but shall be mentioned briefly here again.

Psathyrella acutisquamosa DENNIS has abundant veil as pyramidal warts, equally sized (5–7 × 4–5 µm) but reddish spores (DENNIS 1961).

Psathyrella aequatoriae SINGER is a small species without veil and larger spores (7–8 × 4–5 µm) (SINGER 1978).

Psathyrella avilana DENNIS grows caespitously and terrestrially, has only fugacious veil and less broad spores (6–6.5 × 3.5–4 µm; DENNIS 1961).

Candolleomyces bivelatus (CONTU) D. WÄCHT. & A. MELZER has a different veil structure and larger, thick-walled spores (9–9.5 × 5–5.5 µm in av.; CONTU 1991, MELZER & al. 2018, SAMMUT & MELZER 2012, WÄCHTER & MELZER 2020).

Candolleomyces efflorescens (SACC.) D. WÄCHT. & A. MELZER has also equally sized and pale spores but differs by smaller cheilocystidia (12–15 × 7.5–10 µm), caespitose habit, purplish tints in the pileus and in ITS sequence (see Fig. 2; PEGLER 1977).

Psathyrella glaucescens DENNIS has olive shades on the pileus as well but differs by a larger pileus (1.5–5 cm), an only slightly translucently striate margin, fugacious veil made up of ventricose elements and by truncate spores with a porus (DENNIS 1961, PEGLER 1977).

Psathyrella pallidispора DENNIS has 8–11 × 4–5 µm large and slenderer spores, and sometimes capitate caulocystidia (DENNIS 1970).

Psathyrella pusilla PEGLER has only clavate cheilocystidia, no veil, and spores that are apically truncate by a germ pore (PEGLER 1977).

Candolleomyces subsingeri (T. BAU & J. Q. YAN) D. WÄCHT. & A. MELZER is a slightly larger species with longer and slenderer spores (Q=1.4–2.0) and differs in ITS sequence (see Fig. 2; YAN & BAU 2018).

We would like to thank BRITTA BITTNER and ULRIKE SCHNEIDER for help with lab work. The work was financially supported by the Bundesministerium für Bildung und Forschung (BMBF) - VnmDiv 031B0627 and Ministry of Science and Technology (MOST, Vietnam) - NDT.45.GER/18.

References

- BATTISTIN, E., CHIARELLO, O., VIZZINI, A., ÖRSTADIUS, L., LARSSON, E., 2014: Morphological characterisation and phylogenetic placement of the very rare species *Psathyrella sulcatotuberculosa*. – *Sydowia* **66**(2): 171–181. doi.org/10.12905/0380/sydowia66(2)2014-0171
- CHEVREUX, B., WETTER, T., SUHAI, S., 1999: Genome sequence assembly using trace signals and additional sequence information. – *Computer Science and Biology: Proceedings of the German Conference on Bioinformatics (GCB)* **99**: 45–56.
- COLLEN, B., WHITTON, F., DYER, E. E., BAILLIE, J. E., CUMBERLIDGE, N., DARWALL, W. R., POLLOCK, C., RICHMAN, N. I., SOULSBY, A.-M., BÖHM, M., 2014: Global patterns of freshwater species diversity, threat and endemism. – *Glob. Ecol. Biogeogr.* **23**: 40–51. doi.org/10.1111/geb.12096
- CONTU, M., 1991: *Psathyrella bivelata* spec. nov., une nouvelle espèce sarde de la section *Cystopsathyra*. – *Bull. Trimest. Soc. Mycol. France* **107**: 85–89.
- DE QUEIROZ, J. S., GRISWOLD, D., NGUYEN, D. T., HALL, P., 2013: Vietnam tropical forest and biodiversity assessment. – Quito Sun Mt Int Cadmus Group.
- DENNIS, R. W. G., 1970: Fungus flora of Venezuela and adjacent countries. – *Kew Bull. Add. Ser.* **III**. doi.org/10.2307/3758124
- DENNIS, R. W. G., 1961: Fungi venezuelani: IV. *Agaricales*. – *Kew Bull.* **15**: 67–ii. doi.org/10.2307/4115784
- EDGAR, R. C., 2004: MUSCLE: multiple sequence alignment with high accuracy and high throughput. – *Nucleic Acids Res.* **32**: 1792–1797. https://doi.org/10.1093/nar/gkh340
- FELSENSTEIN, J., 1985: Confidence limits on phylogenies: an approach using the bootstrap. – *Evolution* **39**(4):783–791. doi.org/10.2307/2408678

- GEYER, C. J., 1991: Markov Chain Monte Carlo Maximum Likelihood. – Interface Foundation of North America. Retrieved from the University of Minnesota Digital Conservancy. <http://hdl.handle.net/11299/58440>.
- GUINDON, S., GASCUEL, O., 2003: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. – *Syst. Biol.* **52**: 696–704. doi.org/10.1080/10635150390235520
- HOASHI, Y., 2008: *Psathyrella turcosomarginata*, a new species with cheilocystidia possessing mucoid deposits staining bluish green in ammonia solution. – *Mycoscience* **49**: 385–387. doi.org/10.1007/S10267-008-0437-1
- HUELSENBECK, J. P., RONQUIST, F., 2001: MrBayes: Bayesian inference of phylogenetic trees. – *Bioinformatics* **17**: 754–755. doi.org/10.1093/bioinformatics/17.8.754
- KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK, S., BUXTON, S., COOPER, A., MARKOWITZ, S., DURAN, C., 2012: Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. – *Bioinformatics* **28**: 1647–1649. doi.org/10.1093/bioinformatics/bts199
- KIMURA, M., 1980: A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. – *J. Molec. Evol.* **16**: 111–120. doi.org/10.1007/BF01731581
- KUMAR, S., STECHER, G., LI, M., KNYAZ, C., TAMURA, K., 2018: MEGA X: molecular evolutionary genetics analysis across computing platforms. – *Mol. Biol. Evol.* **35**: 1547–1549. doi.org/10.1093/molbev/msy096
- LUDWIG, E., 2007: Pilzkompodium. 2. Die größeren Gattungen der *Agaricales* mit farbigem Sporenpulver (ausgenommen *Cortinariaceae*). Beschreibungen Abbildungen. – Fungicon.
- MATHENY, P. B., CURTIS, J. M., HOFSTETTER, V., AIME, M. C., MONCALVO, J.-M., GE, Z.-W., YANG, Z.-L., SLOT, J. C., AMMIRATI, J. F., BARONI, T. J., 2006: Major clades of *Agaricales*: a multilocus phylogenetic overview. – *Mycologia* **98**: 982–995. doi.org/10.1080/15572536.2006.11832627
- MELZER, A., KIMANI, V. W., ULLRICH, R., 2018: *Psathyrella aberdarensis*, a new species of *Psathyrella* (*Agaricales*) from a Kenyan National Park. – *Austral. J. Mycol.* **27**: 23–30.
- NAGY, L. G., KOCSUBÉ, S., PAPP, T., VÁGVÖLGYI, C., 2009: Phylogeny and character evolution of the coprinoid mushroom genus *Parasola* as inferred from LSU and ITS nrDNA sequence data. – *Persoonia* **22**: 28–37. doi.org/10.3767/003158509X422434
- NEI, M., KUMAR, S., 2000: Molecular evolution and phylogenetics. – Oxford University Press.
- ÖRSTADIUS, L., RYBERG, M., LARSSON, E., 2015: Molecular phylogenetics and taxonomy in *Psathyrellaceae* (*Agaricales*) with focus on psathyrelloid species: introduction of three new genera and 18 new species. – *Mycol. Prog.* **14**: 25. doi.org/10.1007/s11557-015-1047-x
- PEGLER, D. N., 1977: A preliminary agaric flora of East Africa. – Kew: H. M. Stationery Office.
- PEGLER, D. N., 1983: Agaric flora of the Lesser Antilles. – Kew Bull. Add. Ser. H. M. Stationery Office.
- POYNTON, C., 2003: The CIE system of colorimetry. Digit. Video HDTV Algorithms Interfaces 211–231. doi.org/10.1016/B978-155860792-7/50087-2
- REDHEAD, S. A., VILGALYS, R., MONCALVO, J.-M., JOHNSON, J., HOPPLE Jr, J. S., 2001: *Coprinus* PERS. and the disposition of *Coprinus* species *sensu lato*. – *Taxon* **50**: 203–241. doi.org/10.2307/1224525
- RONQUIST, F., TESLENKO, M., VAN DER MARK, P., AYRES, D. L., DARLING, A., HÖHNA, S., LARGET, B., LIU, L., SUCHARD, M. A., HUELSENBECK, J. P., 2012: MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. – *Syst. Biol.* **61**: 539–542. doi.org/10.1093/sysbio/sys029
- SAMMUT, C., MELZER, A., 2012: *Psathyrellaceae* from Malta, a preliminary survey. – *Veget. Medit.* **27**: 33–44.
- SICOLI, G., PASSALACQUA, N. G., DE GIUSEPPE, A. B., PALERMO, A. M., PELLEGRINO, G., 2019: A new species of *Psathyrella* (*Psathyrellaceae*, *Agaricales*) from Italy. – *MycKeys* **58**: 129. doi.org/10.3897/mycokeys.52.31415
- SINGER, R., 1978: Interesting and new species of basidiomycetes from Ecuador. II. – *Nova Hedwigia* **29**: 1–98.
- SMITH, A. H., 1972: The North American species of *Psathyrella*. – *Mem. New York Bot. Gard.* **24**: 1–633.
- STANKE, M., STEINKAMP, R., WAACK, S., MORGENSTERN, B., 2004: AUGUSTUS: a web server for gene finding in eukaryotes. – *Nucl. Acids Res.* **32**: W309–W312. doi.org/10.1093/nar/gkh379

- VAN WAVEREN, E. K., 1985: The Dutch, French and British species of *Psathyrella*. – *Persoonia* **2**: 1–300.
- WÄCHTER, D., MELZER, A., 2020: Proposal for a subdivision of the family *Psathyrellaceae* based on a taxon-rich phylogenetic analysis with iterative multigene guide tree. – *Mycol. Prog.* **19**: 1151–1265. doi.org/10.1007/s11557-020-01606-3
- YAN, J.-Q., BAU, T., 2018: The Northeast Chinese species of *Psathyrella* (*Agaricales*, *Psathyrellaceae*). – *MycKeys* **33**: 85–102. doi.org/10.3897/mycokeys.33.24704

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Österreichische Zeitschrift für Pilzkunde](#)

Jahr/Year: 2020

Band/Volume: [28](#)

Autor(en)/Author(s): Büttner Enrico, Karich Alexander, Nghi Do Huu, Lange Maximilian, Liers Christiane, Kellner Harald, Hofrichter Martin, Ullrich Rene

Artikel/Article: [Candolleomyces eurysporus, a new Psathyrellaceae \(Agaricales\) species from the tropical Cúc Phýõng National Park, Vietnam 79-92](#)