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A remarkable and widespread new lichenicolous species of *Mycocalicium* (*Sphinctrinaceae*) producing campylidia-like conidiomata and appendiculate conidia

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Abstract. A lichenicolous fungus forming large black, vertically elongate, campylidia-like conidiomata on the thallus of *Ochrolechia* was recently collected in Austria, Mexico and the USA. The conidia are so remarkable in being multiappendiculate that initially no existing fungal genera appeared to be suitable for its description. Nevertheless, molecular phylogenetic analyses of nuITS and nuLSU sequences recovered the species within the genus *Mycocalicium*. To date, no species of *Mycocaliciales* has been reported producing appendiculate conidia. The species is described as new as *M. campylidiophorum*. The new species was also discovered in the type specimen of *Opegrapha chionographa* that was collected in Colombia 163 years ago. This discovery led us to revise *O. chionographa*, originally described as a lichen, and clarify that in fact the name applies to a lichenicolous fungus based on type material that is an admixture of *M. campylidiophorum*, an *Ochrolechia* and an *Opegrapha* species. The name is shown to apply to the *Opegrapha* species and lectotypified as such. *Opegrapha blakii* is treated as synonym of *O. chionographa*.

Key words: *Arthoniales*, fungi, *Mycocaliciales*, *Opegrapha*, phylogeny, taxonomy

Introduction

Lichenicolous fungi grow exclusively on lichens and are distributed among various taxonomic groups, with 2000 non-lichenized, obligately lichenicolous taxa accepted in 2018 worldwide (Diederich et al. 2018). New species are being described at an unprecedented rate suggesting that the real diversity is much higher than the current number of described taxa (e.g., Flakus et al. 2019; Zhurbenko & Ohmura 2020; Zhurbenko et al. 2020; Ertz et al. 2021; Zhurbenko 2021; Diederich et al. 2022a, b; Freire-Rallo et al. 2023).

Many taxa of lichenicolous fungi are known only from the asexual morphs. The classification of these based on morphology alone is often uncertain due to the absence or infrequent co-occurence of sexual morphs and the difficulties in establishing a clear relationship between these two states (e.g., Hawksworth 1979; Tibell 1990; Pérez-Ortega et al. 2011; Muggia et al. 2017). In recent years, the connection between anamorph- and teleomorph-typified taxa of lichenicolous fungi has been successfully demonstrated by DNA-based studies in various taxonomic groups including the connection of *Vouauxiomyces* Dyko & D. Hawksw. with *Abrothallus* De Not. (*Abrothallaceae*, Pérez-Ortega et al. 2011; Suija et al. 2015, 2018), *Phaeosporobolus* D. Hawksw. & Hafellner with *Lichenostigma* Hafellner (*Phaeococcomycetaceae*, Ertz et al. 2014), *Lichenodiplis* Dyko & D. Hawksw. with some *Muellerella*-like teleomorphs (*Chaetothyriales* inc. sedis, Muggia et al. 2015), *Sclerococcum* Fr. with *Dactylospora* Körb. (*Dactylosporaceae*, Diederich et al. 2018), and *Asteroglobulus* Brackel and *Cornutispora* Piroz. with *Spirographa* Zahlbr. (*Spirographaceae*, Flakus et al. 2019). However, the phylogenetic affinity of many genera of lichenicolous coelomycetes and hyphomycetes is still unresolved due to the lack of molecular data for a high percentage of described species (Diederich et al. 2018).

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The subclass *Mycocaliciomycetidae* (*Eurotiomycetes*) with its single order *Mycocaliciales* includes lichenicolous and saprobic fungi having disciform, stipitate or sessile ascomata that are at least in part sclerotized and forming unitunicate and cylindrical asci containing eight ascospores with a pigmented wall (Hibbett et al. 2007). Tibell & Wedin (2000) included two families in the order *Mycocaliciales*: *Mycocaliciaceae* that comprised species with active ascospore dispersal not producing mazaedia and *Sphinctrinaceae* that comprised species with ascospores forming a moderately developed mazaedium. However, Jaklitsch et al. (2016) treated *Mycocaliciaceae* as a synonym of *Sphinctrinaceae* because the separation into these two families was not supported by molecular data, since *Sphinctrina* was found to be nested within members of *Mycocaliciaceae* (e.g., Tibell & Vinuesa 2005; Prieto et al. 2013; Tuovila et al. 2013) and because of their shared morphological characteristics.

In the years 2015–2018, several of the authors (A.H., H.K., J.L.) encountered a very unusual lichenicolous coelomycete on *Ochrolechia* species in Austria, Mexico and the USA. The same species was also discovered by D.E. in the type specimen of *Opegrapha chionographa* Nyl. collected in Colombia and described over a century ago. The morphology of the conidiomata and conidia did not fit any known lichenicolous fungal genus, such that molecular data were used to resolve its systematic position. Here, we provide the description of the material as a new species and establish its phylogenetic position within the genus *Mycocalicium*.

Material and methods

Morphological study

Voucher specimens are deposited in the herbaria BR, CANL, GZU, NY, PC and MEXU. The macroscopic characteristics were studied and measured using a Leica MZ7.5 dissecting microscope. Macroscopic photographs were taken using a Canon 6D camera, Nikon BD Plan 10 objective, and StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field; or with a Keyence VHX-5000 Digital Microscope and a VH-Z20R/W/T lens; or a Canon EOS 60D camera, Canon macro photo lens MP-E 65 mm fixed on a Novoflex focusing rack. Hand-cut sections and squash preparations of the conidiomata were mounted in water, 5% KOH, Phloxine B, Congo Red, Lactophenol Cotton Blue and Lugol's iodine solution, and studied under Leica DMLB and Zeiss Axioscope 40 compound microscopes. The size of the conidiogenous cells, conidia and conidial appendages was measured in water, and the average ($\overline{X}$) and standard deviation (SD) calculated. These measurements are given as $\overline{X} \pm SD$, surrounded by the extreme values (between parentheses), followed by the number of measurements (N).

Molecular techniques

Well-preserved herbarium specimens that were eight months old (specimens from USA), 3 years old (specimens from Mexico) and 5 years old (specimen from Austria) were used for DNA isolation. Hand-cut sections of conidiomata were used for direct PCR as described in Ertz et al. (2015). The material was placed directly in microtubes with 20 µl H_2O. Amplification reactions were prepared for a 50 µl final volume, as detailed in Ertz et al. (2018). The nuITS rDNA (ITS1 + 5.8 S + ITS2) was amplified for all specimens using primers ITS1F and ITS4 (White et al. 1990), and the nuLSU rDNA was amplified for specimens Lendemer 45240, 45253 using primers LIC15R (Miadlikowska et al. 2002) and LR6 (Vilgalys & Hester 1990). The PCR cycling conditions for the nuITS consisted of the following steps: (1) 10 min at 95°C; (2) 35 cycles of 45 s at 95°C, 1 min at 52°C, 75 s at 72°C, and (3) 10 min of final elongation at 72°C, while those for the nuLSU consisted of: (1) 10 min at 95°C; (2) 25 cycles of 45 s at 95°C, 40 s at 52°C, 150 s at 72°C; (3) 14 cycles of 45 s at 95°C, 40 s at 52°C, 150 s at 72°C (+ 5 s per cycle), and (4) 10 min of final elongation at 72°C. Both strands were sequenced by Macrogen® using the amplification primers, and with the additional primers LR3, LR3R, LR5 and LR5R for nuLSU (Vilgalys & Hester 1990). Sequence fragments were assembled with Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to 'megablast' searches to verify their closest relatives and to detect potential contaminations.

Taxon selection and phylogenetic analyses

Two matrices were assembled: first a two-locus dataset of nuLSU and nuITS sequences for placing the newly sequenced taxa in a phylogeny of the order *Mycocaliciales*, and a second dataset of nuITS sequences for providing a detailed phylogeny of *Mycocalicium* s.str.

The closest relatives of the new sequences based on megablast searches were retrieved from GenBank. Additional taxa were selected mainly from Tibell & Vinuesa (2005) and Tuovila et al. (2013), with others notably from Vinuesa et al. (2001), Tuovila et al. (2011a), Prieto et al. (2013), Crous et al. (2016), Beimforde et al. (2017) and Thiyagaraja et al. (2022) in order to include a wide array of taxa belonging to the *Mycocaliciales*. The type species of *Chaenothecopsis*, *C. rubescens*, was not included in the phylogenetic analyses because the only sequence available on GenBank for that species (the unpublished nuITS OQ717807) was difficult to align with those of all other *Sphinctrinaceae*, such that a confirmation of it is needed. The sequences of taxa listed in Table 1 were aligned using MAFFT v.7.505 (Katoh et al. 2002) on the CIPRES Web Portal (Miller et al. 2010) and manually corrected for errors using Mesquite 3.04 (Maddison & Maddison 2015). Terminal ends of sequences, ambiguously aligned regions, and introns were delimited manually and excluded from the datasets.

The resulting matrix of *Mycocaliciales* consisted of 45 terminals and 1461 (1044 for nuLSU and 417 for nuITS) unambiguously aligned sites, while the matrix of *Mycocalicium* s.str. consisted of 37 terminals and 504 unambiguously aligned sites. Three species of *Pyrenulales*, viz. *Pyrenula aspistea* (Ach.) Ach., *P. nitida* (Weigel) Ach. and *Pyrgillus javanicus* (Mont. & Bosch) Nyl. were

Table 1. Species names, voucher specimens and GenBank Accession numbers. The GB Accession numbers of the sequences generated in this study are in bold.

Species	Voucher	ITS	LSU
Brunneocarpos banksiae	CBS 141465	NR_147648	NG_066277
Chaenothecopsis consociata	Tibell 22472	AY795851	DQ008999
Chaenothecopsis debilis	Tibell 16643 (UPS)	AY795852	AY795991
Chaenothecopsis diabolica	Tuovila 06-035 (H)	NR_120164	JX119118
Chaenothecopsis dolichocephala	Tibell 19281 (UPS)	AY795854	AY795993
Chaenothecopsis fennica	Tibell 16024 (UPS)	AY795857	AY795995
Chaenothecopsis golubkovae	Titov 6707	AY795859	AY795996
Chaenothecopsis haematopus	Tibell 16625 (UPS)	AY795861	AY795997
Chaenothecopsis khayensis	JR 04G058 (H)	NR_120165	HQ172895
Chaenothecopsis montana	Tuovila 07-086 (H)	JX119105	JX119114
Chaenothecopsis nigripunctata	Tuovila 06-013 (H)	JX119103	JX119112
Chaenothecopsis pallida	JR 010652 (H)	JX122779	JX122781
Chaenothecopsis pusiola	Tibell 15884 (UPS)	AY795865	–
Chaenothecopsis resinophila	JR 000424 (H)	JX122780	JX122782
Chaenothecopsis savonica	Tibell 15876 (UPS)	AY795868	AY796000
Chaenothecopsis schefflerae	Rikkinen 13183	KY499965	KY499967
Chaenothecopsis sitchensis	Tuovila 06-033 (H)	JX119102	JX119111
Chaenothecopsis subparoica	Tretiach (hb. Tretiach)	AY795869	–
Chaenothecopsis tsugae	JR 07005B (H)	JX119104	JX119113
Chaenothecopsis viridialba	Wedin 6728 (UPS)	JX000103	AY853365
Chaenothecopsis viridireagens	Tibell 22803 (UPS)	AY795872	DQ013257
Cryptocalicium blascoi	Etayo 30875	MW999969	MW999951
Fusichalara minuta	CBS 709.88	KX537754	KX537758
Mycocalicium albonigrum 1	Tibell 19038	AF223966	AY796001
Mycocalicium albonigrum 2	UPSC 2087	AF223967	–
Mycocalicium albonigrum 3	UPSC 2088	AF223968	–
Mycocalicium albonigrum 4	UPSC 2089	AF223969	–
Mycocalicium americanum	Kalb & Nash (UPS)	AY795879	–
Mycocalicium campylidiophorum	Lendemer 45240 (NY)	**OR405878**	**OR416199**
Mycocalicium campylidiophorum	Lendemer 45253 (NY)	**OR405879**	**OR416200**
Mycocalicium campylidiophorum	Huereca 774 (CANL)	**OR405880**	**–**
Mycocalicium campylidiophorum	Huereca 775 (CANL)	**OR405881**	**–**
Mycocalicium campylidiophorum	Komposch 9030 (GZU – holotype)	**OR405882**	**–**
Mycocalicium hyaloparvicellulum	MFLUCC 14-0169	KR920004	–
Mycocalicium subtile 1	Tibell 21020	AF225445	AY796003
Mycocalicium subtile 2	Tibell 16388	AF225438	–
Mycocalicium subtile 3	yuk36b	MW248456	–
Mycocalicium subtile 4	UPSC 1839	AF225429	–
Mycocalicium subtile 5	Tibell 16207	AF225437	–
Mycocalicium subtile 6	Hermansson 3850	AF225435	–
Mycocalicium subtile 7	Tibell 20539	AF225443	–
Mycocalicium subtile 8	UPSC 1904	AF225431	–
Mycocalicium subtile 9	Tibell 17361	AF225439	–
Mycocalicium subtile 10	BIOUG24047-F03	KT695406	–
Mycocalicium subtile 11	Tibell 19319	AF225441	–
Mycocalicium subtile 12	Tibell 20093	AF225442	–
Mycocalicium subtile 13	UPSC 2504	AF225433	–
Mycocalicium subtile 14	Tibell 21003	AF225444	–
Mycocalicium subtile 15	Selva 6747	AF225436	–
Mycocalicium subtile 16	Vinuesa 1	AF225427	–
Mycocalicium subtile 17	Hermansson 3832	AF225434	–
Mycocalicium subtile 18	Goward 1161	AF225428	–
Mycocalicium subtile 19	Tibell 17913	AF225440	–
Mycocalicium aff. *subtile* 1	UPSC 2173	AF225432	–
Mycocalicium aff. *subtile* 2	UPSC 1896	AF225430	–
Mycocalicium victoriae	Boom 21	AF243135	–
Mycocalicium sp. 1	Tibell 17604 (UPS)	AF243133	–
Mycocalicium sp. 2	Goward 975	AF243134	–
Paecilomyces niveus	CBS 100.11	FJ389934	AY176750
Penicillium limosum	CBS 339.97	GU981568	EF411064

Table 1. Continued.

Species	Voucher	ITS	LSU
Phaeocalicium curtisii	BIOUG24047-F02	KT695401	–
Phaeocalicium polyporaeum	ZW-Geo60-Clark	AY789363	AY789362
Phaeocalicium populneum	Tibell 19286 (UPS)	AY795874	AY796009
Phaeocalicium praecedens	Tuovila 09-240 (TUR)	KC590481	KC590486
Pyrenula aspistea	GW1042	JQ927450	EF411063
Pyrenula nitida	F 5929	JQ927458	DQ329023
Pyrgidium montellicum 1	Cáceres & Aptroot 11449	ON979667	OP077215
Pyrgidium montellicum 2	MFLU 21-0135a	ON979674	ON979678
Pyrgillus javanicus	AFTOL-ID 342	DQ826741	DQ823103
Rhopalophora clavispora	CBS 129.74	KX537751	MH872573
Sphinctrina leucopoda	Kalb 33829 (hb. Kalb)	AY795875	AY796006
Sphinctrina turbinata	Tibell 22478 (UPS)	AY795876	AY796007
Stenocybe pullatula	Tibell 17117 (UPS)	AY795878	AY796008

used as the rooting taxa in the *Mycocaliciales* dataset, based on the phylogeny of *Eurotiomycetes* presented in Prieto et al. (2021). For the *Mycocalicium* s.str. dataset, three species of *Sphinctrinaceae*, viz. *Phaeocalicium populneum* (Duby) A.F.W. Schmidt, *P. praecedens* (Nyl.) A.F.W. Schmidt and *Stenocybe pullatula* (Ach.) Stein, were selected to root the tree based on the phylogeny obtained from the *Mycocaliciales* dataset assembled in the current study.

Best-fit evolutionary models were estimated using Akaike Information Criterion (AIC) as implemented in jModelTest v. 2.1.10 (Darriba et al. 2012). For the matrix of *Mycocaliciales*, the TrN+I+G model was selected for the nuLSU dataset and the TIM2ef+I+G model was selected for the nuITS dataset. For the dataset of *Mycocalicium* s.str., the TIM2ef+I+G model was selected.

Analyses for topological incongruence among loci were carried out for the two-locus dataset of the *Mycocaliciales*. The six taxa for which nuLSU sequences were not available were first removed from the nuITS dataset in order to analyze both datasets having the same 39 terminals. The single locus datasets were analyzed with a Maximum Likelihood (ML) approach using the program RAxML v.8.2.12 (Stamatakis 2014) on the CIPRES Web Portal (Miller et al. 2010) with 1,000 ML bootstrap iterations (ML-BS). The GTRGAMMA model was used, and node support was assessed running 1,000 bootstrap replicates. We analyzed the two single locus datasets for their topological incongruence by assuming a conflict significant, when two different relationships (one being monophyletic and the other being non-monophyletic) for the same set of taxa were both supported with bootstrap values ≥ 70% (Mason-Gamer & Kellogg 1996; Reeb et al. 2004). Based on this criterion, no conflict was detected and the nuLSU and nuITS datasets were concatenated.

Bayesian analyses were carried out on the two-locus dataset under the selected models for two partitions (nuLSU, nuITS) and using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes v. 3.2.7a (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) on the CIPRES Web Portal (Miller et al. 2010). Two parallel MCMCMC runs were performed, each using four independent chains and 20 million generations, sampling trees every 1,000th generation. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the 30,002 post-burnin trees of the 40,002 trees sampled by the two MCMCMC runs using the sumt option of MrBayes. Similarly, a Bayesian analysis was carried out on the single locus dataset of *Mycocalicium* s.str. using the same settings as for the *Mycocaliciales* dataset. Convergence between runs were verified using the PSRF (Potential Scale Reduction Factor), where values were all equal or close to 1.000.

In addition, a Maximum Likelihood (ML) analysis was performed on the two locus dataset of *Mycocaliciales* and on the single locus dataset of *Mycocalicium* s.str. using RAxML v.8.2.12 (Stamatakis 2014) on the CIPRES Web Portal (Miller et al. 2010) with 1,000 ML bootstrap iterations (ML-BS) and the GTRGAMMA model. The two-locus dataset of *Mycocaliciales* was divided into two partitions (nuLSU and nuITS).

The ML trees did not contradict the Bayesian tree topologies for the strongly supported branches. Therefore, only the ML trees are shown with the ML-BS values added above or near the internal branches. Internodes with ML-BS ≥ 70 and PP ≥ 0.95 were considered to be significant and represented by thicker lines (Figs 1 & 2). Phylogenetic trees were visualized using FigTree v.1.4.2 (Rambaut 2012).

Results

Phylogenetic analysis

Seven new sequences (two nuLSU and five nuITS) were obtained for this study (Table 1). The RAxML tree obtained from the combined two-locus analysis of the *Mycocaliciales* dataset is shown in Fig. 1. The main well-supported lineages were in accordance with the results obtained by Tibell & Vinuesa (2005) and Tuovila et al. (2013). The order *Mycocaliciales* was strongly supported, but the nodes of the backbone of the *Mycocaliciales* clade were mainly poorly supported. The genera *Chaenothecopsis*, *Mycocalicium* and *Phaeocalicium* were recovered as polyphyletic. The new species, represented by two terminals, was nested in a strongly supported clade together with

M. albonigrum, *M. americanum* and *M. subtile*. The latter being the type species of the genus, this clade is considered here as *Mycocalicium* s.str.

The RAxML tree obtained from the analysis of the nuITS dataset of *Mycocalicium* s.str. is shown in Fig. 2. The five terminals of the new species formed a strongly supported clade, sister to *Mycocalicium subtile*, but with low support. They were also closely related to two terminals named here '*M.* aff. *subtile*'. The specimens of these two terminals were originally identified as *M. subtile*, but eventually considered to represent a morphologially cryptic, undescribed taxon by Vinuesa et al. (2001), because of their nuITS sequences that differed considerably from those of the majority of *M. subtile*. Therefore, we named these two specimens *M.* aff. *subtile* in our phylogenetic tree (Fig. 2).

Little genetic variation exists between the five nuITS sequences obtained from the new species. The sequences from the USA and Austria are identical, while the two sequences from Mexico are identical to each other, but differ from the previous ones by two transitions (T-C and C-T). The overall low levels of nuITS sequence divergence support the conclusion that our material should be treated as a single species.

Mycocalicium hyaloparvicellulum Daranag. & K.D. Hyde was nested within *M. subtile* suggesting that it is conspecific with the latter.

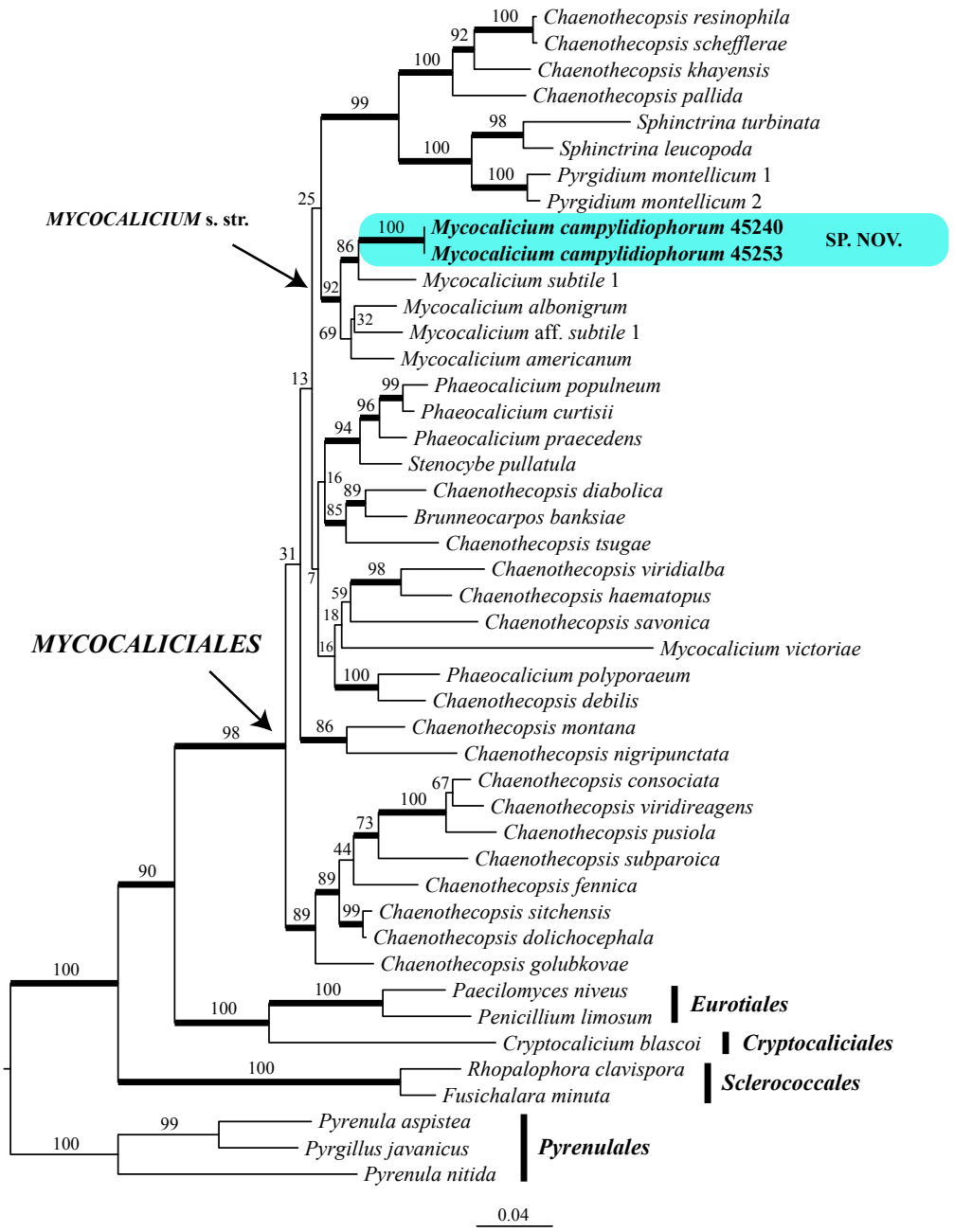

Figure 1. Phylogeny of *Mycocaliciales* (with eight species of other orders as outgroup) based on a data set of nuLSU and nuITS sequences, and that resulted from a RAxML analysis. Maximum Likelihood bootstrap values are shown near internal branches. Internal branches considered as strongly supported by the Bayesian and RAxML analyses are represented by thicker lines. The newly sequenced samples are in bold, and their names followed by collecting numbers of authors, which act as specimen and sequence identifiers. The lineage corresponding to the new species is highlighted.

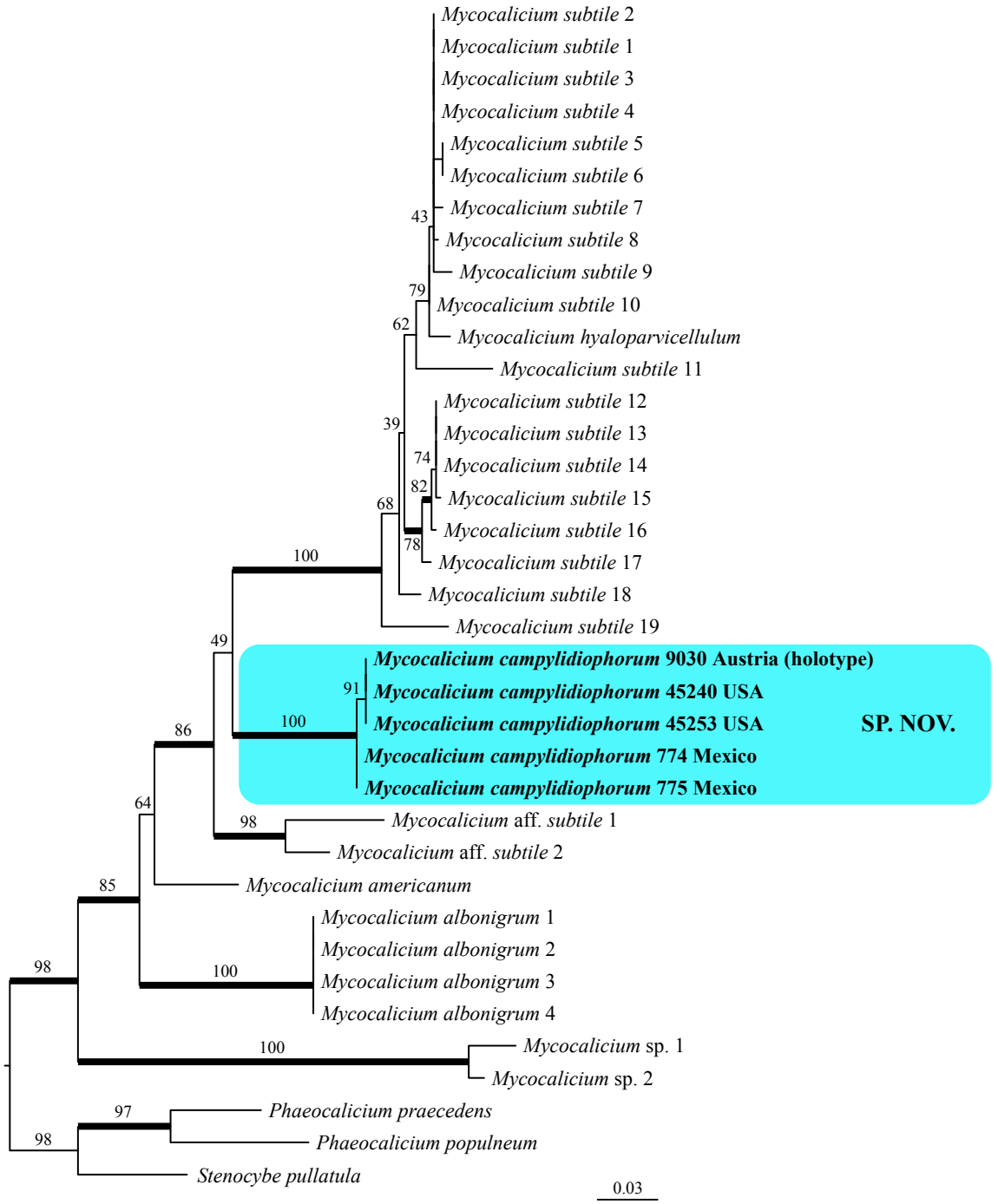

Figure 2. Phylogeny of the genus *Mycocalicium* (with two species of *Phaeocalicium* and *Stenocybe pullatula* as outgroup) based on a data set of nuITS sequences, and that resulted from a RAxML analysis. Maximum Likelihood bootstrap values are shown near internal branches. Internal branches considered as strongly supported by the Bayesian and RAxML analyses are represented by thicker lines. The newly sequenced samples are in bold, and their names followed by collecting numbers of authors, which act as specimen and sequence identifiers, and by the country of collection. The lineage corresponding to the new species is highlighted.

Taxonomy

Mycocalicium campylidiophorum Ertz, Komposch, Huereca, Lendemer & Diederich, sp. nov. (Figs 3–5)

MycoBank MB 849744

Diagnosis: Characterized by large, black, vertically elongate, often laterally flattened, campylidia-like pycnidia, irregularly opening in the upper part, frequently branched conidiophores, holoblastic, elongate ampulliform, percurrently proliferating conidiogenous cells, and brown, aseptate conidia, (4.5–)5–5.5(–6.5) µm diam., (5–)5.5–6(–6.5) µm tall, with one apical and three lateral hyaline, filiform appendages, often with an additional, small, hyaline to brown basal appendage; distinguished from the asexual stage of other *Mycocalicium* species, and from all other known coelomycetous fungi by the campylidia-like conidiomata and the appendiculate conidia.

Type: Austria, Steiermark, Nordalpen, Steirisches Salzkammergut, Mitterndorfer Becken, ~2.17 km SSW Bad Mitterndorf, Hinterberg, 400 m N des Mündungsbereichs der Salza in den Salzastausee, 47°32′8.9″N, 13°55′42.5″E (±5 m, WGS 84), 775 m elev., Grauerlen-Ufergehölzstreifen, auf Lager von

Ochrolechia arborea auf absterbender, glatter Stammborke einer jüngst umgefallenen *Alnus incana* (ehemals Kronenbereich, 10 m hoch), 18 April 2016, H. Komposch 9030 (GZU – holotype!; BR – isotype!).

Description. Sexual stage unknown. Conidiomata pycnidial, black, surface smooth, glossy, first immersed in the host thallus, later superficial, initially subspherical, elongating vertically, often becoming laterally flattened, 350–500 µm diam., 500–800 µm tall, without distinct ostiole, wall splitting in the upper third or half through irregular cracks, eventually conidiomatal wall bending down at the upper edge, resulting in campylidium-like conidiomata with a partly exposed conidiogenous layer, outer conidiomatal layer sometimes covered by conidia when mature. Conidiomatal wall 20–35 µm thick, made of pale to medium brown, densely interwoven and closely packed hyphae, 3–6 µm thick, cell wall gelatinous, 0.8–1.2 µm thick; inner layer in the upper part made of subhyaline, loose, branched, thin-walled hyphae, 1.5–2 µm thick, in the lower part representing the conidiogenous layer. Conidiophores arising from the inner wall of the lower half of the pycnidial cavity, septate, cells cylindrical or irregularly swollen, often branched, 1.5–2.5 µm thick. Conidiogenous cells lateral or terminal, holoblastic, elongate ampulliform with a long narrow neck, percurrently proliferating with up to 8 annellations, usually hyaline, more rarely pale to medium brown when mature, smooth-walled, (9.5–)11–16(–18.5) × (1.5–)2–2.5(–3) µm (N = 18). Conidia acrogenous, dry, arising singly, aseptate, hyaline when young, becoming brown when mature, with four filiform, hyaline appendages, including three lateral appendages a little above the conidial base and one apical appendage, and one additional, minuscule, hyaline to brown, basal appendage (where the conidium was attached to the conidiogenous cell); from above, conidia look like a curved triangle with a constant width (a 'Reuleaux triangle'; rotational symmetry of order 3); in side-view, with one lateral appendage directed towards the observer, conidia look like kites with curved edges (lower triangle smaller than upper; bilateral symmetry); conidia without appendages (4.5–)5–5.5(–6.5) µm diam. (N = 31), (5–)5.5–6(–6.5) µm tall (N = 22); hyaline appendages (3–)4–6(–7.5) µm long (N = 50), 0.6–1 µm thick; basal appendage 1–1.5 µm diam., up to 0.8 µm tall, often indistinct.

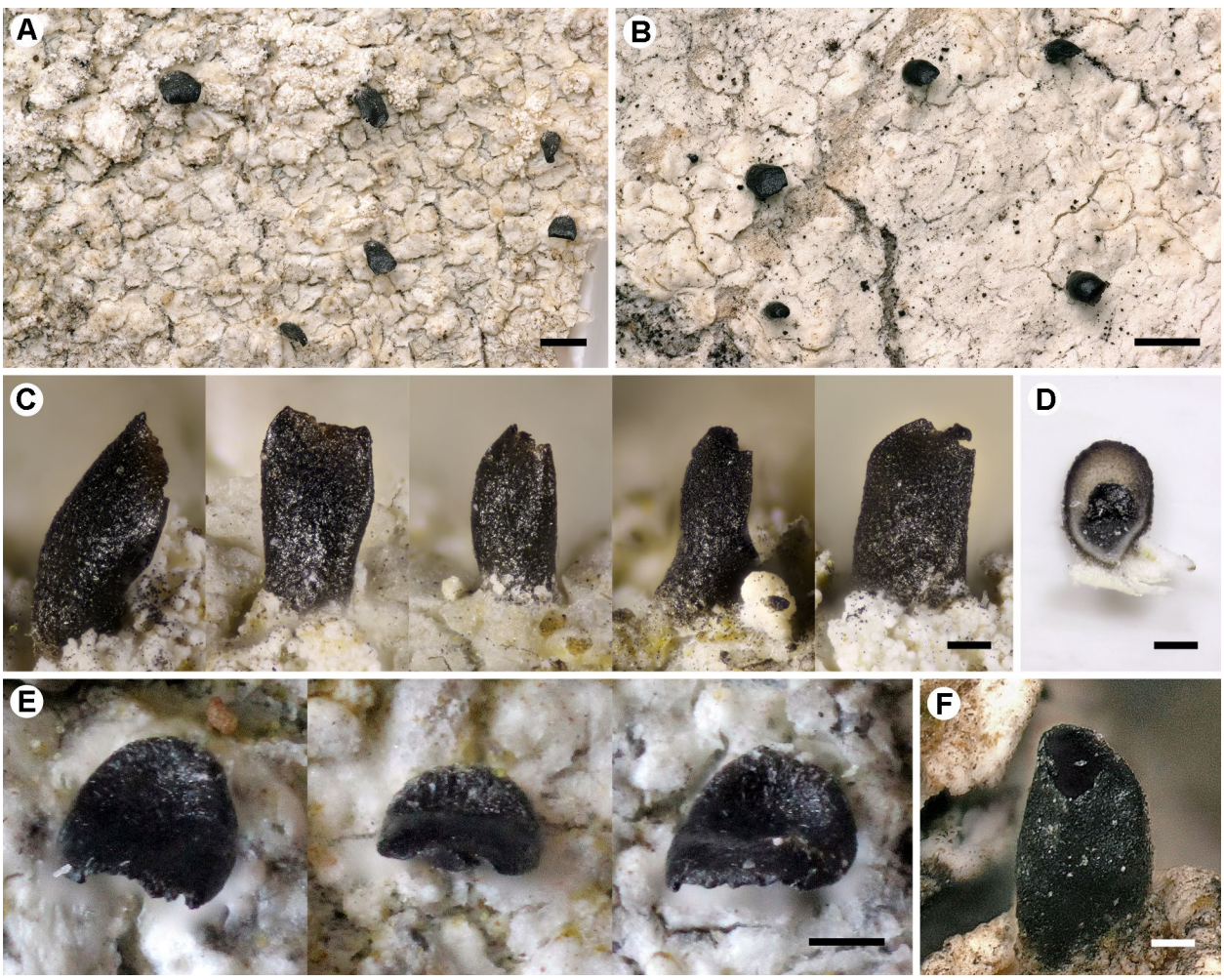

Figure 3. *Mycocalicium campylidiophorum* (A, C–E – holotype; B – Lendemer 45240; F – Huereca 774). A–B – thallus of *Ochrolechia arborea* with lichenicolous conidiomata; C – mature conidiomata with an irregular apical opening releasing conidia; D – section through conidioma, showing the interior cavity filled with brown conidia; the upper conidiomatal wall appears thicker, as the section did not pass through the center of the conidioma; E – older, laterally flattened, campylidia-like conidiomata; F – mature conidioma of *M. campylidiophorum* growing on an apothecium of *Ochrolechia subpallescens*. Scales: A–B = 500 µm; C–E = 200 µm; F = 100 µm.

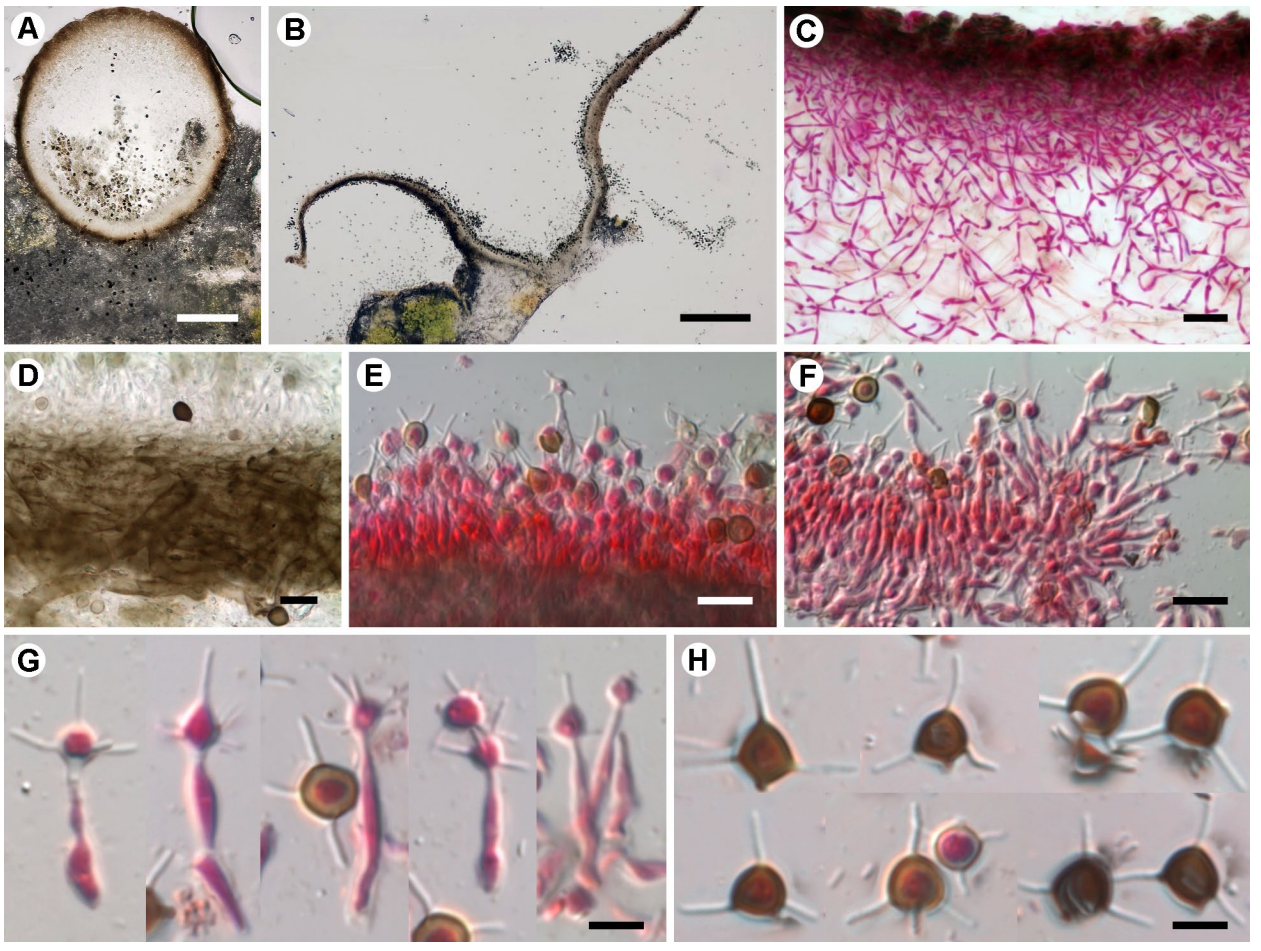

Figure 4. *Mycocalicium campylidiophorum*, holotype. A – section through young pycnidioid conidioma, showing the conidiogenous layer restricted to the lower half; B – section through old campylidioid conidioma (diverging of walls is an artifact of microscopical examination); C – section through sterile conidiomatal wall (in the upper part of the conidioma), showing an outer layer of dense brown hyphae, and an inner layer of loose, branched, hyaline hyphae; D – section through lower conidiomatal wall (below conidiogenous layer), showing thick, brown, interwoven hyphae; E – layer of conidiogenous cells with conidia; F – the same, after pressure on the cover glass; G – conidiogenous cells with conidia; H – mature conidia, with one apical and three lateral hyaline, filiform appendages, the two on the top left with a distinct, brown, basal appendage. A–B: in water; D: in 5% KOH; C, E–H: in a mixture of 5% KOH, Phloxine B and Congo red. Scales: A = 100 µm; B = 200 µm; C = 20 µm; D–F = 10 µm; G–H = 5 µm.

Distribution and ecology. Known from Austria, Colombia, eastern USA and northeastern Mexico. Lichenicolous on the genus *Ochrolechia*. In the USA, it was found on *Ochrolechia arborea* growing on *Acer* and on a *Pinus banksiana* branch in a bog dominated by *Pinus banksiana* with additional hardwoods (*Acer*, *Betula*, *Populus*, *Salix*) and conifers (*Abies*, *Larix*, *Picea*). In Mexico, it was found on *O. subpallescens* growing on *Pinus hartwegii* and on an unidentified sterile *Ochrolechia* growing on *Abies vejarii* in forests with cold-temperate climate at elevations between 2,975–3,350 m. In Colombia, it grew at 2,600 m on an unidentified species of *Ochrolechia*, together with the lichenicolous *Opegrapha chionographa*. In Austria, the fungus was found on *O. arborea* growing on *Alnus incana*.

Etymology. The epithet of the new species refers to the pycnidia resembling campylidia-like conidiomata.

Notes. *Mycocalicium campylidiophorum* is easily recognizable by its black, vertically elongate, often laterally flattened, campylidia-like pycnidia, irregularly opening in the upper part, and brown, aseptate, appendiculate conidia. Zhurbenko et al. (2018) compiled all the lichenicolous fungi species that have been reported exclusively or predominantly from *Ochrolechia*, with no mention of a species with a similar morphology. The only other lichenicolous species growing on *Ochrolechia* and forming pycnidial conidiomata with somewhat appendiculate-like conidia are species of *Spirographa*, but the conidia are then Y-shaped (Zhurbenko et al. 2018, sub '*Cornutispora*'). Moreover, phylogenetic analyses place the genus *Spirographa* (including *Cornutispora*) within the *Ostropales* in *Lecanoromycetes* (Flakus et al. 2019), while *Mycocalicium* belongs to the *Mycocaliciales* in *Eurotiomycetes*.

Only three lichenicolous species have previously been recognized in the genus *Mycocalicium*, viz. *M. chiodectonicola* Aptroot & Etayo, *M. enterographicola* Aptroot & M. Cáceres and *M. rapax* Tibell. *Mycocalicium rapax*, like *M. campylidiophorum*, is the only one that grows on a host belonging to *Pertusariales*, but its host, *Lepra leonina* (Stizenb.) I. Schmitt, B.G. Hodk. & Lumbsch grows on rock in South Africa. *Mycocalicium rapax* is also very different from the new species by having large and sturdy apothecia (0.7–1 mm high, with capitulum 0.3–0.59 mm wide), and more importantly, it forms dark

patches or crescent-shaped zones on the host thallus and is not accompanied by an asexual stage (Tibell 2001), unlike *M. campylidiophorum*. *Mycocalicium chiodectonicola* and *M. enterographicola* were both described from South America on hosts belonging to *Arthoniales* (Aptroot et al. 2016; Etayo & Aptroot 2017). They are very different from *M. campylidiophorum* in having very tiny ascomata (up to 0.1 mm high) producing ornamented ascospores, or green-pruinose ascomata, while no asexual stage has been reported.

Additional specimens examined. COLOMBIA. Lamesa, 2600 m elev., 1860, Lindig 872 (PC0146194 – lectotype of *Opegrapha chionographa*; see below). MEXICO. Nuevo Leon, General Zaragoza, Peña Nevada mountain, small plateau between Picacho San Onofre and Peña Nevada, 23°47′12″N, 99°51′28″W, 3350 m elev., mixed conifer forest dominated by *Pinus hartwegii* and *Pseudotsuga menziensii*, on *Ochrolechia subpallescens* on *Pinus hartwegii*, 26 July 2018, A. Huereca 774, 775 (CANL), 776 (MEXU); Tamaulipas, Miquihuana, Cerro El Nacimiento, trail to the summit, 23°37′53″N, 99°45′42″W, 2975 m elev., conifer forest dominated by *Abies vejarii* and *Pseudotsuga menziensii* with moss understory, on sterile *Ochrolechia* sp., intermixed with *Stenocybe major* on *A. vejarii*, 07 Nov. 2020, A. Huereca 622 (CANL). USA. Michigan, Chippewa county, Hiawatha National forest, FS3343 1.5 mi E of jct w/ MI-123, 1.9 mi NE of Trout Lake, 4.3 mi NW of Old Dick, 46°12′52″N, 84°53′23″W, 860 ft., bog dominated by *Pinus banksiana* with additional hardwoods (*Acer*, *Betula*, *Populus*, *Salix*) and conifers (*Abies*, *Larix*, *Picea*), on *O. arborea* on *Acer*, 22 May 2015, J.C. Lendemer 45240 (NY); ibid., on *O. arborea* on *Pinus banksiana* branch, 22 May 2015, J.C. Lendemer 45253 (NY).

Opegrapha chionographa Nyl., Acta Soc. Sci. Fenn. 7: 475. 1863.

≡ *Melanographa chionographa* (Nyl.) Müll. Arg., Flora, Regensburg 65: 516. 1882. ≡ *Melaspilea chionographa* (Nyl.) Zahlbr., Cat. Lich. Univers. 2: 271. 1923 [1924].

Type: Colombia [Nova Granata], Lamesa, 2600 m elev., coll. Lindig 872, 1860 (PC0146194 – lectotype!, designated here on the ascomata of the opegraphoid lichenicolous fungus, MTB 10014767; PC0146195 – isolectotype!).

= *Opegrapha blakii* Ertz & Diederich, Bot. J. Linn. Soc. 144: 239. 2004, syn. nov.

Type: Venezuela, Tachira, distr. Jauregui, bei El Hato, zwischen Bailadores und Pregonero, 8°05′N, 71°55′W, 2750 m elev., K. & A. Kalb 29389, 13 August 1989 (hb. Kalb – holotype!; BR – isotype!).

Notes. The discovery of *M. campylidiophorum* growing on the thallus of *O. chionographa* in the lectotype specimen of the latter was surprising because the latter was originally described as a lichen in the order *Arthoniales* (Nylander 1863). *Opegrapha chionographa* was combined in the genus *Melanographa*, a genus described to accomodate *Opegrapha* species having brown ascospores, and ultimately in the genus *Melaspilea*. The presence of

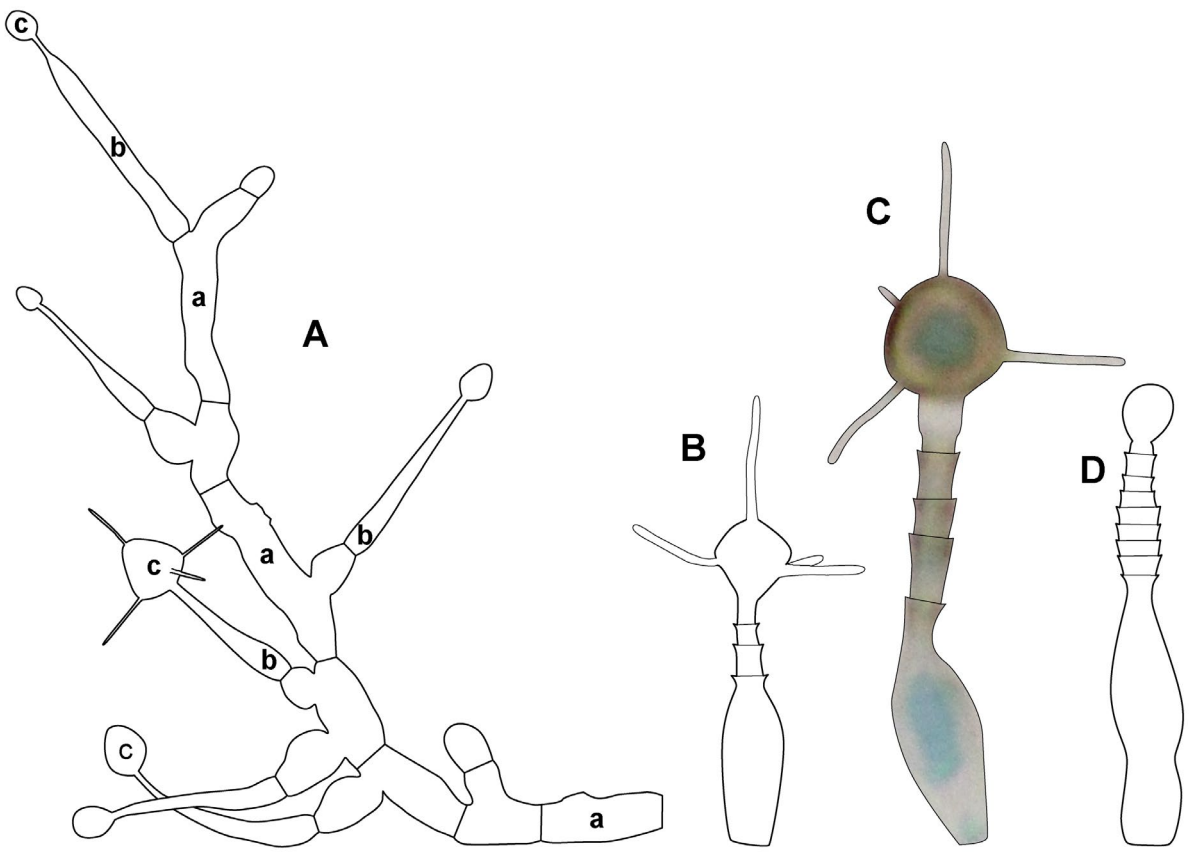

Figure 5. Interpretative schematic drawings of conidiophores, conidiogenous cells, and conidia of *Mycocalicium campylidiophorum* (holotype). A – conidiophores (a), conidiogenous cells (b), and young conidia (c); B – conidiogenous cell with 3 annellations and young, still hyaline conidium, already with appendages; C – schematic drawing combined with photograph (in lactophenol cotton blue) of a mature, brownish conidiogenous cell with 4 annellations and a mature, brown conidium with appendages; annellations are brown, especially at their upper rim; D – old, hyaline conidiogenous cell with multiple annellations producing a young conidium. Scales: 10 µm.

M. campylidiophorum on the white thallus of *O. chionographa* suggests that this white thallus (which is KC+ red) is a species of *Ochrolechia* and that the black ascomata of *Opegrapha* belong to a lichenicolous fungus as well. Moreover, these ascomata are very similar to those of *O. blakii*, a lichenicolous fungus known from *Ochrolechia* (Ertz et al. 2004), supporting this hypothesis. Therefore, we conclude that *O. chionographa* represents a lichenicolous fungus belonging to *Opegrapha* s.lat. and growing on an unknown species of *Ochrolechia*. *Opegrapha blakii* is so similar to *O. chionographa* (lirellate ascomata lacking a carbonized exciple below the hymenium, 4-spored asci, 3-septate ascospores becoming dark brown granulose, 20–22 × 7–8 µm, and apparently the same host lichen genus) that the two species are almost certainly conspecific. The only difference is that *O. chionographa* does not form distinct galls on the host thallus, unlike *O. blakii*, but we consider this to be a variable character. Therefore, we reduce *O. blakii* into synonymy with *O. chionographa*.

Discussion

Mycocalicium campylidiophorum is unique among the *Mycocaliciales* by forming large peculiar pycnidia producing appendiculate conidia (Figs 3–5). Within this order, different types of anamorphic states have been reported, both from axenic cultures and herbarium specimens (Tibell 1997). The anamorph-teleomorph relationship has been established from axenic cultures started from ascospore isolates that produced the anamorphs. Some species develop a hyphomycetous anamorph, such as *Brunneocarpos banksiae* Giraldo & Crous (Crous et al. 2016), *Chaenothecopsis haematopus* Tibell (Tibell & Constantinescu 1991), *C. schefflerae* (Samuels & D.E. Buchanan) Tibell (Samuels & Buchanan 1983; Beimforde et al. 2017), *C. pusiola* (Ach.) Vain. and *C. tasmanica* Tibell (Tibell 1995), and others a coelomycetous anamorph, such as *Chaenothecopsis debilis* (Sm.) Tibell (Tibell 1995), *C. sanguinea* Tibell (Tibell 1997), *Mycocalicium albonigrum* (Nyl.) Fink (Tibell 1990), and *M. subtile* (Pers.) Szatala (Tibell 1990). Both coelomycetous and hyphomycetous anamorphs were even reported for *Chaenothecopsis pusilla* (Ach.) A.F.W. Schmidt (Tibell 1995, 1997), *C. savonica* (Räsänen) Tibell (Tibell 1991) and *C. viridireagens* (Nádv.) A.F.W. Schmidt (Tibell 1993). However, all anamorphs known so far in the *Mycocaliciales* are very different from *M. campylidiophorum* by producing conidia that are not appendiculate.

Seven genera are currently accepted in *Sphinctrinaceae* (including *Mycocaliciaceae*): *Brunneocarpos*, *Chaenothecopsis*, *Mycocalicium*, *Phaeocalicium*, *Pyrgidium*, *Sphinctrina* and *Stenocybe*. As shown by our phylogeny (Fig. 1) and previous molecular studies (Tibell & Vinuesa 2005; Tuovila et al. 2013, 2014; Thiyagaraja et al. 2022), the generic delimitation within the *Mycocaliciales* is still incomplete because the genera *Chaenothecopsis*, *Mycocalicium* and *Phaeocalicium* are polyphyletic. The distinction between these three genera is mainly based on the ascus and ascospores types and on stipe anatomy (Schmidt 1970). However, these characters are very variable in *Chaenothecopsis* and some species are extremely similar to *Mycocalicium* and *Phaeocalicium* in morphology (Tibell 1978; Titov & Tibell 1993: 322; Tibell 1995). Despite these issues of generic delimitation in *Sphinctrinaceae* and the difficulties in assigning some particular species to a genus on the basis of morphological characters, the assignment of the new species to the genus *Mycocalicium* leaves little doubt, since it was recovered with the type species, *M. subtile*, within a strongly supported clade including other species of *Mycocalicium* (Fig. 1).

The closest relative of *Mycocalicium campylidiophorum* is *M. subtile* (Figs 1 & 2), a calicioid species widely distributed in both Hemispheres that grows on lignum and rarely bark of various conifer and deciduous tree species (e.g., Schmidt 1970; Tibell 1987, 1999, 2001; Muñiz & Hladun 2007). Its pycnidia are black, spherical to somewhat ovoid, often with a distinct extended apical part, 0.15–0.20 mm diam. when mature and thus much smaller than in *M. campylidiophorum*. Moreover, the conidia of *M. subtile* are very different from the latter in being more or less curved or irregular, non-appendiculate, 4–5 × 1–1.5 µm (Tibell 1990, 1997, 1999). In culture, brownish multicellular chlamydospores as well as mature spherical conidiomata exudating conidia were obtained from both ascospore and conidia isolations of *M. subtile* (Tibell 1990). The conidia were similar to those produced from specimens of *M. subtile* collected in the field. The close phylogenetic relationship of *M. campylidiophorum* and *M. subtile* is thus surprising since they produce very different types of asexual states. This challenges previous attempts to use asexual stages to support generic relationships in *Mycocaliciales* (e.g., Tibell 1995). The monotypic genus *Brunneocarpos* was even introduced recently based solely on the production of a chlamydospore-like asexual morph in culture (Crous et al. 2016). Nevertheless, conidia in *Mycocalicium* are pale brown, contrasting with the hyaline ones in *Chaenothecopsis* (Tibell 1995, 1999). Therefore, the brown color of the conidial wall in *M. campylidiophorum* supports its placement in *Mycocalicium* so that the pigmentation of the conidial wall (chlamydospores from cultures isolates excluded) might be an important synapomorphy for the genus.

Species of *Mycocaliciales* are ecologically diverse, growing as saprotrophs on bark or dead wood, parasites or commensals on lichens or green algae, or even exclusively on conifer resins or exudates of vascular plants, being sometimes restricted to the exudates of a single tree genus or species (Funk & Kujt 1982; Tibell & Titov 1995; Titov 2001, 2006; Tuovila et al. 2011a, b; Tuovila 2013; Tuovila et al. 2014; Rikkinen et al. 2014; Selva & Tuovila 2016; Beimforde et al. 2017; Gockman et al. 2019). The close relationship of the lichenicolous *M. campylidiophorum* with the saprobic *M. subtile* and *M.* aff. *subtile* suggests that these taxa may have diverged relatively recently from a common ancestor. Multiple switches between the saprobic and lichenicolous life styles have occurred in the *Mycocaliciales* as the lichenicolous species are distributed in different lineages (e.g., *Chaenothecopsis consociata*,

M. campylidiophorum, *Sphinctrina* spp.). Some monophyletic and ecologically homogeneous groups exist. The three lichenicolous species *Chaenothecopsis consociata*, *C. pusiola* and *C. viridireagens* form a strongly supported monophyletic group in our phylogeny (Fig. 1). Previous studies (e.g., Tuovila 2013; Beimforde et al. 2017) revealed that *Chaenothecopsis* species from angiosperm exudates are closely related forming their own well-supported monophyletic clade, but not those on conifer resins as such species are clearly polyphyletic within *Sphinctrinaceae*. A more robust multilocus phylogeny using a wider taxon sampling is needed for a comprehensive study of the evolution of the lifestyles in the family.

The high similarity of the ITS sequences of *M. campylidiophorum* obtained from distantly related populations, together with its spectacular morphology and its lichenicolous habit on species of *Ochrolechia*, clearly supports the recognition as a distinct species. The close relationship with *M. subtile* and *M.* aff. *subtile* is therefore surprising. As pointed out by Muñiz & Hladun (2007) and Tuovila & Huhtinen (2020), the genus *Mycocalicium* is one of the less well-known genera of *Sphinctrinaceae* (as '*Mycocaliciaceae*') and *M. subtile* has become 'a dumping ground for species that do not have some distinctive, clearly discernible characters'. Therefore, more molecular data are needed to improve our knowledge of this genus and of the species complex to which *M. campylidiophorum* belongs. The new species adds to the remarkable diversity of asexual stages in *Sphinctrinaceae*.

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