



# Two new classes of Ascomycota: *Xylobotryomycetes* and *Candelariomycetes*

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## Key words

*Ascomycota*  
*Dothideomycetes*  
*Eurotiomycetes*  
five new taxa  
multigene phylogenetic analyses  
pyrenomycetes  
*Sordariomycetes*

**Abstract** Phylogenetic analyses of a combined DNA data matrix containing nuclear small and large subunits (nSSU, nLSU) and mitochondrial small subunit (mtSSU) ribosomal RNA and the largest and second largest subunits of the RNA polymerase II (*rpb1*, *rpb2*) of representative *Pezizomycotina* revealed that the enigmatic genera *Xylobotryum* and *Cirrosporium* form an isolated, highly supported phylogenetic lineage within *Leotiomyceta*. Acknowledging their morphological and phylogenetic distinctness, we describe the new class *Xylobotryomycetes*, containing the new order *Xylobotryales* with the two new families *Xylobotryaceae* and *Cirrosporaceae*. The two currently accepted species of *Xylobotryum*, *X. andinum* and *X. portentosum*, are described and illustrated by light and scanning electron microscopy. The generic type species *X. andinum* is epitypified with a recent collection for which a culture and sequence data are available. Acknowledging the phylogenetic distinctness of *Candelariomycetidae* from *Lecanoromycetes* revealed in previous and the current phylogenetic analyses, the new class *Candelariomycetes* is proposed.

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## INTRODUCTION

*Xylobotryum* is an enigmatic stromatic ascomycete genus characterised by a unique suite of characters, i.e., perithecioid ascomata on erect, branched or unbranched stromata, a hamathecium of true paraphyses with apically free ends, bitunicate fissitunicate asci with an apically or laterally rupturing ectotunica and a thick endotunica with an inamyloid apical ring, and bicellular brown ellipsoid ascospores with 3–5 longitudinal germ slits per ascospore cell (Ju & Rogers 1994). Currently, two species are accepted in the genus, *Xylobotryum andinum* and *X. portentosum*, of which the former is widely distributed in tropical to subtropical areas worldwide, whereas the latter is so far only known from the tropical to subtropical Americas (Rossman 1976, Ju & Rogers 1994). Due to an unusual combination of morphological characters, its systematic position has historically been controversial, and it has been moved among various ascomycete lineages. In the original description of *Xylobotryum*, Patouillard (in Patouillard & Lagerheim 1895) noted similarities to *Xylaria* and *Kretzschmaria*, and it was placed in the heterogeneous, ill-defined *Sphaeriaceae* by, e.g., Saccardo (1895), Gäumann (1964) and Müller & Von Arx (1973), while Lindau (1897) placed *Xylobotryum* in *Xylariaceae*. Möller (1901) established the genus *Trachyxylaria* for *T. phaeodidyma* (a synonym of *X. portentosum*), again within *Xylariaceae*, while Smith (1901) described *Xyloceras elliottii* as yet another synonym of *Xylobotryum portentosum*. Based on a similar branching pattern of stromata, Lloyd (1920) combined the generic type *Xylobotryum andinum* in *Thamnomycetes* (*Xylariaceae*), but accepted *Xylobotryum* for *X. portentosum* and *X. rickii* (Lloyd 1925). Rodway (1926) established the new

genus and species *Melanobotrys tasmanicus*, a synonym of *Xylobotryum andinum*, without a familial affiliation. Miller (1949) excluded *Xylobotryum* from *Xylariaceae* due to 2-celled ascospores and asci lacking a xylariaceous apical apparatus, but did not propose an alternative classification. Acknowledging substantial morphological differences, Müller & Von Arx (1962) considered the similarities to *Xylaria* to be superficial and classified the genus in *Diatrypaceae* (*Xylariales*, *Sordariomycetes*), which was also followed by Dennis (1970). Rossman (1976) did not assign *Xylobotryum* to a family but assumed pyrenomycetous affinity as well, based on her observations of unitunicate asci and true unbranched paraphyses. Conversely, Rogerson et al. (1990) observed fissitunicate ascus dehiscence and placed *Xylobotryum* in *Pleosporales* ('Loculoascomycetes'; now *Dothideomycetes*). Barr (1987, 1990) tentatively classified the genus within the *Didymosphaeriaceae* (*Dothideomycetes*) based on asci and 1-septate ascospores she considered being similar to *Didymosphaeria*, but highlighting its unique stipitate stromata within the family. Huhndorf (1994) hypothesised that *Xylobotryum* might be affiliated with *Hypsostromataceae* (*Dothideomycetes*). After detailed morphological and pure culture studies, Ju & Rogers (1994) did not assign the genus to a family or order but tentatively also assumed loculoascomycetous affinities in light of the functionally bitunicate asci and the stromatic nature of the ascomata. This treatment was subsequently followed by Eriksson & Hawksworth (1988) and Kirk et al. (2008), who placed *Xylobotryum* in *Dothideales* and *Dothideomycetes* incertae sedis, respectively. Finally, Eriksson & Hawksworth (1995) and Lumbsch & Huhndorf (2007, 2010) referred it to *Ascomycota* incertae sedis, a position which, in lack of new data, has not changed since. No asexual morph is known for *Xylobotryum*.

The peculiar monotypic coelomycetous genus *Cirrosporium* has been described from New Zealand by Hughes (1980) and was beautifully illustrated in the detailed study of Réblová & Seifert (2012). It is characterised by large, cylindrical, tubular, dark brown pycnidia up to 4–5 mm long with vertical ribs and a unique meristem arthric conidium ontogeny producing dark

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brown, 3-septate conidia, which are aggregated in conspicuous, compact, up to 2.5 mm long columnar cirrhi at the pycnidial apex (Hughes 1980, Réblová & Seifert 2012). In their molecular phylogenetic analyses of a multigene sequence matrix, Réblová & Seifert (2012) revealed a phylogenetic position of *Cirrosporium* within *Eurotiomycetes* as sister-group to *Mycocaliciales*; however, this placement received only low support. Owing to its isolated phylogenetic position, Réblová & Seifert (2012) concluded that *Cirrosporium* may warrant placement in a family and order of its own, but they did not formally describe them.

Recent fresh collections made by the second author enabled us to study the morphology of both *Xylobotryum* species in detail, to isolate *X. andinum* in pure culture and to obtain sequence data for *X. andinum* and *X. portentosum* to investigate their phylogenetic affinities. Remarkably and unexpectedly, in phylogenetic analyses *Xylobotryum* and *Cirrosporium* formed a highly supported monophyletic lineage in an isolated position. We here give detailed descriptions and illustrations of both accepted *Xylobotryum* species, and we propose a new formal higher-level classification for the genera *Xylobotryum* and *Cirrosporium* according to the results of the molecular phylogenies.

The above analyses attracted our attention to a group of lichenised *Ascomycota*, which has recently been elevated to a subclass of *Lecanoromycetes*, the *Candelariomycetidae* (Lücking et al. 2017). This subclass contains the single order *Candelariales* with 2 families, *Candelariaceae* and *Pycnoraceae*. As characterised by Jaklitsch et al. (2016), members of this group are mostly epiphytic on bark and rock, they have mostly bright yellow crustose to squamulose thalli, apotheciate ascomata with amyloid paraphyses, clavate amyloid asci, hyaline ellipsoid to citriform, 0–1-septate ascospores, chlorococcoid photobionts, and pycnidial asexual morphs having aseptate hyaline conidia. *Candelaria* is among the most widespread urban lichens.

Owing to our phylogenetic analyses, the *Candelariales* are separate from the *Lecanoromycetes*. We therefore propose a new formal higher-level classification for this order.

## MATERIALS AND METHODS

### Culture preparation, isolates and specimens

Single ascospore isolates of *X. andinum* were prepared on 2 % malt extract agar (MEA) and grown on MEA and 2 % corn meal agar (CMA, Sigma-Aldrich) supplemented with 2 % w/v dextrose (CMD). The isolate of *X. andinum* obtained in the present study has been deposited at the Westerdijk Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands; strain identifiers including NCBI GenBank accession numbers of gene sequences used to generate the phylogenetic trees are listed in Table 1. Details of the specimens used for morphological investigations are listed in the Taxonomy section under the respective descriptions. The following specimen was sequenced for the phylogenetic analyses but is not further treated here: *Pycnora sorophora*: AUSTRIA, Oberösterreich, Bez. Schärding, St. Ägidi, Flenkental 6, 560 masl, N48°30'01" E13°43'10", on old board of a barn, 4 July 2016, *F. Berger 30916* (WU 39968). Specimens have been deposited in the Fungarium of the Institute of Botany, University of Vienna (WU).

### Morphological observations

For light microscopy, hand sections of ascomata were made using a razor blade and mounted in heated chloralactophenol on a microscope slide and covered with a cover slip. Ascomatal contents containing asci and paraphyses were transferred to a drop of 1 % sodium dodecyl sulfate (SDS), gently torn apart with a preparation needle when necessary and observed directly in 1 % SDS or transferred to a drop of Congo red in 1 % SDS, chlo-

razol black or diluted India ink. Amyloidity of asci was assessed using both Melzer's reagent and Lugol's solution. Photomicrographs were taken with a Nikon Coolpix 995 digital camera either directly mounted on a stand or, for higher magnifications, through the eyepiece of an Olympus SZ60 stereomicroscope, by the means of a 30 mm diam adapter. Photomicrographs were taken with the same camera mounted on the trinocular port of a Leitz Orthoplan microscope. The digitalised photographs were processed with Adobe Photoshop Elements 10. Measurements are reported as maxima and minima in parentheses and the range representing the mean plus and minus the standard deviation of a number of measurements given in parentheses.

For scanning electron microscopy (SEM), ascospores were prepared according to the method described in Voglmayr & Mehrabi (2018) and examined in a Jeol JSM-6390 scanning electron microscope at 10 kV.

### DNA extraction and sequencing methods

The extraction of genomic DNA from pure culture was performed as reported previously (Voglmayr & Jaklitsch 2011, Jaklitsch et al. 2012) using the DNeasy Plant Mini Kit (QIAGEN GmbH, Hilden, Germany). DNA extraction from ascomata followed the method described in Voglmayr et al. (2012). The following loci were amplified and sequenced: the complete internal transcribed spacer region (ITS1-5.8S-ITS2) and a c. 0.9 kb fragment of the large subunit nuclear ribosomal DNA (nLSU rDNA), amplified and sequenced as a single fragment with primers V9G (De Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990), or as two separate fragments for ITS and LSU with primer pairs V9G/ITS4 (White et al. 1990) and LR0R (Vilgalys & Hester 1990)/LR5, respectively; a c. 1.7 kb fragment of the small subunit nuclear ribosomal DNA (nSSU rDNA) with primers SL1 (Landvik et al. 1997) and NS24mod (Voglmayr & Jaklitsch 2011); partial mitochondrial small subunit ribosomal DNA (mtSSU) with forward primers mrSSU1 (Zoller et al. 1999) or MSU1 and reverse primers MSU2 or MSU7 (Zhou & Stanosz 2001); a c. 1.2 kb fragment of the RNA polymerase II subunit 2 (*rpb2*) gene with primers dRPB2-5f and dRPB2-7r (Voglmayr et al. 2016) or fRPB2-7cr (Liu et al. 1999); and a c. 1.2 kb fragment of the RNA polymerase II subunit 1 (*rpb1*) gene with primers RPB1-6R1asc (Hofstetter et al. 2007) and RPB1-Af (Stiller & Hall 1997). For *Pycnora sorophora*, an additional fragment covering the terminal part of the nSSU and the complete ITS region was amplified with primers nSSU1088 (Kauff & Lutzoni 2002) and ITS4 to confirm the correctness of the nSSU, and the nLSU was additionally amplified with primers LIC24Rm (5'-GAAAAGAAACCAACAGGGATTG-3', a modification of LIC24R of Miadlikowska & Lutzoni 2000) and LIC2044 (Kauff & Lutzoni 2002). PCR products were purified using an enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) with the same primers as in PCR; in addition, primers ITS4, LR2R-A (Voglmayr et al. 2012) and LR3 (Vilgalys & Hester 1990) were used for the complete ITS-LSU region and nSSU1088 (Kauff & Lutzoni 2002) for the SSU region. Sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems).

### Phylogenetic analyses

For phylogenetic analyses, combined matrices of nLSU, nSSU, mtSSU, *rpb1* and *rpb2* sequences were produced. GenBank sequences of the classes of *Leotiomyceta* were selected from Réblová & Seifert (2012), Prieto et al. (2013) and Réblová et al. (2017) and supplemented with additional GenBank sequences.

**Table 1** Sources/Strains and NCBI GenBank accessions used in the phylogenetic analyses of the combined multigene matrix of selected *Pezizomycotina*. Sequences in **bold** were generated during the present study.

Taxon	Source/Strain	GenBank accession no.				
		nLSU	nSSU	mtSSU	<i>rpb1</i> (A–F)	<i>rpb2</i> (5–7)
<i>Acarospora schleicheri</i>	AFTOL-ID 345/1005	AY640945	AY640986	AY584694	DQ782859	AY641026
<i>Acarosporina microspora</i>	AFTOL-ID 78 = CBS 338.39	AY584643	AY584667	AY584612	DQ782818	AY584682
<i>Aleuria aurantia</i>	AFTOL-ID 65	AY544654	AY544698	–	DQ471120	DQ247785
<i>Arachnomyces glareosus</i>	CBS 116129	FJ358273	FJ358341	FJ225785	FJ358405	–
<i>Arthonia dispersa</i>	UPSC 2583	AY571381	AY571379	AY571383	–	–
<i>Ascosphaera apis</i>	CBS 402.96	FJ358275	FJ358343	–	FJ358406	–
<i>Aspergillus fumigatus</i>	INFU/Jc/KF/6, F-A, Af293	FM179606	GU980961	JQ346808	XM_741647	XM_741647
<i>Baeomyces placophyllus</i>	AFTOL-ID 347	AF356658	AF356657	AY584695	DQ870936	AY641028
<i>Buellia stilingiana</i>	AFTOL-ID 571	–	DQ912319	DQ912287	DQ912368	DQ912391
<i>Bulgaria inquinans</i>	AFTOL-ID 916 = CBS 118.31	DQ470960	DQ471008	–	DQ471152	DQ470910
<i>Caliciopsis pinea</i>	AFTOL-ID 1869 = CBS 139.64	DQ678097	DQ678043	FJ190653	–	EF411067
<i>Caloplaea flavorubescens</i>	AFTOL-ID 2090/2379	AF279887	AF241540	AY143403	DQ915593	–
<i>Calosphaeria pulchella</i>	CBS 115999	AY761075	AY761071	–	–	GU180661
<i>Camarops ustulinoides</i>	AFTOL-ID 72 = CBS 122033	DQ470941	DQ470989	FJ190588	DQ471121	DQ470882
<i>Candelaria concolor</i>	AFTOL-ID 1706/2388	DQ986791	–	DQ986806	EF436462	DQ992419
<i>Candelariella aurella</i>	AFTOL-ID 2390/2389	AY853361	–	AY853313	DQ915594	–
<i>Candelariella reflexa</i>	AFTOL-ID 1271	DQ912331	DQ912309	DQ912272	DQ912354	DQ912380
<i>Candelariella terrigena</i>	AFTOL 227	DQ986745	DQ986730	–	DQ986816	DQ992427
<i>Capnodium coffeae</i>	AFTOL-ID 939 = CBS 147.52	DQ247800	DQ247808	FJ190609	DQ471162	DQ247788
<i>Catolechia wahlenbergii</i>	AFTOL-ID 1743	DQ986794	DQ986704	DQ986811	KJ766824	DQ992424
<i>Cetraria islandica</i>	AFTOL-ID 211	DQ912334	DQ912311	DQ912277	DQ912356	DQ912382
<i>Chaenotheca furfuracea</i>	Wedin 6366 (UPS)	JX000087	JX000068	JX000121	JX000137	–
<i>Chaenothecopsis savonica</i>	Tibell 15876 (UPS)	AY796000	U86691	–	–	–
<i>Chaetosphaeria ciliata</i>	ICMP 18253	GU180637	GU180614	–	–	GU180659
<i>Chlorociboria aeruginosa</i>	AFTOL-ID 151	AY544669	AY544713	AY544734	DQ471125	DQ470886
<i>Cirrosporium novae-zelandiae</i>	CBS 123236	HQ878612	HQ878613	JQ437441	JQ437440	HQ878614
<i>Cladonia caroliniana</i>	AFTOL-ID 3	AY584640	AY584664	AY584614	DQ782816	AY584684
<i>Coenogonium lepreurii</i>	AFTOL-ID 351	AF465442	AF465457	AY584698	–	AY641032
<i>Colletotrichum gloeosporioides</i>	MCA2498, CBS 114054	DQ286199	M55640	FJ190626	AY489659	DQ858455
<i>Corynelia uberata</i>	UME 31276	–	AF242262	–	–	–
<i>Dactylospora haliotrepha</i>	AFTOL-ID 758	FJ176855	FJ176802	KJ766382	–	FJ238344
<i>Dactylospora mangrovei</i>	AFTOL-ID 2108	FJ176890	FJ176836	KJ766383	KJ766849	FJ238375
<i>Dermatocarpon miniatum</i>	AFTOL-ID 91, Wedin 6362 (UPS)	AY584644	AY584668	AY853319	DQ782821	DQ782863
<i>Dermea acerina</i>	AFTOL-ID 941 = CBS 161.38	DQ247801	DQ247809	DQ976373	DQ471164	DQ247791
<i>Diaporthe phaseolorum</i>	AFTOL-ID 357 = NRRL 13736	U47830	L36985	AY584703	FJ238426	AY641036
<i>Dibaeis baeomyces</i>	AFTOL-ID 358/3475	AF279385	AF085473	AY584704	DQ842011	AY641037
<i>Diploschistes actinostomus</i>	AFTOL-ID 98	AF279389	AF279388	AY584692	DQ870943	AY641039
<i>Dirinaria applanata</i>	AFTOL-ID 839	DQ973035	DQ973011	DQ972983	–	DQ973098
<i>Dolabra nepheliae</i>	CBS 122120	GU332517	–	GU332519	GU332521	–
<i>Dothiora cannabinae</i>	AFTOL-ID 1359 = CBS 737.71	DQ470984	DQ479933	FJ190636	DQ471182	DQ470936
<i>Elaphomyces granulatus</i>	AFTOL-ID 436	KT232217	KT232240	KT232222	–	KT232234
<i>Endocarpon pallidulum</i>	AFTOL-ID 661	DQ823097	DQ823104	FJ225674	DQ840552	DQ840559
<i>Eremascus albus</i>	UCB 50-026, CBS 975.69	AY004345	M83258	–	FJ358410	–
<i>Exophiala dermatitidis</i>	AFTOL-ID 668 = CBS 207.35	DQ823100	X79312	FJ225740	DQ840555	DQ840562
<i>Fusichalara minuta</i>	CBS 709.88	KX537758	KX537773	KX537762	KX537766	KX537770
<i>Gelasinospora tetrasperma</i>	AFTOL-ID 1287	DQ470980	DQ471032	FJ190627	DQ471178	DQ470932
<i>Geoglossum nigrinum</i>	AFTOL-ID 56	AY544650	AY544694	AY544740	DQ471115	DQ470879
<i>Graphis scripta</i>	AFTOL-ID 2091/2525/7428	AY640029	AF038878	AY853322	DQ870947	HM244793
<i>Gyalecta jenensis</i>	AFTOL-ID 361, Spribille s.n. (GZU)	AF279391	AF279390	AY584705	KR017455	AY641043
<i>Gyromitra californica</i>	AFTOL-ID 176	AY544673	AY544717	AY544741	DQ471130	DQ470891
<i>Icmadophila ericetorum</i>	AFTOL-ID 875	DQ883694	DQ883704	DQ986897	DQ883723	DQ883711
<i>Lasallia pustulata</i>	AFTOL-ID 554	DQ883690	DQ883700	DQ986889	DQ883719	DQ883707
<i>Lecanora contractula</i>	AFTOL 877	DQ986746	DQ986741	DQ986898	DQ986817	DQ992428
<i>Lecidea fuscoatra</i>	AFTOL-ID 589/4523	DQ912332	AF088239	DQ912275	DQ912355	DQ912381
<i>Leptophlemma polyanthes</i>	AFTOL-ID 367	AF356691	AF356690	AY584709	–	AY641050
<i>Leotia lubrica</i>	AFTOL-ID 1	AY544644	L37536	AY544746	DQ471113	DQ470876
<i>Leptosphaeria maculans</i>	AFTOL-ID 277 = DAOM 229267	DQ470946	DQ470993	–	DQ471136	DQ470894
<i>Lichina pygmaea</i>	Schultz 04011/04069	–	AF282909	KX984061	–	–
<i>Lobarina scrobiculata</i>	AFTOL-ID 128	AY584655	AY584679	AY584621	DQ883736	DQ883749
<i>Microglossum rufum</i>	AFTOL-ID 1292	DQ470981	DQ257358	–	DQ471179	DQ470933
<i>Monascus purpureus</i>	AFTOL-ID 426 = CBS 109.07	AF364966	DQ782881	FJ225780	DQ842012	DQ782869
<i>Monilochaetes infuscans</i>	CBS 379.77	GU180645	GU180619	–	–	GU180658
<i>Mycocalicium subtile</i>	Wedin 6889 (UPS), Wedin 6353 (S), Wedin 8492 (S)	AY853379	JX000072	AY853330	JX000141	–
<i>Orceolina kerguelensis</i>	AFTOL-ID 296	AF274116	DQ366257	AY212853	DQ366255	DQ366256
<i>Peltigera degenii</i>	AFTOL-ID 134	AY584657	AY584681	AY584628	DQ782826	AY584688
<i>Peltula umbilicata</i>	AFTOL-ID 891	DQ832334	DQ782887	AY584711	DQ782855	DQ832335
<i>Penicillium freii</i>	AFTOL-ID 378 = DOAM 216705	AY640958	AY640998	AY584712	–	AY641058
<i>Phaeomoniella chlamydospora</i>	CBS 229.95/UCR-PC4	AF353609	–	genome <sup>1</sup>	genome <sup>1</sup>	genome <sup>1</sup>
<i>Phyllobaeis imbricata</i>	AFTOL-ID 852	DQ986781	DQ986739	DQ986895	–	DQ992472
<i>Placopsis perrugosa</i>	AFTOL-ID 383	AF356660	AF356659	AY584716	–	AY641063
<i>Pleopsidium chlorophanum</i>	AFTOL-ID 1004	DQ842017	DQ525540	DQ991756	DQ782858	DQ525442
<i>Preussia terricola</i>	AFTOL-ID 282 = DAOM 230091	AY544686	AY544726	AY544754	DQ471137	DQ470895
<i>Pseudonectria roussseliana</i>	AFTOL-ID 191 = CBS 114049	U17416	AF543767	FJ713627	AY489670	DQ522459
<i>Pycnora praestabilis</i>	AFTOL-ID 4927	KJ766644	–	KJ766478	KJ766886	–
<i>Pycnora sorophora</i>	F. Berger 30916 = WU 39968	<b>MH468790</b>	<b>MH468790</b>	<b>MH468796</b>	<b>MH468797</b>	<b>MH468793</b>

**Table 1** (cont.)

Taxon	Source/Strain	GenBank accession no.				
		nLSU	nSSU	mtSSU	<i>rpb1</i> (A–F)	<i>rpb2</i> (5–7)
<i>Pycnora xanthococca</i>	Hermansson 11849	AY853388	–	AY853339	–	–
<i>Pyrenula reebiae</i>	AFTOL-ID 387	AY640962	AY641001	AY584720	DQ840558	AY641068
<i>Pyrgillus javanicus</i>	AFTOL-ID 342	DQ823103	NG013194	FJ225774	DQ842010	DQ842009
<i>Pyxine subcinerea</i>	AFTOL-ID 686	DQ883802	DQ883793	DQ912292	DQ883745	DQ883758
<i>Ramichloridium anceps</i>	AFTOL-ID 659 = CBS 181.65	DQ823102	AY554292	FJ225752	DQ840557	DQ840564
<i>Rhizocarpon oederi</i>	AFTOL-ID 1372	–	DQ983486	DQ986788	–	DQ992477
<i>Rhopalophora clavispora</i>	CBS 637.73	KX537757	KX537772	KX537761	KX537765	KX537769
<i>Roccellographa cretacea</i>	AFTOL-ID 93	DQ883696	DQ883705	FJ772240	DQ883716	DQ883713
<i>Schismatomma decolorans</i>	AFTOL-ID 307	AY548815	AY548809	AY548816	–	DQ883715
<i>Sclerophora farinacea</i>	Wedin 6414 (UPS)	JX000095	JX000078	JX000130	JX000144	–
<i>Solorina bispora</i>	AFTOL-ID 127	DQ973044	DQ973021	DQ972994	–	DQ973082
<i>Sordaria fimicola</i>	SMH 4106, MUCL 937, CBS 723.96	AY780079	X69851	–	–	DQ368647
<i>Spathularia velutipes</i>	AFTOL-ID 1291	FJ997861	FJ997860	–	–	FJ997863
<i>Sphinctrina turbinata</i>	AFTOL-ID 1721, Lofgren 637	EF413632	U86693	FJ713611	–	EF413633
<i>Spiromastix warcupii</i>	AFTOL-ID 430	DQ782909	AB015768	FJ225794	EF413613	–
<i>Stemphylium vesicarium</i>	AFTOL-ID 940 = CBS 191.86	DQ247804	DQ247812	FJ190610	DQ471163	DQ247794
<i>Stenocybe pullatula</i>	Tibell 17117	AY796008	U86692	–	–	–
<i>Sticta beauvoisii</i>	AFTOL-ID 1242	DQ986769	DQ986713	DQ986867	–	DQ992456
<i>Stictis radiata</i>	AFTOL-ID 398	AF356663	U20610	AY584727	–	AY641079
<i>Symbiotaphrina kochii</i>	CBS 250.77	AY227719	AY227717	genome <sup>1</sup>	genome <sup>1</sup>	genome <sup>1</sup>
<i>Talaromyces flavus</i>	CBS 310.38 = NRRL 2098, FRR 2386	EU021596	GU733356	L14508	–	EU021620
<i>Thamnia vermicularis</i>	AFTOL-ID 2071/3340	AY853395	AF085472	AY853345	DQ915599	AY485634
<i>Thelotrema lepadinum</i>	AFTOL 83/2025	AY300866	–	DQ972997	DQ973067	DQ973085
<i>Trapelia placodioides</i>	AFTOL 962	AF274103	AF119500	AF431962	DQ366259	DQ366260
<i>Trichocoma paradoxa</i>	CBS 788.83	FJ358290	FJ358354	FJ225782	–	JN121550
<i>Trichoglossum hirsutum</i>	AFTOL-ID 64/408	AY544653	AY544697	AY584733	DQ471119	DQ470881
<i>Trinosporium guianense</i>	CBS 132537	JX069853	–	–	genome <sup>1</sup>	genome <sup>1</sup>
<i>Umbilicaria arctica</i>	AFTOL-ID 1266	DQ986772	DQ986717	DQ986872	DQ986841	DQ992460
<i>Usnea antarctica</i>	AFTOL-ID 813	DQ883692	DQ883702	DQ990920	DQ883721	DQ883709
<i>Varicellaria hemisphaerica</i>	AFTOL-ID 959	AF381556	DQ902340	DQ973000	DQ902341	DQ902342
<i>Verrucaria muralis</i>	AFTOL-ID 2265	EF643803	EF689878	FJ225708	EF689805	–
<i>Xanthoria parietina</i>	Gaya 8	JQ301589	JQ301641	JQ301530	JQ301734	JQ301784
<i>Xylaria hypoxylon</i>	AFTOL-ID 51	AY544648	AY544692	AY544760	DQ471114	DQ470878
<i>Xylobotryum andinum</i>	XA1 = CBS 144327 = WU 39969	<b>MH468791</b>	<b>MH468791</b>	–	<b>MH468798</b>	<b>MH468794</b>
<i>Xylobotryum portentosum</i>	XP = WU 33543	<b>MH468792</b>	<b>MH468792</b>	–	<b>MH468799</b>	<b>MH468795</b>
<i>Xylona heveae</i>	TC161	JQ838237	JQ838238	genome <sup>1</sup>	genome <sup>1</sup>	genome <sup>1</sup>

<sup>1</sup> Sequence retrieved from genome deposited at JGI-DOE (<http://genome.jgi.doe.gov/>).

For some strains for which the whole genome data are available, sequences were retrieved from JGI-DOE (<http://genome.jgi.doe.gov/>). The sequence selection of *Lecanoromycetes* was cross-checked with the supplemental table S1 of Miadlikowska et al. (2014) to exclude misidentified taxa or obvious contaminant sequences. Two taxa of *Pezizomycetes* (*Aleuria aurantia* and *Gyromitra californica*) were added as outgroup according to Réblová & Seifert (2012). All alignments were produced with the server version of MAFFT ([www.ebi.ac.uk/Tools/mafft/](http://www.ebi.ac.uk/Tools/mafft/)), checked and refined using BioEdit v. 7.0.9.0 (Hall 1999). For the protein-coding genes (*rpb1*, *rpb2*), a preliminary sequence alignment was produced and single-base inserts (sequencing errors) causing reading frame shifts in some GenBank sequences and introns were removed from well-aligned blocks. Subsequently, the amino acid codons were determined, and the nucleotide alignment was then manually refined according to the reading frame of the amino acid alignment. After exclusion of ambiguously aligned regions, long gaps and introns, the final matrix contained 5949 nucleotide characters, i.e., 1158 from the nLSU, 1652 from the nSSU, 772 from the mtSSU, 1140 from *rpb1* and 1227 from *rpb2*.

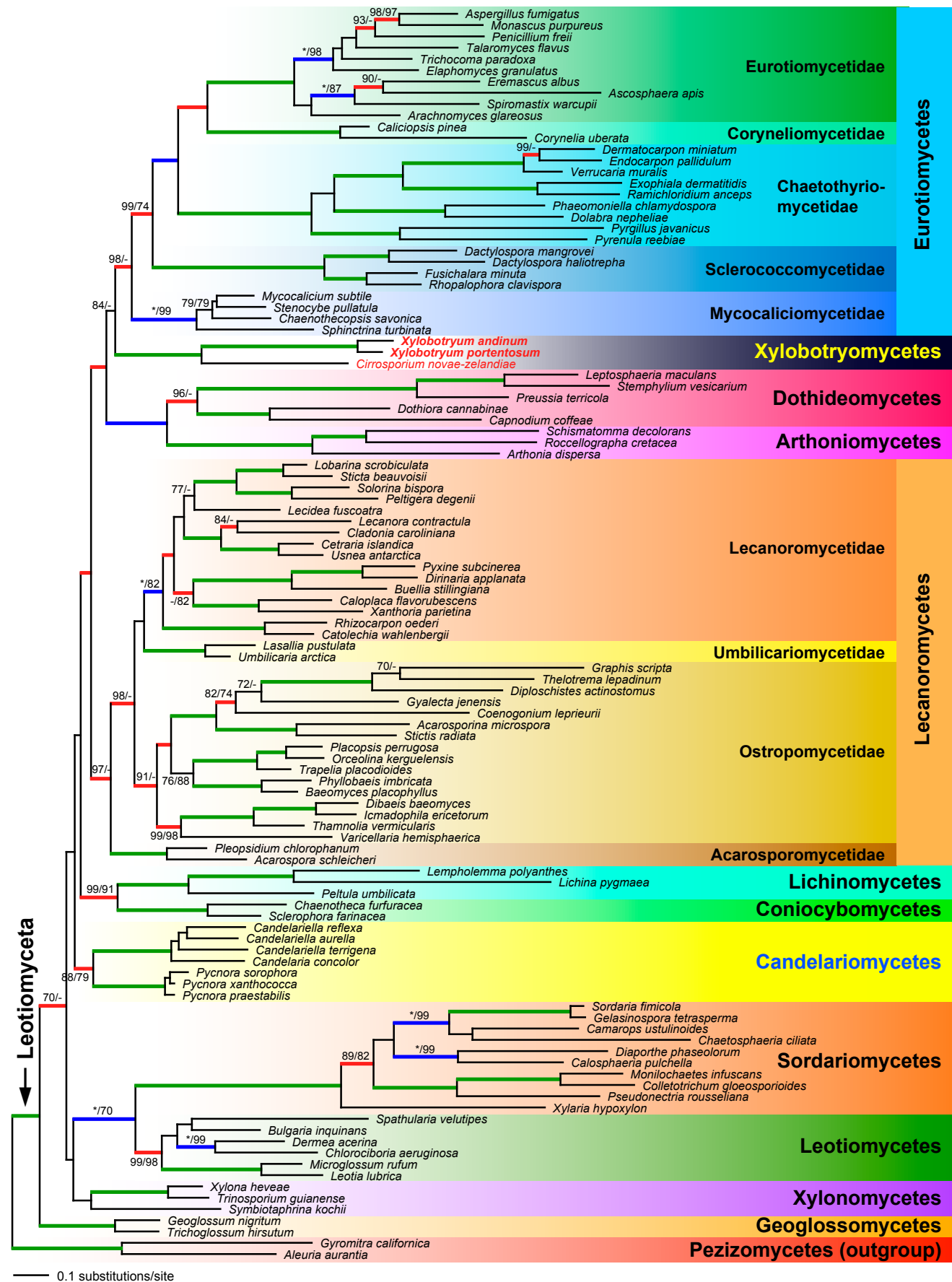
In Maximum likelihood (ML) and Bayesian analyses (BI), substitution model parameters were calculated separately for the different gene regions included in the combined analyses, and for the protein-coding genes (*rpb1*, *rpb2*) also separately for the first, second and third codon positions. Maximum likelihood analyses were performed with RAXML 8.2.10 (Stamatakis 2006) via the CIPRES Science Gateway v. 3.3 (Miller et al. 2010) using the ML + rapid bootstrap setting and the GTRCAT substitution model with 1000 bootstrap replicates.

The substitution models for Bayesian analyses were selected using the Bayesian Information Criterion (BIC) as implemented in MEGA7 (Kumar et al. 2016). The GTR model (Rodríguez et al. 1990) with an estimated proportion of invariable sites and with a gamma distribution (GTR+I+G) was selected for all loci. Bayesian analyses were performed with the computer program MrBayes (v. 3.2.6; Huelsenbeck & Ronquist 2001) via the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). Two parallel runs of four incrementally heated, simultaneous Markov chains were performed over 6.5 million generations of which every 500th tree was sampled in each run. The first 2000 trees sampled of each run were discarded and a 90 % majority rule consensus of the remaining trees was computed to obtain posterior probabilities.

Maximum parsimony (MP) bootstrap analysis was performed with PAUP v. 4.0a161 (Swofford 2002), with 1000 bootstrap replicates using five rounds of heuristic search replicates with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect) during each bootstrap replicate. All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COL-LAPSE command was set to minbrlen.

In the phylogenetic tree (Fig. 1), bootstrap support below 70 % was not considered, and from Bayesian analyses only maximum posterior probabilities (1.0) were added. In the Results and Discussion, bootstrap values below 70 % are considered low, between 70–90 % medium and above 90 % high.





**Fig. 1** Phylogram of the best ML tree (lnL = -170318.7567) revealed by RAXML from an analysis of the combined nLSU-nSSU-mtSSU-*rpb1-rpb2* matrix of selected *Pezizomycotina*, showing the phylogenetic position of *Xylobotryum* and *Cirrosporium* (*Xylobotryomycetes*) and *Candelariomycetes*. Thickened nodes in red denote maximum support by BI (1.0 PP), in blue by BI and ML (1.0 PP/100 %) and in green by BI, ML and MP (1.0 PP/100 %/100 %); ML and MP bootstrap support between 70 and 99 % is given at the first and second position, respectively, above or below the branches; with asterisks (\*) denoting ML bootstrap support of 100 %.

## RESULTS

### Molecular phylogeny

Despite extensive PCR trials using various primer combinations, annealing temperatures and DNA polymerases, no clean mtSSU rDNA sequences could be obtained for the two *Xylobotryum* species. As no nSSU sequences are available for *Pycnora* in GenBank, the nSSU sequence obtained for *Pycnora sorophora* could not be verified as correct by BLAST searches which revealed *Symbiotaphrina kochii* (*Xylonomycetes*) as closest match (99 % sequence identity), followed by various *Leotiomyces*. However, the sequence of the overlapping nSSU-ITS fragment amplified with primers nSSU1088/ITS4 completely matched the separately amplified nSSU and ITS-nLSU sequences in its respective parts, confirming that the correct nSSU fragment was amplified.

Of the 5949 nucleotide characters of the combined matrix, 3049 are parsimony informative (484 of nLSU, 493 of nSSU, 457 of mtSSU, 795 of *rpb1* and 820 of *rpb2*). Fig. 1 shows the phylogram of the best ML tree (lnL = -170318.7567) obtained by RAxML.

Phylogenetic relationships of classes within *Leotiomyceta* were mostly unsupported in all analyses (Fig. 1). However, except *Xylonomycetes* which were consistently unsupported, all classes received maximum support in Bayesian analyses, and almost all also high to maximum support in ML analyses (Fig. 1). MP bootstrap support was commonly lower, but most classes also supported by ML and Bayesian analyses still received support above 70 % (Fig. 1); notable exceptions are the *Dothideomycetes* (56 %), *Eurotiomycetes* (61 %) and *Lecanoromycetes* (57 %). *Candelariales* consistently did not cluster with *Lecanoromycetes* and are thus recognised as a class of their own, *Candelariomycetes*, which received maximum support in Bayesian analyses and medium support in ML (88 %) and MP (79 %) analyses. The genera *Xylobotryum* and *Cirrosporium* were revealed as closest relatives with maximum support and were sister group to the *Eurotiomycetes* with low (57 % MP) to medium (84 % ML) support. However, in Bayesian analyses the *Xylobotryum*-*Cirrosporium* clade was placed outside *Eurotiomycetes* as sister clade to the *Dothideomycetes*-*Arthoniomycetes* clade with 0.93 PP. Monophyly of the clade containing *Arthoniomycetes*, *Dothideomycetes*, *Eurotiomycetes* and *Xylobotryomycetes* received maximum support in Bayesian analyses, but no support in MP and ML bootstrap analyses. Due to this result and their unique morphological features, *Xylobotryum* and *Cirrosporium* are here classified in the new class *Xylobotryomycetes* within *Pezizomycotina* and placed in two monotypic families *Xylobotryaceae* and *Cirrosporiaceae*, respectively, within the order *Xylobotryales*.

### Taxonomy

***Candelariomycetes*** Voglmayr & Jaklitsch, *class. nov.* — MycoBank MB826790

*Holotype order.* *Candelariales* Miadl., Lutzoni & Lumbsch.

*Holotype genus.* *Candelaria* A. Massal.

A new class of the phylum *Ascomycota* containing the single order *Candelariales* with the two families *Candelariaceae* and *Pycnoraceae*. *Thallus* crustose to squamulose, peltate-subumbilicate, or microfoliose, rarely lichenicolous and without thallus, often bright yellow; photobiont chlorococcoid; cephalodia absent. *Ascomata* apothecial, lecanorine or rarely biatorine (*Candelariaceae*) or lecideine (*Pycnoraceae*). *Hamathecium* consisting of

unbranched to slightly branched paraphyses, amyloid. *Asci* with apical tholus, clavate; outer wall amyloid, tholus weakly amyloid except for a darker amyloid, ring-shaped structure in the lower part; containing 8 to many (64) ascospores (*Candelariaceae*). *Ascospores* hyaline, non-septate to (indistinctly) 1–3-septate, ellipsoid to citriform, non-amyloid. *Asexual morphs* where known pycnidial. *Conidia* hyaline, non-septate, ellipsoid to bacillar, sometimes curved. *Secondary chemistry*: pulvinic acid derivatives (*Candelariaceae*) or depsides (alectorialic acid; *Pycnoraceae*).

*Habitat* — On rock (many *Candelariaceae*), rarely on bryophytes or soil or epiphytic on bark, or typically on wood (*Pycnoraceae*).

*Notes* — The diagnosis above was slightly modified from the diagnosis of subclass *Candelariomycetidae* published in Lücking et al. (2017).

***Xylobotryomycetes*** Voglmayr & Jaklitsch, *class. nov.* — MycoBank MB826791

*Holotype order.* *Xylobotryales* Voglmayr & Jaklitsch.

*Holotype genus.* *Xylobotryum* Pat.

A new class of the phylum *Ascomycota* containing the single order *Xylobotryales* with the two families *Cirrosporiaceae* and *Xylobotryaceae*. Saprobic or possibly parasitic. *Sexual morphs* where known stromatic. *Stromata* branched or unbranched, bearing superficial ascomata. *Ascomata* perithecioid. *Ostiole* canal periphysate. *Hamathecium* consisting of filiform, septate paraphyses with free ends. *Asci* bitunicate, fissitunicate, apex with an apical ring. *Ascospores* pigmented, septate, with longitudinal germ slits. *Asexual morphs* where known pycnidial, unilocular. *Conidiogenesis* meristem arthric. *Conidia* pigmented, septate.

***Xylobotryales*** Voglmayr & Jaklitsch, *ord. nov.* — MycoBank MB826792

*Etymology.* Referring to the name of the type genus, *Xylobotryum*.

*Holotype family.* *Xylobotryaceae* Voglmayr & Jaklitsch.

*Other family.* *Cirrosporiaceae* Voglmayr & Jaklitsch.

Saprobic or possibly parasitic on wood or bark. *Sexual morphs* where known stromatic. *Stromata* large, upright, stipitate, branched or unbranched, bearing superficial ascomata. *Ascomata* perithecioid, sessile or stipitate. *Ostioles* periphysate. *Paraphyses* filiform, with free ends. *Asci* bitunicate, fissitunicate; apex with an apical ring. *Ascospores* 2-celled, brown, with longitudinal germ slits. *Asexual morphs* where known pycnidial, cylindrical, unilocular, consisting of a sterile basal part and a fertile upper part with an apical ostiole. *Conidiogenous cells* forming a meristem, producing pigmented, septate arthroconidia.

***Cirrosporiaceae*** Voglmayr & Jaklitsch, *fam. nov.* — MycoBank MB826793

*Etymology.* Referring to the name of the type genus.

*Type genus.* *Cirrosporium* S. Hughes.

Saprobic on wood or bark. *Sexual morphs* unknown. *Pycnidia* large, dark brown, upright, cylindrical, elongate, unilocular, consisting of a sterile basal part and a fertile tubular upper part with a wide apical ostiole. *Conidiogenous cells* growing as a meristem from the basis of the pycnidial cavity upwards, hyaline, producing brown to black, transversely septate, catenate arthroconidia being ejected in black cirrhi.

**Xylobotryaceae** Voglmayr & Jaklitsch, *fam. nov.* — MycoBank MB826794

*Etymology.* Referring to the name of the type genus.

*Type genus.* *Xylobotryum* Pat.

Saprobic or possibly parasitic on wood or bark. *Stromata* dark brown to black, upright, stipitate, branched or unbranched, bearing superficial ascomata. *Ascomata* perithecioid, subglobose to ellipsoid, sessile or stipitate, black. *Ostioles* inconspicuous to papillate; ostiolar canal lined with periphyses. *Paraphyses* abundant, filiform, with free ends, septate, hyaline, thin-walled, embedded in a gelatinous matrix. *Asci* bitunicate, fissitunicate, with a long stipe; containing 8 (sometimes 4 fully developed and 4 aborted) ascospores; apex with an inamyloid apical ring; ectotunica firm, elastic, rupturing apically, laterally or basally; endotunica swelling after dehiscence. *Ascospores* ellipsoid to fusiform, 2-celled, brown, with longitudinal germ slits. *Asexual morphs* unknown.

***Xylobotryum*** Pat., in Patouillard & De Lagerheim, Bull. Herb. Boissier 3 (1): 69. 1895.

*Type species:* *Xylobotryum andinum* Pat.

*Synonyms.* *Melanobotrys* Rodway, Pap. & Proc. Roy. Soc. Tasmania 1925: 168. 1926.

*Trachyxylaria* Möller, Bot. Mitt. Tropen 9: 308. 1901.

*Xyloceras* A.L. Sm., J. Linn. Soc., Bot. 35 (no. 242): 16. 1901.

Saprobic or possibly parasitic on wood or bark. *Stromata* large, dark brown to black, upright, stipitate, branched or unbranched, bearing numerous superficial ascomata. *Ascomata* perithecioid, subglobose to ellipsoid or oval, sessile or short-stipitate, occasionally laterally collapsed; surface black, texture leathery when dry, rubbery when moist; contents hyaline, slightly gelatinous when rehydrated. *Peridium* pseudoparenchymatous, 3-layered: outermost layer of dark brown, thick-walled angular cells; median layer subhyaline, of thin-walled hyphal to prismatic cells; inner layer pale brown, of similar, more pigmented cells. *Stromatic tissues* continuous with the ascomatal walls, with an outermost dark brown layer similar to that of ascomata; inner layer composed of subhyaline prismatic to elongate cells; hyphae of the innermost tissue loosely intertwined. *Ostioles* inconspicuous to papillate; ostiolar canal densely lined with hyaline periphyses with bluntly rounded ends. *Paraphyses* abundant, filiform, with free ends, 1–3 µm wide, hyaline, thin-walled, remotely septate, unbranched in the upper part, embedded in a gelatinous matrix. *Asci* bitunicate, fissitunicate, peripheral, narrowly clavate to slightly fusiform, apically broadly rounded, straight to contorted at base, with a long stipe and a swollen furcate base; containing 8 (sometimes 4 fully developed and 4 aborted) obliquely uniseriate to irregularly biseriate ascospores; apex with an apical ring stained by Congo red but not reacting in Lugol and Melzer's reagent; ectotunica slightly refractive, firm, elastic, rupturing apically, laterally or basally; endotunica swelling after dehiscence. *Ascospores* ellipsoid to slightly fusiform, equilateral, with broadly rounded ends, 2-celled, pale brown, with several longitudinal germ slits in each cell extending over the entire length, without appendages or hyaline sheath visible in India ink. *Asexual morph* unknown.

*Notes* — Six species have been described in *Xylobotryum*, of which two, *X. andinum* and *X. portentosum*, are currently accepted in the genus and two (*X. coralloides*, *X. dussii*) are considered synonyms of *X. andinum* (Müller & Von Arx 1962, Rossman 1976). The remaining two *Xylobotryum* species belong elsewhere. After type studies, Dennis (1977) recognised *X. caespitosum* as a calicioid fungus that is now classified as *Chaenothecopsis caespitosa* in *Mycocaliciaceae* (Hawksworth

1980, Hawksworth et al. 2014), and Rossman (1976) confirmed *Xylobotryum rickii* to be a true *Xylaria* in which it had originally been described.

*Xylobotryum* seems to be confined to tropical and subtropical regions, where it is widely distributed. It is commonly considered a saprobe, but Agnihothrudu & Barua (1960) assumed that *X. andinum* could be a parasite of tea bushes in India, and Rodway (1926) recorded growth on galls of *Nothofagus*. Collection MJF 07074 of *X. andinum* likewise comes from a living stump.

Both currently accepted *Xylobotryum* species are indistinguishable by ascus and ascospore characters, but their stromata are markedly distinct. While *X. andinum* shows branched stromata with branches terminally bearing clusters of 2–5 ascomata, *X. portentosum* has unbranched, erect, fusiform stromata with a large fertile apical part densely covered by numerous superficial ascomata.

***Xylobotryum andinum*** Pat., in Patouillard & De Lagerheim, Bull. Herb. Boissier 3 (1): 69. 1895. — Fig. 2, 3

*Synonyms.* *Thamnomycetes andinus* (Pat.) Lloyd, Mycol. Writings 6 (Letter 62): 908. 1920.

*Xylobotryum dussii* Pat., in Duss, Enum. Champ. Guadeloupe (Lons-le-Saunier): 77. 1903.

*Melanobotrys tasmanicus* Rodway, Pap. & Proc. Roy. Soc. Tasmania 1925: 168. 1926.

*Xylobotryum coralloides* Syd., Ann. Mycol. 36 (4): 297. 1938.

*Typification.* ECUADOR, San Jorge, on decorticated wood, July 1892, G. Lagerheim (FH 01146514, holotype). — FRANCE, French West Indies, Martinique, Fort-de-France, forest trail of Fond Baron, hygrophilous rainforest, on a dead corticated trunk, 10 Aug. 2016, J. Fournier MJF 16201 (WU 39969, epitype designated here; ex-epitype culture CBS 144327 = XA1; MBT382221).

*Stromata* corymbose, 1–4 mm high, on a dark brown to black roughened stipe 0.5–0.8 mm diam, straight to furcate, ramifying upwards into 1–4 branches bearing a cluster of most often 2–5 fully exposed ascomata standing roughly at the same level; stromata either separate, upright and scattered, but more commonly compound, arising radially from a central common stipe 1–1.3 mm diam, forming dense, convex clusters 1–4.5 mm high × 2–8.5 mm diam. *Ascomata* subglobose to oval, 0.35–0.85 mm high and 0.3–0.5 mm diam, occasionally laterally collapsed; apex rounded to bluntly conical; surface black, slightly roughened, texture leathery when dry, rubbery when moistened; contents hyaline, gelatinous when rehydrated. *Peridium* 55–100 µm thick, pseudoparenchymatous, 3-layered: outermost layer 22–54 µm thick, of dark brown angular cells 7–20 µm in their greatest dimension, unevenly thick-walled, wall 0.8–1.5 µm thick, with clusters of cells protruding outwardly; median layer 18–35 µm thick, subhyaline, of hyphal to prismatic thick-walled cells, wall to 2 µm thick; inner layer 14–23 µm thick, pale brown, merging with the median layer, of similar, more pigmented prismatic cells. *Stipe* with dark brown outermost layer similar to that of ascomata and interior solid, pale brown, textura oblita, composed of vertically oriented moderately thick-walled hyphae 5.5–7.5 µm wide, continuous with those of the median layer of ascomatal wall. *Ostioles* inconspicuous to conic-papillate, ostiolar canal densely periphysate, periphyses 25–35 µm long and 2.5–3.0 µm wide, hyaline, with bluntly rounded ends. *Paraphyses* abundant, filiform, with free ends, 1.5–2.2 µm wide, hyaline, thin-walled, remotely septate, unbranched, embedded in a gelatinous matrix, filling the ascomatal centre and converging upwards beneath the ostiole. *Asci* peripheral, bitunicate, fissitunicate, narrowly clavate to slightly fusiform, apically broadly rounded, pars sporifera 24–34 × 6–7 µm, stipe 18–62 µm long, straight to most often contorted at the base, with a swollen furcate base; containing 8 or 4 obliquely uniseriate to irregularly biseriate ascospores at maturity with remnants of

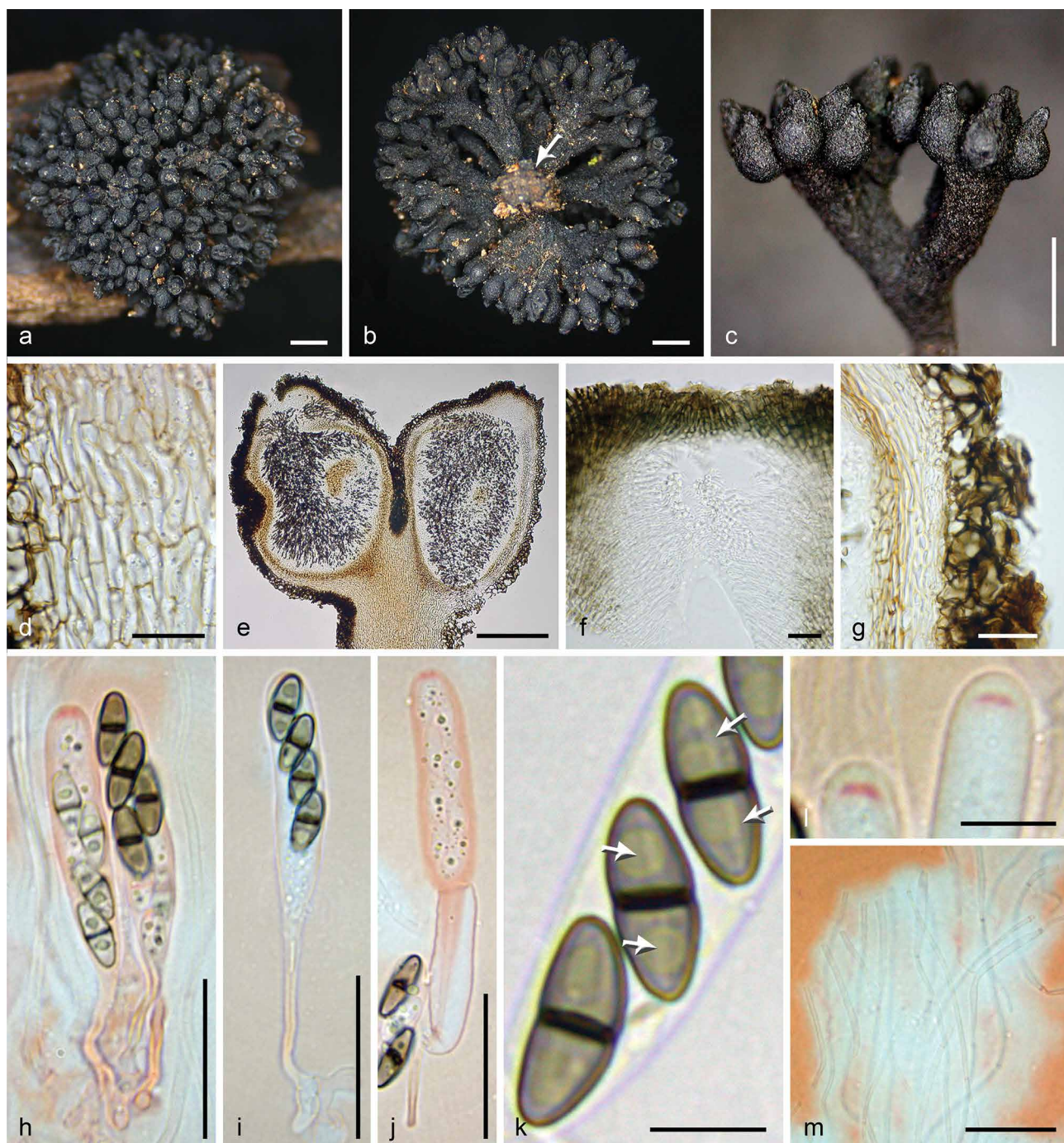


4 aborted hyaline ascospores usually still visible; apex with an inconspicuous discoid thickening  $0.7\text{--}1.0 \times 2.0\text{--}2.5\text{ }\mu\text{m}$  ( $n = 10$ ) stained by Congo red, not reacting in Melzer's reagent nor in Lugol's solution; ectotunica slightly refractive, firm, elastic, not easily ruptured, rupturing apically or basally under pressure on the cover slip; endotunica including the apical thickening swelling after dehiscence. *Ascospores* ellipsoid to slightly fusiform, equilateral, with broadly rounded ends,  $(8.0\text{--})9.0\text{--}9.5\text{--}(10.0) \times (2.9\text{--})3.3\text{--}3.5\text{--}(3.7)\text{ }\mu\text{m}$ ,  $l/w = (2.3\text{--})2.6\text{--}2.8\text{--}(3.0)$  ( $n = 120$ ), equally 2-celled, slightly constricted at the black,  $0.9\text{--}1.2\text{ }\mu\text{m}$  thick, occasionally slightly obliquely inserted septum, smooth-

walled, pale olivaceous brown, with 3–5 thin longitudinal germ slits in each cell extending over the whole length, without appendages or hyaline sheath visible in India ink.

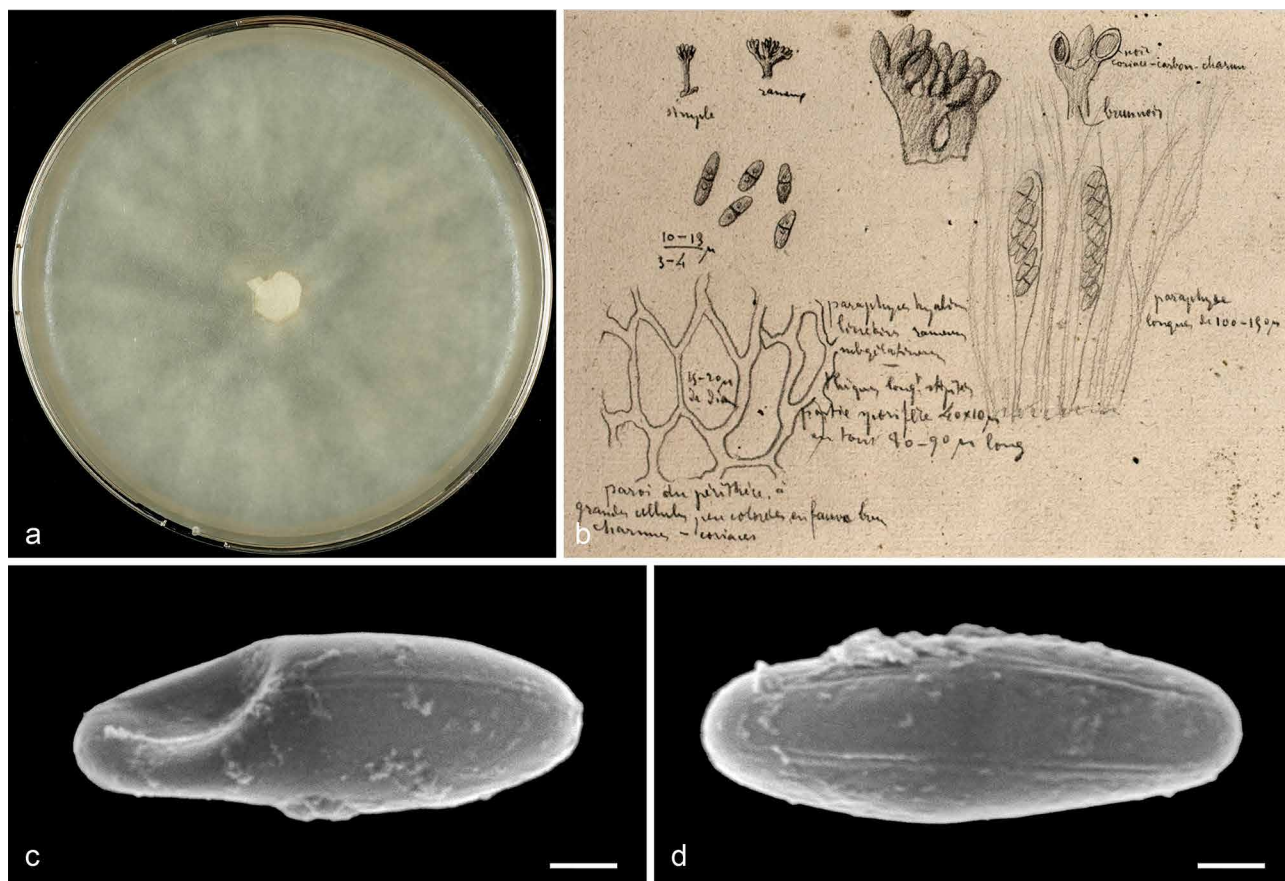
*Colonies* on CMD reaching 60 mm diam after 5 d at  $22\text{ }^{\circ}\text{C}$ , covering the entire plate after 7 d, first colourless and translucent, later becoming cream. *Mycelium* immersed, cobweb-like, of widely spaced, radially growing tortuous hyphae; aerial hyphae absent (Fig. 3a). *Colonies* on 2 % MEA inconspicuous. *Mycelium* immersed, of very widely spaced radially growing tortuous hyphae. No asexual morph observed.

*Habitat* — On dead, rarely living bark and wood of various trees and shrubs.



**Fig. 2** *Xylobotryum andinum*. a. Corymbose compound stroma in top view; b. reverse side of the compound stroma showing a central stipe (arrow); c. heads of branched stroma showing ascomata; d. stipe in vertical section; e. two adjacent ascomata in vertical section; f. ostiolar region in vertical section showing the periphyses; g. ascumatal wall in vertical section showing the 3-layered peridium; h. 4-spored immature and mature asci lined by paraphyses, showing contorted stipes, aborted hyaline ascospores and apical thickening; i. mature ascus with remnants of aborted ascospores; j. dehiscing immature ascus showing the entire endotunica containing eight hyaline ascospores; k. mature ascospores, two of which showing faint longitudinal germ slits (arrows); l. apical thickenings of two immature asci; m. paraphyses embedded in gel matrix (d–g. in chloral-lactophenol; h–j, l, m. in Congo red in 1 % SDS; k. in 1 % SDS) (a, b, d–m: WU 39969 (epitype); c: MJF 07074). — Scale bars: a–c = 1 mm; d, f–j = 20  $\mu\text{m}$ ; e = 0.2 mm; k, l = 5  $\mu\text{m}$ ; m = 10  $\mu\text{m}$ .





**Fig. 3** *Xylobotryum andinum*. a Colony on CMD (22 °C, 28 d); b. line drawings by Patouillard attached to the holotype (FH 01146514); c, d. SEM pictures of ascospores showing the longitudinal germ slits (WU 39969, epitype). — Scale bars: c, d = 1 µm.

**Distribution** — Widely distributed in tropical, subtropical to warm temperate, humid areas throughout the world; recorded from Australia, China, Dominica, Ecuador, Grenada, Guadeloupe, Guyana, India, New Zealand, Puerto Rico, Taiwan, Trinidad, USA, Venezuela (Rossman 1976, Rogerson et al. 1990, Ju & Rogers 1994, MyCoPortal 2018).

**Other material examined.** FRANCE, French Guiana, Régina, Nouragues natural reserve, Inselberg field centre, entrance of the eastern track, on a dead corticated branch on the ground, 16 June 2012, *J. Fournier* GYJF 12007 (WU 33542); Roura, Cacao, Molocoï trail, hygrophilous tropical rainforest, on a dead corticated trunk, 8 May 2008, *C. Lechat* CLL 8133 (WU 39970); French West Indies, Martinique, Le Morne-Rouge, forest trail of La Propreté, hygrophilous tropical rainforest, on a living corticated stump, 24 Aug. 2007, *C. Lécuru* MJF 07074; *ibid.*, on a dead corticated branch on the ground, 29 Aug. 2007, *J. Fournier* MJF 07199 (immature).

**Notes** — The ascospores of our collections are slightly smaller than recorded in the literature (e.g., 10–13 × 3–4 µm in Patouillard & De Lagerheim 1895; 10–12.5(–13) × (3–)3.5–4 µm in Rossman 1976). In addition, in our collections usually only four ascospores reached maturity in the ascus, the other four being aborted. Although in the literature mainly asci with eight fully developed ascospores were recorded (Patouillard & De Lagerheim 1895, Müller & Von Arx 1962, Rossman 1976), we do not consider this feature to be taxonomically important as all other features are in line with the 8-spored collections. Also, Ju & Rogers (1994) reported the occurrence of asci with four normal and four incompletely developed ascospores in their investigations. This phenomenon may be connected to the peculiar mating system recorded by Ju & Rogers (1994), which involves the production of four homothallic and four heterothallic ascospores per ascus. The original line drawings of the holotype (FH 01146514) by Patouillard are reproduced here as Fig. 3b. The longitudinal germ slits are scarcely noticeable in LM (Fig. 2k), but clearly seen in SEM (Fig. 3c, d).

***Xylobotryum portentosum* (Mont.) Pat., Bull. Soc. Mycol. France 16 (4): 185. 1900. — Fig. 4**

**Basionym.** *Sphaeria portentosa* Mont., Ann. Sci. Nat., Bot., sér. 2, 8: 358. 1837.

**Synonyms.** *Sphaeria antilopea* Lév., Ann. Sci. Nat., Bot., sér. 3, 5: 256. 1846.

*Xylaria portentosa* (Mont.) Mont., Syll. Gen. Sp. Crypt. (Paris): 201. 1856.  
*Xyloceras elliottii* A.L. Sm. (as '*elliotti*'), J. Linn. Soc., Bot. 35 (no. 242): 16. 1901.

*Trachyxylaria phaeodidyma* Möller, Bot. Mitt. Tropen 9: 308. 1901.

**Stromata** upright, unbranched, terete to flattened, straight to curved, 18–43 mm high including the 4–8 mm long and 1.8–6 mm wide stipe, tapering into a fertile, flattened, obtuse or acuminate apex; surface black, roughened by superficial ascomata, stipe glabrous; interior loosely fibrous to woolly, fulvous. **Ascomata** subglobose, 0.25–0.55 mm diam, sessile or short-stipitate, occasionally laterally collapsed, apically papillate; surface black, slightly roughened, texture leathery when dry, rubbery when moist; contents hyaline, slightly gelatinous when rehydrated. **Peridium** 35–60 µm thick, pseudoparenchymatous, 3-layered: outermost layer 18–30 µm thick, of dark brown, angular cells 7–22 µm in their greatest dimension, unevenly thick-walled, wall 1–2 µm thick; median layer 7–16 µm thick, subhyaline, of elongate to prismatic thin-walled cells; inner layer 7–20 µm thick, pale brown, merging with the median layer, of similar, more pigmented prismatic cells; wall to 1 µm thick. **Stromatal crust** continuous with the ascomatal walls, with an outermost dark brown layer 20–38 µm thick, homologous with that of ascomata; inner layer 180–270 µm thick, composed of very thick-walled prismatic cells up to 60 µm in their greatest dimension, wall 3.5–4.5 µm thick, subhyaline, becoming gradually reddish brown inwardly; hyphae of the internal tissue loosely intertwined, 5.5–10 µm wide, reddish brown,





**Fig. 4** *Xylobotryum portentosum* (WU 33543). a. Stromata in side view, three attached to their substrate; b. apex of a stroma showing the acuminate tip and the surface roughened by superficial ascomata; c. stromatal surface in close-up showing globose ascomata with raised-diskoid ostioles and laterally collapsed ascomata (arrows); d. stroma in vertical section showing the black outer crust bearing superficial ascomata, some short-stipitate (arrows), and the fulvous, loosely fibrous to woolly interior; e. sessile ascoma in vertical section; f. lateral ascomatal wall in vertical section; g. stromatal crust in vertical section; h. 4- and 7-spored asci; i, j. 8-spored asci featuring large and smaller ascospores in equal parts; k. dehiscence of an immature ascus; l. branched base of a paraphysis; m. apical thickenings of two immature asci; n. mature ascospore showing faint longitudinal germ slits (arrows) (e–g. in chloral-lactophenol; h, n. in 1 % SDS; i, k–m. in Congo red in 1 % SDS; j. in chlorazol black). — Scale bars: a = 10 mm; b = 1 mm; c, e = 0.2 mm; d = 0.5 mm; f = 20 µm; g = 100 µm; h–l = 10 µm; m, n = 5 µm.

septate, thin-walled, wall to 0.8  $\mu\text{m}$  thick. *Ostioles* conspicuous, bluntly papillate to raised-discoïd, 80–170  $\mu\text{m}$  diam at the base; ostiolar canal densely periphysate, periphyses 25–40  $\mu\text{m}$  long and 2–2.8  $\mu\text{m}$  wide, hyaline, with bluntly rounded ends. *Paraphyses* abundant, basally 2–3  $\mu\text{m}$  wide and occasionally branched, filiform between and above asci and 1–1.8  $\mu\text{m}$  wide, with free ends, hyaline, thin-walled, remotely septate, embedded in slightly gelatinous matrix, filling the ascomatal centre and converging upwards beneath the ostiole. *Asci* peripheral, bitunicate, fissitunicate, narrowly clavate to slightly fusiform, apically broadly rounded, pars sporifera 27–32  $\times$  5.5–6.5  $\mu\text{m}$ , stipe 18–45  $\mu\text{m}$  long, straight to most often contorted at base, with a swollen furcate base; containing 8 obliquely uniseriate to irregularly biseriate ascospores, with four significantly smaller ascospores randomly distributed in the ascus (GYJF 12004); apex with an inconspicuous discoïd thickening 0.8–0.9  $\times$  2.0–2.5  $\mu\text{m}$  ( $n = 6$ ) faintly stained by Congo red, darker in fresh material, not reacting in Melzer's reagent nor in Lugol's solution; ectotunica slightly refractive, firm, elastic, not easily ruptured, rupturing apically or basally under pressure on the cover slip; endotunica swelling after dehiscence. *Ascospores* ellipsoid to slightly fusiform, equilateral, with broadly rounded ends, (7.8–)8.8–10.2(–11.7)  $\times$  (3.0–)3.3–3.8(–4.1)  $\mu\text{m}$ ,  $l/w = (2.2–)2.6–2.7(–3.1)$  ( $n = 120$ ), equally 2-celled, slightly constricted at the black, 0.8–1  $\mu\text{m}$  thick, occasionally slightly obliquely inserted septum, smooth-walled, pale olivaceous brown, with 3–5 thin longitudinal germ slits in each cell extending over the whole length, without appendages or hyaline sheath visible in India ink. No asexual morph observed.

**Habitat** — On dead bark and wood of various trees and shrubs; commonly recorded from tree ferns (*Cyathea* spp.).

**Distribution** — Tropical and subtropical Central and South America; recorded from Brazil, Chile, Colombia, Costa Rica, Dominica, Ecuador, French Guiana, Guadeloupe, Guyana, Jamaica, Martinique, Nicaragua, Panama, Peru, Puerto Rico (Rossman 1976, Trierveiler-Pereira et al. 2008, Guzmán & Piepenbring 2011, Mushroom Observer 2018, MyCoPortal 2018).

**Specimens examined.** FRANCE, French Guiana, Régina, Nouragues Nature Reserve, track K, 50 m upstream from 'pont ficelle', on a corticated moss-covered branch on the ground, 16 June 2012, J. Fournier GYJF 12004 (WU 33543 = XP); French West Indies, Guadeloupe, without place and exact date, 1998, on *Cyathea* sp., leg. J. Vivant, comm. F. Candoussau, det. G. J. Samuels, JF 98169 (WU 39971).

**Notes** — The collection from Guadeloupe features a larger stroma and larger ascomata than GYJF 12004. Its asci contain eight ascospores of roughly the same dimensions, unlike those of the Guianese collection. Smaller ascospores 6–7  $\times$  2.6–3.2  $\mu\text{m}$  (mean = 6.7  $\times$  2.9  $\mu\text{m}$ ,  $n = 30$ ) occurring in groups of four in most asci of collection GYJF 12004 were not taken into account in the above measurements. This is in comparison with *X. andinum* in which asci are either 8-spored (in literature) or 4-spored with four aborted hyaline ascospores (JF, pers. obs.; see above). Likewise (see above), ascospore dimensions of *X. portentosum* are fairly variable, those recorded here being slightly smaller than usually reported (e.g., 10–13  $\times$  3–4  $\mu\text{m}$  in Patouillard 1900; (9.5–)10–13(–15)  $\times$  3–4(–4.5)  $\mu\text{m}$  in Trierveiler-Pereira et al. 2008). Based on co-occurrence of both *Xylobotryum* species at the same localities, Rogerson et al. (1990) suspected *X. portentosum* to be conspecific with *X. andinum*; however, our molecular and morphological data clearly disprove this. For additional illustrations of fresh collections of *X. portentosum* see Læssøe & Petersen (2008) and Guzmán & Piepenbring (2011).

## DISCUSSION

### *Phylogenetic relationships and classification of Xylobotryum and Cirrosporium*

The unique suite of characters of *Xylobotryum* not matching any other lineage of *Ascomycota*, in combination with mostly superficial morphological investigations and the lack of molecular data, resulted in uncertainties about its systematic affiliations, which up to now precluded a comprehensible higher-level classification within *Ascomycota*. In the past, the genus has been either attributed to groups now classified in *Sordariomycetes* (e.g., Müller & Von Arx 1962, Dennis 1970, Rossman 1976) or *Dothideomycetes* (e.g., Barr 1987, 1990, Rogerson et al. 1990, Huhndorf 1994, Ju & Rogers 1994), but mostly without convincing evidence. For instance, Barr (1987) concluded that *Xylobotryum* should be best accommodated in the *Didymosphaeriaceae* based on ascus characters and 1-septate ascospores; however, at the same time she expressed doubts about her tentative disposition due to the unique stipitate stromata unparalleled in *Dothideomycetes*. In their detailed pure culture, light and electron microscopy study, which was the first thorough investigation of the micromorphology and life cycle of *Xylobotryum*, Ju & Rogers (1994) revealed the asci to be functionally bitunicate, indicating dothideomycetous affinities. However, in light of the unique character combination of *Xylobotryum*, they remained uncertain about its systematic affiliation. Since then, no new data on the genus *Xylobotryum* have become available.

Our molecular phylogenetic results, which place *Xylobotryum* neither in *Sordariomycetes* nor in *Dothideomycetes* but near the *Eurotiomycetes*, shed new light on the apparently conflicting evidence of the studies cited above. Considering that in the pre-molecular era part of the *Eurotiomycetes* (e.g., *Chaetothyriomycetidae*) were considered to have dothideomycetous affinities due to their fissitunicate asci, the results of our molecular phylogenies are not surprising. The *Eurotiomycetes* are a morphologically extremely heterogeneous lineage, which makes it impossible to give diagnostic features for the whole class (Geiser et al. 2015, Jaklitsch et al. 2016). They currently contain the five subclasses *Chaetothyriomycetidae*, *Coryneliomycetidae*, *Eurotiomycetidae*, *Mycocaliciomycetidae* and *Sclerococcomycetidae* (Réblová et al. 2017). Ascomata of *Eurotiomycetes* can be gymno- or cleistothecial (*Eurotiomycetidae*), perithecioid or rarely apothecial (*Chaetothyriomycetidae*, *Coryneliomycetidae*), apothecial (*Sclerococcomycetidae*) or apothecial-mazaediate (*Mycocaliciomycetidae*). Also the asci are highly variable: globose, thin-walled and evanescent in *Eurotiomycetidae*; unitunicate, non-amyloid with an external amyloid gelatinous cap ('*Dactylospora*-type' fide Bellemère & Hafellner 1982) in *Sclerococcomycetidae*; unitunicate, cylindrical, non-amyloid, with or without an apical thickening and sometimes evanescent in *Mycocaliciomycetidae*; initially bitunicate with early deliquescing ectotunica and an endotunica deliquescing at maturity in *Coryneliomycetidae*; and fissitunicate in *Chaetothyriomycetidae*. In their extensive analyses of *Ascomycota*, Schoch et al. (2009) resolved the ancestor of the *Eurotiomycetes* as fissitunicate, which is in line with our analyses where *Xylobotryum* and *Cirrosporium* are revealed as basal to the *Eurotiomycetes* (Fig. 1). A classification of *Cirrosporium* within *Eurotiomycetes*, close to the *Mycocaliciales*, possibly in a new order, was suggested by Réblová & Seifert (2012). They discussed and noted that morphological characters such as stipitate ascomata of the *Coryneliales* and *Mycocaliciales* and a dark brown ascospore wall of some species of the *Mycocaliciales*, bear some superficial similarity to the large, cylindrical pycnidia and the dark brown conidial cell walls with thick septa of *Cirrosporium*. They also hypothesized that the meristematic hyphal



growth in some species of the *Chaetothyriales* and *Onygenales*, and the coelomycetous asexual morphs in the *Mycocaliciales* and *Coryneliales* support a relationship between *Cirrosporium* and the *Eurotiomycetes*. However, the extreme morphological and ecological heterogeneity of the *Eurotiomycetes* facilitates detection of convergences or similarities. Also, a discussion of each morphological character does not make much sense as, e.g., both the characters longitudinal germ slit (*Xylobotryum*) and meristem arthroconidia (*Cirrosporium*) are polyphyletic and occur in several unrelated classes of *Ascomycota*. Longitudinal germ slits in brown ascospores are particularly common in *Sordariomycetes* (e.g., *Cainiaceae*, *Coniooeciaceae*, *Coniochaetaceae*, *Lopadostomataceae*, *Xylariaceae*), but also occur in *Dothideomycetes* (e.g., *Delitschiaceae*, *Hypsostromataceae*, *Sporormiaceae*) and *Leotiomycetes* (*Bulgaria*; see Jaklitsch et al. 2014, 2016). Meristem arthroconidia occur, e.g., also in *Leotiomycetes* (*Erysiphe*, *Trimmatostroma*), *Dothideomycetes* (*Hysterium*) and other genera of unknown affinities (Réblová & Seifert 2012).

Our phylogenetic tree (Fig. 1), which contains a more representative taxon selection and more DNA data than the earlier analyses of Réblová & Seifert (2012), shows a position of the *Xylobotryum*-*Cirrosporium* clade near the *Mycocaliciomycetidae*. However, the former is basal to the whole class *Eurotiomycetes* and does not exhibit a convincingly strong affiliation to this class. An affiliation of the clade to the *Eurotiomycetes* is only moderately supported by ML analyses (84 %), weakly supported by MP analyses (57 %) and unsupported in Bayesian analyses. In the latter, the *Xylobotryum*-*Cirrosporium* clade is located outside the *Eurotiomycetes* as sister to the *Dothideomycetes*-*Arthoniomycetes* clade with insignificant support (0.93 PP). Only if the results of ML analyses are considered alone, it may be possible to define this clade as a subclass of *Eurotiomycetes*, but this would only increase the extreme heterogeneity of this large class even more. Thus, low and inconsistent phylogenetic support and the unparalleled morphological traits of *Xylobotryum* and *Cirrosporium* warrant classification of these genera in a new class, the *Xylobotryomycetes*.

The close phylogenetic relationship of *Xylobotryum* and *Cirrosporium* is unexpected and remarkable. Morphological comparison of these genera is difficult, as neither an asexual morph of *Xylobotryum* nor a sexual morph of *Cirrosporium* is known. The large, cylindrical, tubular, dark brown pycnidia in combination with a unique meristem arthric conidium ontogeny producing brown, 3-septate conidia aggregated in conspicuous columnar cirrhi in *Cirrosporium* is unparalleled in ascomycetes (Hughes 1980, Réblová & Seifert 2012). Also, the combination of characters in *Xylobotryum*, viz. upright stipitate stromata bearing superficial ascomata, apically free paraphyses, fissitunicate asci and bicellular brown ascospores with several longitudinal germ slits, is unique. Considering the differences of these character combinations, it is unlikely that *Cirrosporium* and *Xylobotryum* are only distinct at the generic level, but they justify their classification as two distinct families *Cirrosporiaceae* and *Xylobotryaceae* within an order *Xylobotryales*.

### Phylogenetic placement of *Candelariales*

There is some disagreement and incongruence about the phylogenetic placement, circumscription and classification of the *Candelariales*. In all phylogenetic analyses (e.g., Wedin et al. 2005, Miądlikowska et al. 2006, 2014, Hofstetter et al. 2007, Lumbsch et al. 2007, Schoch et al. 2009, Prieto et al. 2013), the *Candelariales* occupy an isolated phylogenetic position outside the core *Lecanoromycetes*, which, however, varies substantially, depending on the selection of taxa included in the analyses. In acknowledging their phylogenetic distinctness,

Miądlikowska et al. (2006) informally introduced the '*Candelariomycetidae*', which was formally established as a subclass of *Lecanoromycetes* by Lücking et al. (2017). In the extensive analyses of Miądlikowska et al. (2014), the *Candelariales* formed a rather basal clade within *Lecanoromycetes* and were revealed as sister group of *Dactylospora*. This *Candelariales*-*Dactylospora* clade was placed between the more basal *Acarosporomycetidae* clade and the core *Lecanoromycetes* clade consisting of the subclasses *Ostropomycetidae*, *Umbilicariomycetidae* and *Lecanoromycetidae*. However, in their analyses the *Pycnoraceae*, which were revealed as sister group of *Candelariaceae* within *Candelariales* by Bendiksby & Timdal (2013), were not only placed outside the *Candelariales* but even outside the *Lecanoromycetes*. It has to be noted that the analyses of Miądlikowska et al. (2014), like most other studies focussing on *Lecanoromycetes* (e.g., Miądlikowska et al. 2006, Hofstetter et al. 2007, Lumbsch et al. 2007), seriously suffer from an insufficient outgroup selection that evidently greatly influences the topologies of the basal nodes of the trees. Miądlikowska et al. (2014) only included few species from *Leotiomycetes*, *Lichinomycetes* and *Geoglossomycetes*, but did not include any representatives of other classes of *Leotiomycetes* like *Sordariomycetes*, *Dothideomycetes*, and, most notably, *Eurotiomycetes*. This is well illustrated by the position of *Dactylospora*, which was revealed as a member of *Eurotiomycetes* by Réblová et al. (2017) where it was placed in their new subclass *Sclerococcomycetidae* with maximum support, a position fully matching our analyses (Fig. 1). Therefore, although the analyses of Miądlikowska et al. (2014) are highly conclusive for the core *Lecanoromycetes*, the highly biased outgroup selection does not allow for an evaluation of the phylogenetic position of basal lineages like *Candelariales* and *Dactylospora* in their tree.

Our analyses confirm the results of Schoch et al. (2009) and Prieto et al. (2013) that the *Candelariales* are phylogenetically separate from *Lecanoromycetes*. We argue that the results of the multigene analyses of Schoch et al. (2009), Prieto et al. (2013) and our analyses are more conclusive concerning the remote phylogenetic position of *Candelariales* due to the more significant taxon selection, including representatives from most (Schoch et al. 2009, Prieto et al. 2013) to almost all (our data set; all except *Laboulbeniomycetes*) currently accepted classes of *Leotiomycetes*. In our analyses, the *Pycnoraceae* are sister group to *Candelariaceae* with medium (88 % ML and 79 % MP) to maximum (1.0 PP) support (Fig. 1), confirming a close relationship and classification of both families within *Candelariales*. By providing new sequences for all five genes for *Pycnora sorophora*, the sequence data were significantly extended for a member of *Pycnoraceae*, compared to previous analyses which were, with the exception of a short *rpb1* sequence for *P. praestabilis*, restricted to nLSU and mtSSU data, adding to increasing support for a sister group relationship of *Pycnoraceae* and *Candelariaceae*. The phylogenetic position of *Candelariales* is unstable and shifting around in the various analyses depending on the taxon selection and analysis method, but it was never revealed as belonging to *Lecanoromycetes* when a representative matrix of *Leotiomycetes* was included. We therefore argue that it should be recognised as a class of its own, *Candelariomycetes*.

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