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ORIGINAL ARTICLE

Novel fungi from an ancient niche: lachnoid and chalara-like fungi on ferns

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Abstract A survey was conducted in Brazil to collect fungi on ferns. Based on morphology and inferred phylogeny from DNA sequences of two loci, namely the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (LSU), several species belonging to chalara-like genera and lachnoid fungi were recognized. Eighteen fungal isolates, collected from five host species, representing 10 different localities were studied. Three novel genera (*Lachnopsis*, *Scolecolachnum* and *Zymochalara*), and six novel species (*Bloxamia cyatheicola*, *Lachnopsis catarinensis*, *Lachnopsis dicksoniae*, *Scolecolachnum pteridii*, *Zymochalara lygodii* and *Zymochalara cyatheae*) are introduced. Furthermore, two new combinations (*Erioscyphella euterpes* and *Erioscyphella lushanensis*) are proposed. Two novel taxa (*Lachnopsis catarinensis* and *Lachnopsis dicksoniae*) may be included in the list of potentially endangered fungal species in Brazil, if proven to be restricted to their tree-fern host, *Dicksonia sellowiana*, which is included in the official list of endangered plant species in Brazil.

Keywords *Bloxamia* · *Chalara* · Conservation · Endangered species · *Lachnum* · Tropical ferns

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Introduction

Numerous fungal taxa have been published from Brazil in recent years. These represent additions to its rich, but rather underexplored, fungal biodiversity. Surveys for Brazilian fungi have followed biome-based approaches such as for the Cerrado (Hernández-Gutiérrez and Dianese 2014; Hernández-Gutiérrez et al. 2014; Armando et al. 2015) and the Caatinga (Almeida et al. 2012; Fiuza et al. 2015; Izabel et al. 2015), crop-based approaches such as for *Eucalyptus* (Cândido et al. 2014; Rodrigues et al. 2014; Alfenas et al. 2015; Oliveira et al. 2015), and weed-based approaches (Guatimosim et al. 2015a; Macedo et al. 2013, 2016), among others. A plethora of mycological novelties emerged from such systematic surveys, particularly when these involved groups of host-plants that were poorly studied by mycologists. An example of a poorly studied niche for fungi is the tropical Brazilian fern flora. Ferns are members of Pteridophyta (= 'Monilophyta'), and represent some of the oldest lineages of vascular plants (Smith et al. 2008). In recent classifications (e.g., Smith et al. 2008), the division includes 37 families, approximately 300 genera and more than 9000 species. Around 48 fungal species have been recorded on ferns from Brazil (Farr and Rossman 2015; Mendes and Urben 2015). This is a very small number of species, especially considering that the number of fern species in Brazil is estimated to be more than 1110 (Forzza et al. 2015). If

the postulated 5–6 unique fungal species per plant species (Hawksworth 1991) holds true for ferns, thousands of undescribed fungi may wait to be named from this group of hosts. In a recent study focused on cercosporoid fungi causing frond diseases on Brazilian ferns, 1 new genus, 15 new species, 11 new combinations and 9 new host records were published (Guatimosim et al. 2016), confirming that this group of plants harbors a highly diverse and overlooked mycobiota.

The present work also aimed at contributing to the field of fungal conservation in Brazil, representing an expansion of a theme covered in two previously published studies (Rocha et al. 2010; Silva et al. 2016). Throughout the survey, collections were systematically made of fungal pathogenic, or seemingly pathogenic, to *Dicksonia sellowiana*. This large and slow-growing tree fern species (known in Brazil as 'xaxim' or 'samambaiaçu') used to be a common component of the Brazilian Atlantic rainforest, but progressively became rare, owing to biome destruction and unsustainable exploitation by the vase and substrate industry (Windisch 2002). It is included in the official list of Brazilian species threatened with extinction (Pillar et al. 2009). This and previous publications involved the search of unique, specialized and potentially host-specific fungi, therefore under threat of co-extinction simultaneously with their sole host-species. As in the previous studies, it was recognized that, before any attempt to include such organisms in a novel list of endangered fungi from Brazil, surveying the fungi associated with the selected hosts and clarifying their taxonomy would be critical.

Lachnoid fungi are members of the Hyaloscyphaceae *sensu lato*, which is considered the largest family in Helotiales, comprising about 933 species belonging to 74 genera (Kirk et al. 2008). Species in this family are small discomycetes, with brightly colored apothecial ascomata that are ornamented with more or less conspicuous hairs along the apothecial margins and the outside of the receptacle (Han et al. 2014). Earlier studies by Cantrell and Hanlin (1997) suggested that the family was probably monophyletic, and, based on this premise, mycologists have considered the presence of hairs on the ascomata as a synapomorphic character (Han et al. 2014).

Based on morphology, Hyaloscyphaceae was subdivided into three tribes: Arachnopezizeae, Hyaloscypheae, and Lachneae (Nannfeldt 1932). Arachnopezizeae includes species with apothecia formed on a well-developed subiculum or in a false subiculum-like hyphal layer. Hyaloscypheae has species with tiny apothecia bearing hairs that have highly diverse shapes, and mostly cylindrical paraphyses, while Lachneae includes species with relatively large apothecia, multiseptate, usually granulate hairs, and lanceolate paraphyses (Nannfeldt 1932).

Raitviir (2004) employed morphological characters to elevate Lachneae to familial level—Lachnaceae, Baral (2015) elevated Arachnopezizeae to familial level—Arachnopezizaceae, and Hosoya et al. (2010), using morphology and multi-locus DNA sequence data, confirmed this hypothesis. The latter authors, however, acknowledged that low taxon sampling was a barrier to an adequate understanding of the taxonomy of Lachanaceae. In the most recent work dealing with taxonomy of Hyaloscyphaceae *sensu lato*, Han et al. (2014) examined the morphological characteristics in the context of multi-locus DNA sequence data and, based on 70 species belonging to each of the formerly accepted tribes, demonstrated Hyaloscyphaceae to be polyphyletic; they rejected the presence of hairs as a synapomorphic feature for the family. Additionally, Hyaloscyphaceae *sensu stricto* was tentatively restricted to the genus *Hyaloscypha*, although a more extensive sampling within this family is recognized as required for further confirmation of this hypothesis (Han et al. 2014).

Chalara asexual morphs are relatively poor in distinctive features that would allow a natural subdivision of this polyphyletic group (Cai et al. 2009). The monograph by Nag Raj and Kendrick (1975) is just a first step in its resolution. Since DNA sequencing became available to properly evaluate the evolutionary relationships among fungi, only some representative taxa of the chalara-like complex have been thoroughly studied (Réblová 1999; Coetsee et al. 2000). The segregation of the *Ceratocystis*-related taxa (Microascales) has become well established (Paulin-Mahady and Harrington 2000; Paulin-Mahady et al. 2002), while the bulk of the genus in the Helotiales is still poorly resolved, and the type species of the genus is not yet available in culture.

Species within *Bloxamia* are sporodochial, having scattered or gregarious, black, disciform sporodochia, with pale brown superficial stromata composed of subhyaline to pale brown cells, arranged in dense palisades, from which the conidiophores emerge and produce catenulate, hyaline conidia (Nag Raj and Kendrick 1975). The genus is based on *Bl. truncata* occurring on dead decorticated wood of *Ulmus* sp. from England (Pirozynski and Morgan-Jones 1968). Presently seven species are recognized within *Bloxamia*, as summarized in Table 2 (below).

A morphological and phylogenetic-based study involving inference of two DNA regions (ITS and LSU) was performed on chalara-like genera and lachnoid species collected on ferns in Brazil, and the results are presented here.

Materials and methods

Specimens and isolates

Frond samples of five fern species bearing fungal colonies were collected in Brazil from different biomes, including the Amazon, Atlantic rainforest, Caatinga and Cerrado between 2011 and 2014. These were examined under a Nikon SMZ1500 stereo-microscope (Nikon Instruments, Tokyo, Japan) and later dried in a plant press. Conidia were scraped

from a single frond spot, and single-conidial colonies were established on potato carrot agar (PCA; Crous et al. 2009). Ascospore-grown colonies were obtained by excising fragments from selected ascomata and fastening them on the inner side of Petri dish lids. Such dishes contained PCA and were inverted. Ascospores shot onto the surface of the medium were transferred individually onto fresh plates after germination, and observed under an Olympus SZX7 stereo microscope (Olympus, Tokyo, Japan). Freehand sections of fungal colonies were prepared and fungal structures mounted in water, clear lactic acid, lactofuchsin, Melzer's reagent and Lugol. Whenever necessary, sections were made using a Microm HM520 freezing microtome (Microm, Neuss, Germany). Observations and images were made with a Nikon Eclipse 80i (Nikon Instruments) compound microscope with differential interference contrast illumination fitted with a Nikon DS-Fi1 camera. Images were processed with NIS-Elements imaging software (Nikon Instruments). Colony descriptions were based on observations of colonies formed on potato dextrose agar (PDA; Crous et al. 2009) and PCA, incubated at 25 °C in the dark, and under a 12-h light regime. After 30 days, the colony diameter was measured and the colony color was described following the terminology of Rayner (1970). Representative fungarium specimens were deposited at the Fungarium of the Universidade Federal de Viçosa (VIC). Axenic cultures were deposited at the working collection of P.W. Crous (CPC), housed at the CBS-KNAW Fungal Biodiversity Centre, and at the Coleção Octávio de Almeida Drumond (COAD, Universidade Federal de Viçosa). A complete list of the isolates used in this study is presented in Table 1.

Scanning electron microscopy

Samples of dried material containing fungal structures were mounted on stubs with double-sided adhesive tape and gold-coated using a Balzer's FDU 010 sputter coater (Optics Balzers, Neugrüt, Liechtenstein). A LEO VP 1430 scanning electron microscope (SEM; Carl-Zeiss, Jena, Germany) was used to analyze and generate images from the samples.

DNA isolation, amplification and sequencing

Isolates were grown on 2 % malt extract agar (MEA; Crous et al. 2009) for 20 days at 25 °C on the laboratory bench. Genomic DNA was extracted from mycelium scraped from colonies of each isolate using the Wizard® Genomic DNA Purification Kit (Promega, WI, USA) following the manufacturer's instructions. For *Bloxamia* species, fronds harboring fertile stromata were examined under a dissecting microscope to check for possible contamination by other fungi, including yeasts. The fronds were then soaked in sterile water for 1 h in order to hydrate the specimens and facilitate removal of the stromata. Thirty fertile stromata were removed from the fronds with a sterile fine-pointed needle, and placed into a microcentrifuge tube (1.5 mL). Total genomic DNA was extracted as described above in addition to the steps described by Pinho et al. (2012). The DNA samples were subsequently diluted 50–100 times in preparation for further DNA amplification reactions. Three partial nuclear genes were targeted for PCR amplification and sequencing, namely, the 18S nrRNA gene (SSU), the 28S nrRNA gene (LSU) and the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrDNA operon. The primer pair NS1 + NS4 (White et al. 1990) was used to amplify and sequence the SSU locus, the primer pair LR0R + LR5 (Vilgalys and Hester 1990) was used to amplify and sequence the LSU locus, whereas the ITS locus was amplified and sequenced with the primer pair ITS5 + ITS4 (White et al. 1990). The PCR amplifications were performed in a total volume of 12.5 μL solution, containing 10–20 ng of template DNA, 1× PCR buffer, 0.63 μL DMSO (99.9 %), 1.5 mM $MgCl_2$, 0.5 μM of each primer, 0.25 mM of each dNTP, 1.0 U BioTaq DNA polymerase (Bioline, Luckenwalde, Germany). PCR conditions were set as follows: an initial denaturation temperature of 95 °C for 5 min, followed by 35 cycles of denaturation temperature of 95 °C for 30 s, primer annealing at 52 °C for 30 s, primer extension at 72 °C for 1 min, and a final extension step at 72 °C for 1 min. The resulting fragments were sequenced using the PCR primers and the BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA, USA) following the protocol of the manufacturer. DNA sequencing amplicons were purified through Sephadex® G-50 Superfine columns (Sigma Aldrich, St. Louis, MO, USA) in MultiScreen HV plates (Millipore, Billerica, MA, USA). Purified sequence reactions were run on an ABI Prism 3730xl DNA Sequencer (Life Technologies, Carlsbad, CA, USA).

IDNA sequence data were analyzed in Molecular Evolutionary Genetics Analysis v.6.0 (MEGA; Tamura et al. 2013). Consensus sequences were generated and imported into MEGA for initial alignment and the construction of sequence datasets. Initially, sequences obtained from the datasets of Crous et al. (2014, TreeBASE S16625), Han et al. (2014, TreeBASE S12034), Hosoya et al. (2010), Perić and Baral (2014), from GenBank (www.ncbi.nlm.nih.gov), and the novel sequences generated on this study, were aligned using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/index.html; Katoh et al. 2002) and, whenever indicated, manually improved in MEGA. After a preliminary analysis, the datasets were trimmed down to Brazilian isolates and their direct neighbors.

Table 1 Collection details and GenBank accession numbers of isolates included in this study

Species	Culture / specimen accession numbers[a]	Host/isolation source	Country	Collector	GenBank accession numbers[b]			Reference
					ITS	LSU	SSU	
Albotricha acutipila	TNS-F-16740	Stem of bamboo	Japan	–	AB481234	AB481317	–	Hosoya et al. 2010
Al. albotestacea	TNS-F-16497	*Miscanthus* sp.	Japan	–	AB481235	AB481303	–	Hosoya et al. 2010
Ambrosiella beaveri	CBS 121753 = CMW 26179	*Vitus rotundifolia*	USA	D. Six	–	KM495315	–	de Beer et al. 2014
Am. hartigii	CBS 403.82 = CMW 25525	*Acer* sp.	Germany	–	–	KM495317	–	de Beer et al. 2014
Am. xylebori	CBS 110.61 = CMW 25531	*Coffea canephora*	Ivory Coast	L. Brader	–	KM495318	–	de Beer et al. 2014
Arachnopeziza aurata	JHH 2210	–	USA	J.H. Haines	U57496	–	–	Cantrell and Hanlin 1997
Bloxamia cyatheicola	**VIC 42563^T**	*Cyathea delgadii*	Brazil	R.W. Barreto	KU597790	KU597757	KU597775	This study
	VIC 42579	*Cyathea delgadii*	Brazil	R.W. Barreto	KU597789	KU597756	KU597774	This study
	VIC 42574	*Cyathea atrovirens*	Brazil	R.W. Barreto	KU597788	KU597755	KU597773	This study
	VIC 42584	*Cyathea delgadii*	Brazil	R.W. Barreto	KU597791	KU597758	KU597776	This study
	VIC 42460	*Cyathea delgadii*	Brazil	E. Guatimosim	KU597792	KU597759	KU597777	This study
Brunnipila clandestina	JHH 4676	–	USA	J.H. Haines	U58636	–	–	Cantrell and Hanlin 1997
Br. fuscescens	JHH 4035	–	USA	J.H. Haines	U58637	–	–	Cantrell and Hanlin 1997
	TNS-F-16637	*Lindera obtusiloba*	Japan	R. Sasagawa	AB481254	–	–	Hosoya et al. 2010
	TNS-F-16635	*Lindera obtusiloba*	Japan	R. Sasagawa	AB481255	AB481311	–	Hosoya et al. 2010
Brunnipila sp.	TNS-F-16535	*Quercus crispula*	Japan	R. Sasagawa	AB481283	AB481301	–	Hosoya et al. 2010
	TNS-F-16691	*Fallopia* sp.	Japan	R. Sasagawa	AB481273	AB481320	–	Hosoya et al. 2010
Calycina citrina	F115889	*Fagus sylvatica*	Spain	–	KC412004	–	–	Baral et al. 2013
	F118000	*Quercus robur*	Spain	–	KC412005	–	–	Baral et al. 2013
	Andy 9-27-03	–	–	–	AY789385	AY789386	–	Wang et al. 2005
Cal. claroflava	F132983	*Quercus ilex*	Spain	–	KC412006	–	–	Baral et al. 2013
Cal. herbarum	isolate 1549	–	–	–	AY348594	–	–	Zhang and Zhuang 2004
	KUF-F51458	*Boehmeria* sp.	Korea	–	JN033390	JN086693	–	Han et al. 2014
	KUS-F52362	unidentified herb	Korea	–	JN033407	JN086710	–	Han et al. 2014
Cal. languida	F116599	*Fagus sylvatica*	Spain	–	KC412002	–	–	Baral et al. 2013
	F116600	*Fagus sylvatica*	Spain	–	KC412003	–	–	Baral et al. 2013
Cal. populina	CBS 247.62 = KACC45615	–	France	–	JN033382	JN086685	–	Han et al. 2014
Capitotricha bicolor	JHH 4596	–	USA	J.H. Haines	U59005	–	–	Cantrell and Hanlin 1997

Table 1 (continued)

Species	Culture / specimen accession numbers[a]	Host/isolation source	Country	Collector	GenBank accession numbers[b]			Reference
					ITS	LSU	SSU	
Chalara acuaria	HKUCC OC0014	–	–	–	–	FJ176248	–	Cai et al. 2009
Ch. alabamensis	HKUCC OC0005	–	–	–	–	FJ176247	–	Cai et al. 2009
Ch. aspera	HKUCC OC0004	–	–	–	–	FJ176244	–	Cai et al. 2009
	HKUCC OC0009	–	–	–	–	FJ176245	–	Cai et al. 2009
Ch. austriaca	CBS 264.94	*Hordeum vulgare*	Finland	T. Tuomi	–	FJ176255	–	Cai et al. 2009
Ch. breviclavata	HKUCC OC0021	–	–	–	–	FJ176243	–	Cai et al. 2009
Ch. constricta	CBS 248.76	decaying wood	Belgium	W. Gams	–	FJ176256	–	Cai et al. 2009
Ch. crassipes	CBS 829.71	*Pteridium aquilinum*	Germany	W. Gams	–	FJ176254	–	Cai et al. 2009
Ch. fungorum	CBS 942.72	*Picea abies*	Sweden	L. Beyer	–	FJ176252	–	Cai et al. 2009
	HKUCC OC0033	–	–	–	–	FJ176251	–	Cai et al. 2009
Ch. holubovae	CCF 3977	–	–	–	FR667221	FR667868	–	Koukol 2011
	CCF 3978	–	–	–	FR667222	FR667869	–	Koukol 2011
Ch. hyalocuspica	CCF 3975	–	–	–	FR667220	FR667867	–	Koukol 2011
	CCF 3976	–	–	–	FR667221	FR667868	–	Koukol 2011
Ch. longipes	CCF 3973	–	–	–	FR667213	FR667862	–	Koukol 2011
	CCF 3974	–	–	–	FR667214	FR667863	–	Koukol 2011
Ch. microspora	CBS 131.74	*Pinus sylvestris*	Netherlands	W. Gams	FR667228	FR667875	–	Koukol 2011
	CCF 3980	–	–	–	FR667226	FR667873	–	Koukol 2011
Ch. parvispora	CBS 385.94	–	Czech Republic	V. Holubová-Jechová	–	FJ176253	–	Cai et al. 2009
Ch. piceae-abietis	CCF 3982	–	–	–	FR667230	FR667877	–	Koukol 2011
Ch. pseudoaffinis	CBS 261.75	*Cedrus atlantica*	France	W. Gams	FR667872	FR667872	–	Koukol 2011
	CCF 3979	*Pinus sylvestris*	Czech Republic	O. Koukol	FR667224	FR667871	–	Koukol 2011
Ch. pulchra	HKUCC OC0030	–	–	–	–	FJ176242	–	Cai et al. 2009
Ch. selaginellae	HKUCC OC0011	–	–	–	–	FJ176241	–	Cai et al. 2009
Cistella acuum	CBS 605.77	*Picea abies*	Switzerland	E. Müller	GU727552	GU727552	–	Bogale et al. 2010
	CCF 3970	*Picea abies*	Norway	O. Koukol	FR667211	FR667860	–	Koukol 2011
	JHH 3966	–	USA	J.H. Haines	U57492	–	–	Cantrell and Hanlin 1997
Ci. albidolutea	KUS-F52678	*Carex* sp.	Korea	–	JN033429	JN086732	–	Han et al. 2014
Ci. grevillei	JHH 1602	–	USA	J.H. Haines	U57089	–	–	Cantrell and Hanlin 1997
Ci. spicicola	CBS 731.97		Finland	S. Huhtinen	GU727553	GU727553	–	Bogale et al. 2010

Table 1 (continued)

Species	Culture / specimen accession numbers[a]	Host/isolation source	Country	Collector	GenBank accession numbers[b]			Reference
					ITS	LSU	SSU	
		Diphasiastrum complanatum						
Cistella sp.	KUS-F52527	–	Korea	–	JN033419	JN086722	–	Han et al. 2014
Coccomyces dentatus	AFTOL-ID 147	*Berberis nervosa*	USA	K. Hosaka	DQ491499	AY544657	–	James et al. 2006
Dasyscyphella montana	TNS-F-16527	unidentified wood	Japan	–	AB481242	AB481293	–	Hosoya et al. 2010
Davidsoniella australis	CMW 2333	*Nothofagus cunninghamii*	Australia	M. Hall	–	KM495396	–	de Beer et al. 2014
Dav. eucalypti	CMW 3254	*Eucalyptus sieberi*	Australia	M.J. Dudzinski	–	KM495338	–	de Beer et al. 2014
Endoconidiophora fujiensis	CBS 100208 = CMW 1955	*Larix kaempferi*	Japan	M.J. Wingfield &Y. Yamaoka	–	KM495345	–	de Beer et al. 2014
En. pinicola	CBS 100199 = CMW 29499	*Pinus sylvestris*	UK	J. Gibbs	–	KM495364	–	de Beer et al. 2014
En. polonica	CBS 100205 = CMW 20930	*Picea abies*	Norway	H. Solheim	–	KM495367	–	de Beer et al. 2014
En. rufipennis	CMW 11661	*Picea engelmannii*	Canada	H. Solheim	–	KM495373	–	de Beer et al. 2014
Erioscyphella abnormis	Dumont VE 57	Unidentified wood	Venezuela	K. P. Dumont	U59002	–	–	Cantrell and Hanlin 1997
	TNS-F-16617	Twig	Japan	R. Sasagawa	AB481250	AB481309	–	Hosoya et al. 2010
	TNS-F-16582	Unidentified wood	Japan	R. Sasagawa	AB481249	–	–	Hosoya et al. 2010
	KUS-F52080	Unidentified wood	–	–	JN033395	JN086698	–	Han et al. 2014
Er. brasiliensis	HMAS 75520	–	China	P. Zhao & W.Y. Zhuang	JF937579	–	–	Zhao and Zhuang 2011
	HMAS 78490	–	China	P. Zhao & W.Y. Zhuang	JF937580	–	–	Zhao and Zhuang 2011
	F7IIT4	–	Taiwan	M.L. Wu	U59000	–	–	Cantrell and Hanlin 1997
	PR 129	–	Puerto Rico	S.A. Cantrell	U58999	–	–	Cantrell and Hanlin 1997
Er. euterpes	PR 147	*Euterpe globosa*	Puerto Rico	S.A. Cantrell	U58640	–	–	Cantrell and Hanlin 1997
Er. lushanensis	HMAS 81575	*Miscanthus* sp.	China	P. Zhao & W.Y. Zhuang	JF937582	–	–	Zhao and Zhuang 2011
Er. sclerotii	HMAS 78499	–	China	P. Zhao & W.Y. Zhuang	JF937584	–	–	Zhao and Zhuang 2011
	PR 102	–	Puerto Rico	S. A. Cantrell	U59001	–	–	Cantrell and Hanlin 1997
Erioscyphella sp.	TNS-F-16442	Unidentified wood	Japan	R. Sasagawa	AB481270	AB481305	–	Hosoya et al. 2010
	TNS-F-16841	Unidentified wood	Japan	T. Hosoya	AB481281		–	Hosoya et al. 2010

Table 1 (continued)

Species	Culture / specimen accession numbers[a]	Host/isolation source	Country	Collector	GenBank accession numbers[b]			Reference
					ITS	LSU	SSU	
	TNS-F-16838	Unidentified wood	Japan	R. Sasagawa	AB481280	AB481327	–	Hosoya et al. 2010
Erioscyphella sp.	HMAS 78572	–	China	W.Y. Zhuang & Z.H. Yu	JF937583	–	–	Zhao and Zhuang 2011
Geoglossum uliginosum	SAV 10162	–	Czech Republic	V. Kučera	KJ152695	KJ152696	–	Hustad et al. 2014
Graphium fabiforme	CBS 124921 = CMW 30626	*Adansonia rubrostipa*	Madagascar	J. Roux & M.J. Wingfield	–	KM495387	–	de Beer et al. 2014
Huntiella decipiens	CBS 129736 = CMW 30855	*Eucalyptus saligna*	South Africa	G.K. Nkuekam & J. Roux	–	KM495333	–	de Beer et al. 2014
Hu. moniliformis	CBS 118127 = CMW 10134	*Eucalyptus grandis*	South Africa	M. van Wyk	–	KM495355	–	de Beer et al. 2014
Hu. tribiliformis	CBS 115866 = CMW 13013	*Pinus merkusii*	Indonesia	M.J. Wingfield	–	KM495381	–	de Beer et al. 2014
Hyaloscypha albohyalina var. *spiralis*	TNS-F-31133	Unidentified wood	Japan	T. Hosoya	AB546941	–	–	Hosoya et al. 2010
	KUS-F52652	–	Korea	–	JN033426	JN086729	–	Han et al. 2014
Hya. aureliella	KUS-F52070	Unidentified wood	Korea	–	JN033394	JN086697	–	Han et al. 2014
	NY1	–	USA	S.A. Cantrell	U57495	–	–	Cantrell and Hanlin 1997
	M234	–	UK	Leonard	EU940228	EU940152	–	Baral et al. 2009
Hya. fuckelii	M233	–	UK	Leonard	EU940230	EU940154	–	Baral et al. 2009
Hya. hepaticola	M171	–	Finland	Nieminen	EU940194	EU940118	–	Baral et al. 2009
	M339	–	Finland	Nieminen	EU940226	EU940150	–	Baral et al. 2009
Hya. albohyalina var. monodictys	TNS-F-5013	–	Japan	–	JN033456	JN086756	–	Han et al. 2014
Hya. vitreola	M39	–	Finland	Söderholm	EU940231	EU940155	–	Baral et al. 2009
Hyphodiscus hymeniophilus	CBS 602.77	*Alnus viridis*	Switzerland	P. Raschle	DQ227264	DQ227264	–	Untereiner et al. 2006
	CBS 687.74	*Quercus pubescens*	France	W. Gams	–	DQ227262	–	Untereiner et al. 2006
Hyp. otanii	TNS-F-7099	Unidentified wood	Japan	T. Hosoya	AB546949	AB546947	–	Hosoya et al. 2010
Hyp. theiodeus	TNS-F-31803	Decaying wood	Japan	–	AB546953	AB546952	–	Hosoya et al. 2010
	TNS-F-32000	–	Japan	T. Hosoya	AB546953	AB546952	–	Hosoya et al. 2010
Incrucipulum ciliare	TNS-F-16759	*Quercus crispula*	Japan	R. Sasagawa	AB481253	–	–	Hosoya et al. 2010
	TNS-F-16758	*Quercus crispula*	Japan	R. Sasagawa	AB481252	AB481324	–	Hosoya et al. 2010
I. radiatum	TNS-F-16764	*Fagus crenata*	Japan	R. Sasagawa	AB481262	–	–	Hosoya et al. 2010
	TNS-F-16769	*Fagus crenata*	Japan	R. Sasagawa	AB481261	AB481322	–	Hosoya et al. 2010
Knoxdaviesia cecropiae	CBS 120015 = CMW 997	*Protea longifolia*	South Africa	M.J. Wingfield	–	KM495391	–	de Beer et al. 2014
K. serotecta	CBS 129738 = CMW 36767		South Africa		–	KM495394	–	de Beer et al. 2014

Table 1 (continued)

Species	Culture / specimen accession numbers[a]	Host/isolation source	Country	Collector	GenBank accession numbers[b]			Reference
					ITS	LSU	SSU	
		Grow on insect (*Cossonus* sp.) found in *Euphorbia ingens*		J.A. van der Linde & J. Roux				
K. ubusi	CBS 129742 = CMW 36769	Insect tunnels in *Euphorbia tetragona*	South Africa	J. Roux	–	KM495395	–	de Beer et al. 2014
Lachnellula subtilissima	CBS 196.66	*Abies alba*	Switzerland	E. Müller	KC464640	KC492978	–	Unpublished
	CBS 197.66	*Picea abies*	Switzerland	E. Müller	KC464641	KC492979	–	Unpublished
La. willkommii	CBS 172.35	–	USA	G.G. Hahn	KC464644	KC492982	–	Unpublished
	CBS 200.66	–	Switzerland	E. Müller	KC464645	KC492983	–	Unpublished
Lachnopsis catarinensis	**CPC 24723 = COAD 2006^T**	*Dicksonia sellowiana*	Brazil	E. Guatimosim	KU597793	KU597760	KU597778	This study
	CPC 24714 = COAD 2003	*Dicksonia sellowiana*	Brazil	E. Guatimosim	KU597795	KU597762	–	This study
	CPC 24713	*Dicksonia sellowiana*	Brazil	E. Guatimosim	KU597794	KU597761	KU597779	This study
Lachno. dicksoniae	**CPC 24742 = COAD 1429^T**	*Dicksonia sellowiana*	Brazil	P.B. Schwartsburd	KU597796	KU597763	KU597780	This study
Lachno. cf. *pteridophylli*	PR 148	*Dicksonia* sp.	Puerto Rico	S.A. Cantrell	U58635	–	–	Cantrell and Hanlin 1997
Lachno. cf. *varians*	TNS-F-17631	Unidentified fern	Japan	T. Hosoya	AB481267	–	–	Hosoya et al. 2010
Lachnum asiaticum	TNS-F-16494	Unidentified bamboo	Japan	R. Sasagawa	AB481251	AB481297	–	Hosoya et al. 2010
Lachnum controversum	JHH 4611	–	USA	J.H. Haines	U58638	–	–	Cantrell and Hanlin 1997
Lachnum cf. *hyalopus*	HMAS 81586	–	China	P. Zhao & W.Y. Zhuang	JF937581	–	–	Zhao and Zhuang 2011
Lachnum nudipes	JHH 4644	–	USA	J.H. Haines	U59003		–	Cantrell and Hanlin 1997
	TNS-F-16651	*Fallopia* sp.	Japan	T. Hosoya	AB481257	AB481314	–	Hosoya et al. 2010
Lachnum pudibundum	TNS-F-16501	Unidentified wood	Japan	R. Sasagawa	AB481259	AB481298	–	Hosoya et al. 2010
Lachnum rhytismatis	TNS-F-16545	*Symplocos coreana*	Japan	R. Sasagawa	AB481263	–	–	Hosoya et al. 2010
	TNS-F-16544	*Symplocos coreana*	Japan	R. Sasagawa	AB481264	–	–	Hosoya et al. 2010
Lachnum soppittii	TNS-F-16551	Unidentified wood	Japan	T. Hosoya	AB481266	AB481308	–	Hosoya et al. 2010
Lachnum sp.	TNS-F-16634	*Fagus crenata*	Japan	T. Hosoya	AB481274	AB481312	–	Hosoya et al. 2010
Lachnum spartinae	RTH 1078	–	USA	R.T. Hanlin	U58639	–	–	Cantrell and Hanlin 1997
Lachnum virgineum	JHH 4312	–	USA	J.H. Haines	U59004	–	–	Cantrell and Hanlin 1997
	TNS-F-16583	Unidentified wood	Japan	R. Sasagawa	AB481268	–	–	Hosoya et al. 2010
	TNS-F-16588	Unidentified wood	Japan	R. Sasagawa	AB481269	–	–	Hosoya et al. 2010

Table 1 (continued)

Species	Culture / specimen accession numbers[a]	Host/isolation source	Country	Collector	GenBank accession numbers[b]			Reference
					ITS	LSU	SSU	
	HMAS 81601	–	China	P. Zhao & W.Y. Zhuang	JF937586	–	–	Zhao and Zhuang 2011
	HMAS 81599	–	China	–	AF505518	–	–	Zhao and Zhuang 2011
Lasiobelonium lonicerae	TNS-F-16667	Unidentified wood	Japan	R. Sasagawa	AB481284	AB481284	–	Hosoya et al. 2010
Neodasyscypha cerina	JHH 3916	–	USA	J.H. Haines	U57812	–	–	Cantrell and Hanlin 1997
Perrotia flammea	JHH 4497	–	Switzerland	J.H. Haines	U57988	–	–	Cantrell and Hanlin 1997
Proliferodiscus alboviridis	GA 34	–	USA	S.A. Cantrell	U57990	–	–	Cantrell and Hanlin 1997
Pr. distinctus	JHH 1114	–	USA	J.H. Haines	U57989	–	–	Cantrell and Hanlin 1997
Proliferodiscus sp.	KUS-F52660	–	Korea	–	JN033427	JN086730	–	Han et al. 2014
	TNS-F-17436	–	Japan	–	JN033452	JN086752	–	Han et al. 2014
Pr. tricolor	CBS 122000	*Quercus robur*	Germany	H.O. Baral	KC464643	KC492981	–	Unpublished
Psilachnum chrysostigma	isolate 14793	–	–	–	JF908572		–	Osmundson et al. 2013
Ps. ellisii	JHH 4253	–	USA	J.H. Haines	U57493	–	–	Cantrell and Hanlin 1997
	KUS-F52663	*Carex* sp.	Korea	–	JN033428	JN086731	–	Han et al. 2014
	KUS-F52489	*Carex* sp.	Korea	–	JN033418	JN086721	–	Han et al. 2014
Ps. staphyleae	KUS-F52105	*Staphylea bumalda*	Korea	–	JN033396	JN086699	–	Han et al. 2014
Psilachnum sp.	KUS-F52448	*Philadelphus schrenckii*	Korea	–	JN033415	JN086718	–	Han et al. 2014
Rommelaarsia flavovirens	HB 9951b	*Equisetum arvense*	France	P. Tanchaud	KT958773	KT958770	–	Baral 2015
	HB 9951c	*Equisetum arvense*	France	P. Tanchaud	KT958774	KT958771	–	Baral and Haelewaters 2015
	HB 9684	*Equisetum arvense*	Netherlands	L. Rommelaars	KT958772	KT958769	–	Baral and Haelewaters 2015
Saccharomyces cerevisiae	DAOM 216365	–	–	–	JN942842	JN938921	–	Schoch et al. 2012
Scolecolachnum pteridii	**CPC 25778 = COAD 1796^T**	*Pteridium arachnoideum*	Brazil	D.J. Soares	KU597798	KU597765	–	This study
	CPC 24666	*Pteridium arachnoideum*	Brazil	R.W. Barreto	KU597797	KU597764	–	This study
Solenopezia solenia	JHH 4169	–	USA	J.H. Haines	U57991	–	–	Cantrell and Hanlin 1997

Table 1 (continued)

Species	Culture / specimen accession numbers[a]	Host/isolation source	Country	Collector	GenBank accession numbers[b]			Reference
					ITS	LSU	SSU	
Thielaviopsis ethacetica	CMW 37775	*Ananas comosus*	Malaysia	A. Johnson	–	KM495337	–	de Beer et al. 2014
Th. musarum	CMW 1546	*Musa* sp.	New Zealand	T.W. Canter-Vissche	–	KM495357	–	de Beer et al. 2014
Th. paradoxa	CBS 130761 = CMW 36689	*Theobromae cacao*	Cameroon	M. Mbenoun & J. Roux	–	KM495363	–	de Beer et al. 2014
Trichopeziella nidulus	JHH 4485	–	Switzerland	J.H. Haines	U57813	–	–	Cantrell and Hanlin 1997
Trichopeziza mollissima	TNS-F-16763	unidentified herb	Japan	R. Sasagawa	AB481286		–	Hosoya et al. 2010
Trichopeziza sulphurea	JHH 4513	–	Switzerland	J.H. Haines	U58634	–	–	Cantrell and Hanlin 1997
Vibrissea flavovirens	MBH 39316	–	–	–	AY789427	–	–	Wang et al. 2005
V. truncorum	CUP-62562	–	USA	–	AY789403	–	–	Wang et al. 2005
Xenochalara juniperi	CBS 670.75^{ET} = CMW 1099	*Juniperus communis*	Netherlands	W. Gams	AF184887	–	–	Coetsee et al. 2000
	CMW 2547	*Juniperus communis*	Netherlands	W. Gams	AF184888	–	–	Coetsee et al. 2000
	CMW 1901	*Juniperus communis*	Netherlands	W. Gams	AF184889	–	–	Coetsee et al. 2000
Zymochalara cyatheae	**CPC 24665 = COAD 1092^{T}**	*Cyathea delgadii*	Brazil	R.W. Barreto	KU597799	KU597766	KU597781	This study
	CPC 24690	*Cyathea delgadii*	Brazil	R.W. Barreto	KU597800	KU597767	KU597782	This study
	CPC 24735	*Cyathea delgadii*	Brazil	E. Guatimosim	KU597802	KU597769	KU597784	This study
	CPC 24736 = COAD 2013	*Cyathea delgadii*	Brazil	E. Guatimosim	KU597803	KU597770	KU597785	This study
	CPC 25072 = COAD 1758	*Cyathea delgadii*	Brazil	R.W. Barreto	KU597801	KU597768	KU597783	This study
Z. lygodii	**CPC 24710 = COAD 2001^{T}**	*Lygodium volubile*	Brazil	E. Guatimosim	KU597805	KU597772	KU597787	This study
	CPC 24699 = COAD 1992	*Lygodium volubile*	Brazil	R.W. Barreto	KU597804	KU597771	KU597786	This study

Newly generated sequences are in bold

[a] *CBS* CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands; *CCF* Culture Collection of Fungi, Charles University in Prague, Faculty of Science, Prague, Czech Republic; *CMW* Culture collection of Mike Wingfield, housed at Forestry and Agricultural Biotechnology Institute at University of Pretoria, South Africa; *COAD* Coleção Octávio de Almeida Drumond, Viçosa, Minas Gerais, Brazil; *CPC* Culture collection of Pedro Crous, housed at CBS; *F* Fundación Medina's Fungal Culture collection; *HB* private herbaria of Hans-Otto Baral, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain; *HKUCC* The University of Hong Kong culture collection, Hong Kong, Japan; *HMAS* Herbarium of Mycology, Institute of Microbiology, Chinese Academy of Sciences, China; KUS Korea University Herbarium, Seoul, Korea; *MUCL* Mycothèque del'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; *TNS* National Museum of Nature and Science, Tsukuba, Japan; *UPS* Botanical Museum, Uppsala University, Sweden; *VIC* Herbário da Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil; *T*: ex-type cultures

[b] *ITS* internal transcribed spacers and intervening 5.8S nrDNA; *LSU* 28S nrRNA gene; *SSU* 18S rRNA gene

Phylogenetic analysis

Appropriate gene models were selected using MrModeltest v.2.3 (Nylander 2004) and applied to each gene partition. Based on the results of MrModeltest, a Bayesian phylogenetic analysis was performed with MrBayes v.3.2.3 applying the GTR+I+G substitution model for ITS and LSU, through Cipres Gateway (Miller et al. 2010). *Coccomyces dentatus* AFTOL-ID 147 and *Graphium fabiforme* CMW 30626 served as outgroup for the chalara-like ITS and LSU analyses, respectively, while *Saccharomyces cerevisiae* DAOM 216365 and *Geoglossum uliginosum* SAV 10162 served as outgroup for the lachnoid ITS and LSU analyses, respectively. Posterior probabilities were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v.3.2.3 (Ronquist et al. 2012). Six simultaneous Markov chains were run for 10,000,000 generations, trees were sampled every 100th generation, and 10,000 trees were obtained. The first 2000 trees, representing the burn-in phase were discarded, while the remaining 8000 trees were used for calculating posterior probabilities. Bayesian posterior probabilities are presented on the left of each node, on each tree. Sequences derived in this study were lodged in GenBank, the alignment in TreeBASE (http://www.treebase.org; S19782), and taxonomic novelties in MycoBank.

Results

Phylogenetic results

The four datasets consisted of 446 characters, representing 34 taxa (including the outgroup) for the chalara-like ITS tree, 793 characters, representing 48 taxa (including the outgroup) for the chalara-like LSU tree, 477 characters, representing 94 taxa (including the outgroup) for the lachnoid ITS tree, and 788 characters, representing 57 taxa (including the outgroup) for the lachnoid LSU tree.

The respective alignments included 185 and 229 unique site patterns for the chalara-like ITS and LSU trees, respectively, and 226 and 406 unique site patterns for the lachnoid ITS and LSU trees, respectively. After topological convergence of the Bayesian runs, the following numbers of trees were generated and subsequently sampled (using a burn-in fraction of 0.25 and indicated after the slash) in order to generate the four Bayesian phylogenies: 1710/1368, 903/722 for the chalara-like ITS and LSU trees (Figs. 1, 2), respectively, and 2093/1674, 2130/1704 for the lachnoid ITS and LSU trees (Figs. 3, 4), respectively. The resulting phylogenetic trees of the individual datasets could not be concatenated, as sequences for both loci were not available for all isolates. The results are presented below.

Taxonomy

The phylogenetic analyses employed, aiming to distinguish species boundaries of the fungi studied, revealed a rich diversity among the fungi collected on Brazilian ferns. Six isolates of lachnoid and 12 isolates for chalara-like fungi, collected from five host species, representing 10 different localities were studied. The Bayesian analyses resulted in a total of six frond-related taxa, belonging to four genera, including *Bloxamia*, and three new genera that are introduced below. Additionally, six species are newly described.

Bloxamia cyatheicola Guatimosim, R.W. Barreto & Crous, **sp. nov.** (Fig. 5).

MycoBank MB813045

Etymology: Name refers to the host tree fern genus *Cyathea*.

Frond spots amphigenous, irregular, starting as small chlorotic areas, becoming pale brown and necrotic, affecting scattered pinnulae. *Internal hyphae* not observed. *External hyphae* absent. *Conidiomata* sporodochial, hypophyllous, erumpent, either solitary or crowded along the margins of the pinnule, discoid, up to 1000 × 2000 μm, solitary, when wet pulvinate, slimy, amber-colored, when dry, flattened, pulvinate and tough, black. In vertical section, sporodochia with a basal stroma of *textura intricata*, 190–205 μm deep in the centre of the conidioma, composed of 4–5 μm diam cells, dark brown towards the host tissue, and paler towards the external side. *Coniodiophores* often reduced to the conidiogenous cells. *Phialides* arising from the stroma surface in a densely packed palisade, discrete, subcylindrical, 17–41 × 1.5–3.5 μm, rarely 1-septate, pale brown, becoming paler towards the apex, smooth. *Phialoconida* endogenous, basipetal, extruded in short easily fragmenting chains, cylindrical, truncate at both ends, 2.5–8 × 1–3 μm, aseptate, hyaline, with small guttules, smooth. *Ascomata* apothecial, hypophyllous, sometimes associated with the conidioma on the same pinnula, erumpent, scattered at the margin of the pinnulae, discoid or cupulate (when dry), up to 500 μm diam and 1900 μm high, solitary, sessile, slimy, tough, black. In vertical section, apothecia with a basal stroma of *textura intricata*, 103–198 μm deep, composed of 3 μm diam cells. *Medullary excipulum* of *textura epidermoidea*, up to 250 μm thick, composed of thin-walled hyphae, 1–1.5 μm diam, sub-hyaline to hyaline. *Paraphyses* unbranched, filiform, swollen at the tip, 1–2.5 μm wide, septate, hyaline, smooth. *Asci* unitunicate, subcylindrical or clavate, without croziers, straight to curved, 68–113 × 6.5–14 μm, 8-spored, with small euamyloid apical ring, hyaline, smooth. *Ascospores* uniseriate, rarely biseriate, fusoid, straight, 10–18 × 4–7 μm, 1-septate, with one cell slightly larger, biguttulate, hyaline, smooth.

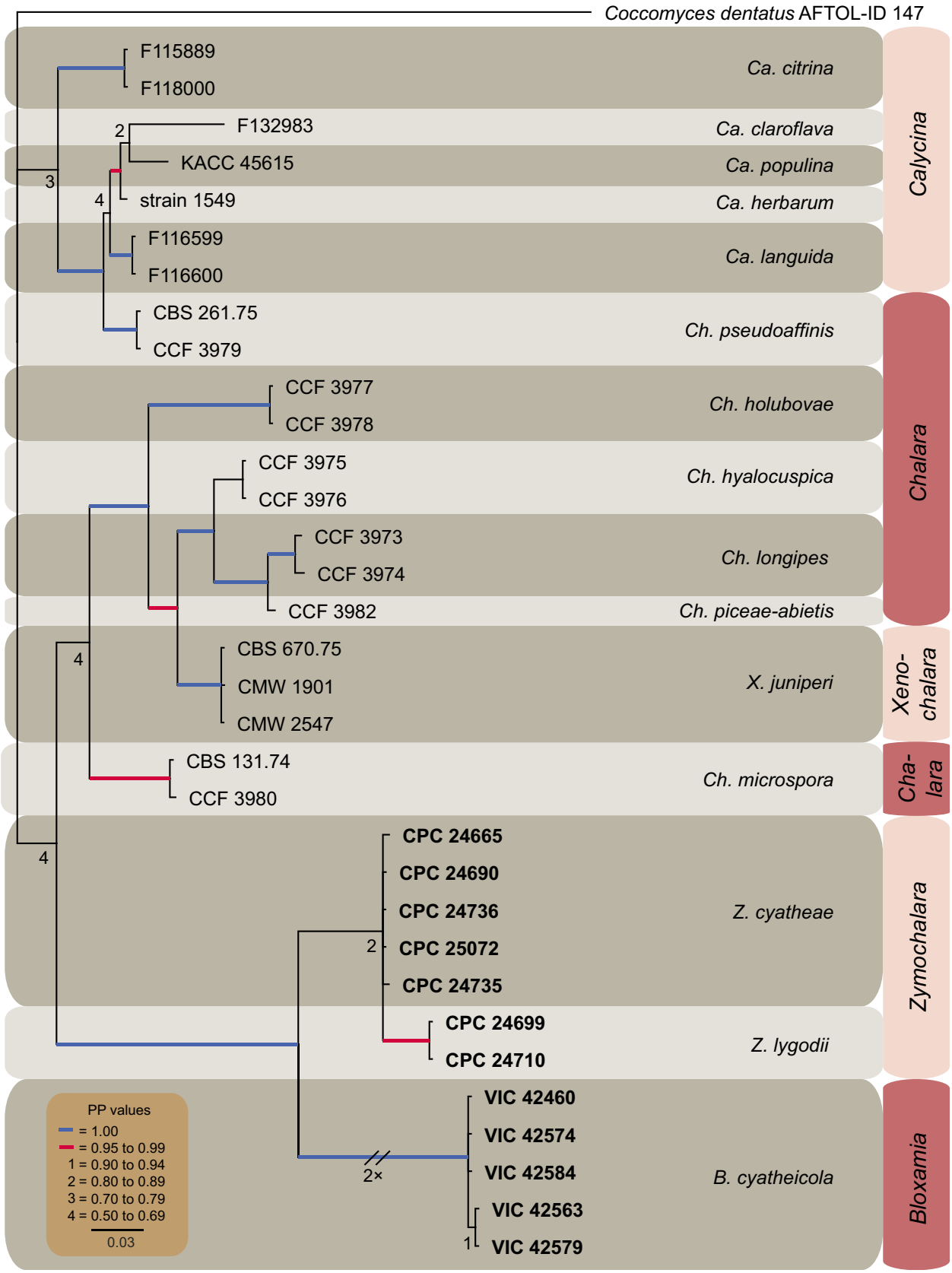

Fig. 1 Consensus phylogram (50 % majority rule) of chalara-like species, from a Bayesian analysis of the ITS sequence alignment. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend) and the *scale bar* indicates 0.03 expected changes per site. Isolates from Brazil are indicated in *bold*. The tree was rooted to *Coccomyces dentatus* (isolate AFTOL-ID 147)

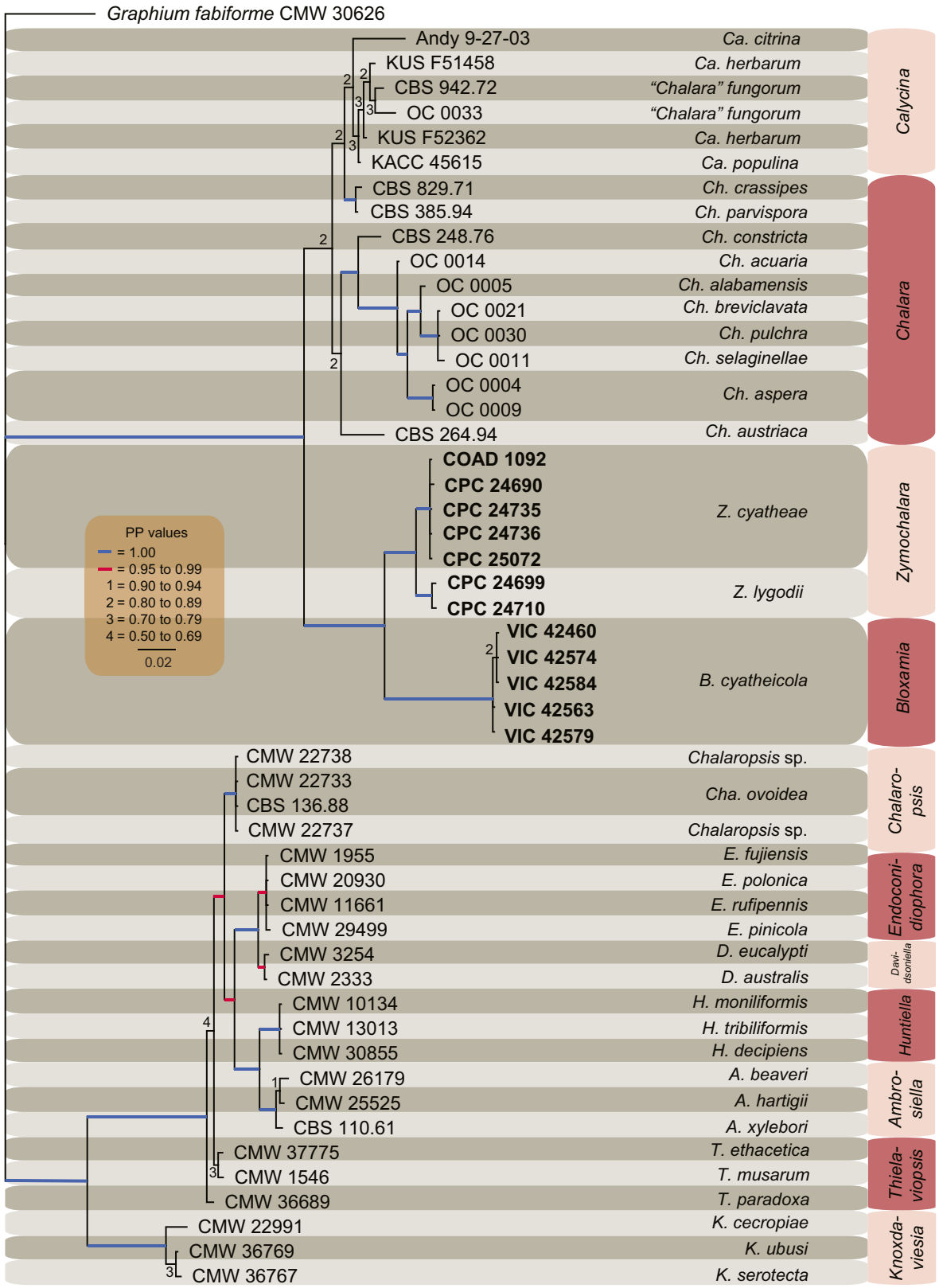

Fig. 2 Consensus phylogram (50 % majority rule) of chalara-like species, from a Bayesian analysis of the LSU sequence alignment. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend) and the *scale bar* indicates 0.02 expected changes per site. Isolates from Brazil are indicated in *bold*. The tree was rooted to *Graphium fabiforme* (isolate CMW 30626)

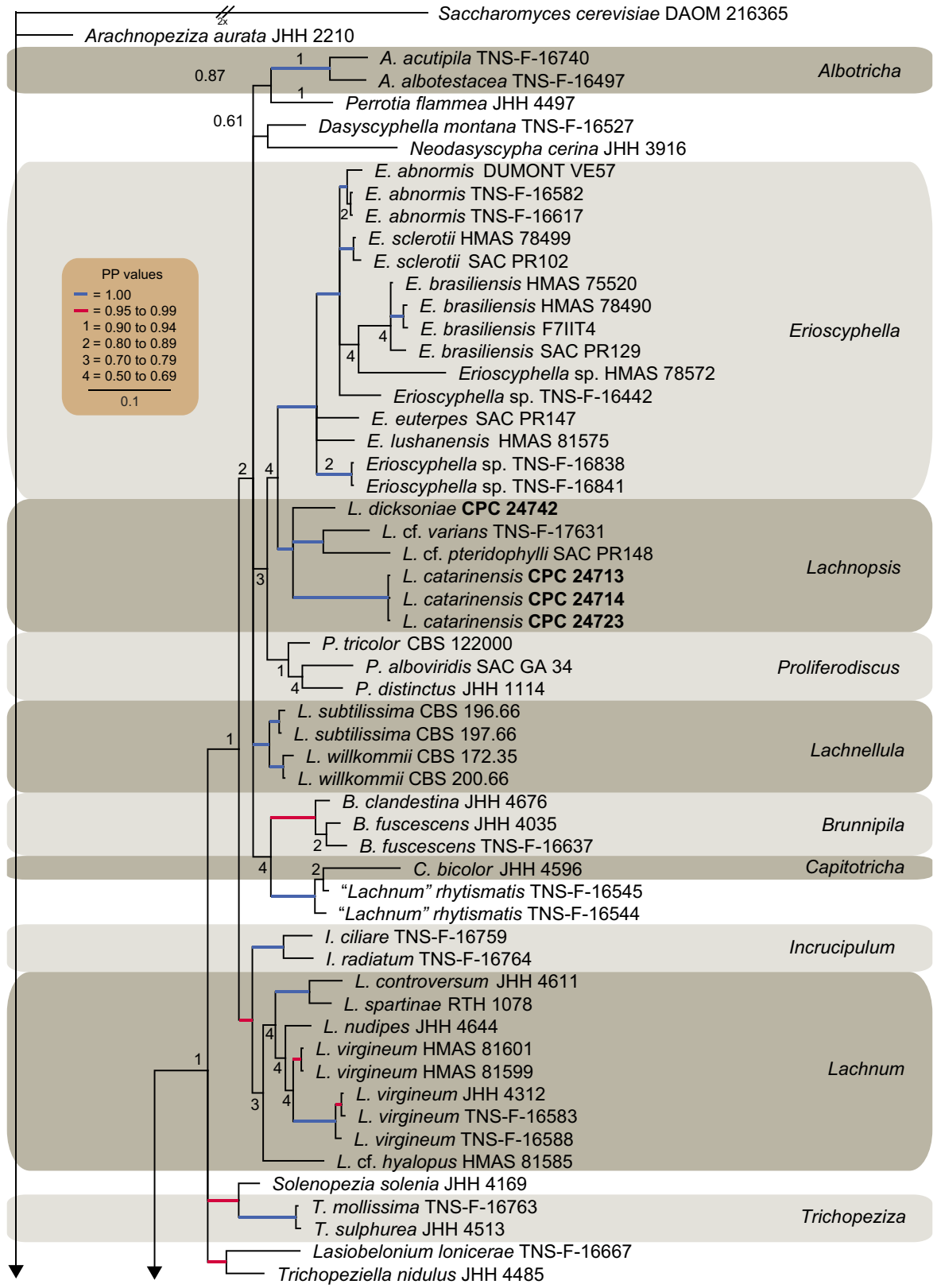

Fig. 3 Consensus phylogram (50 % majority rule) of lachnoid species, from a Bayesian analysis of the ITS sequence alignment. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend) and the *scale bar* indicates 0.1 expected changes per site. Isolates from Brazil are indicated in *bold*. The tree was rooted to *Saccharomyces cerevisiae* (isolate DAOM 216365)

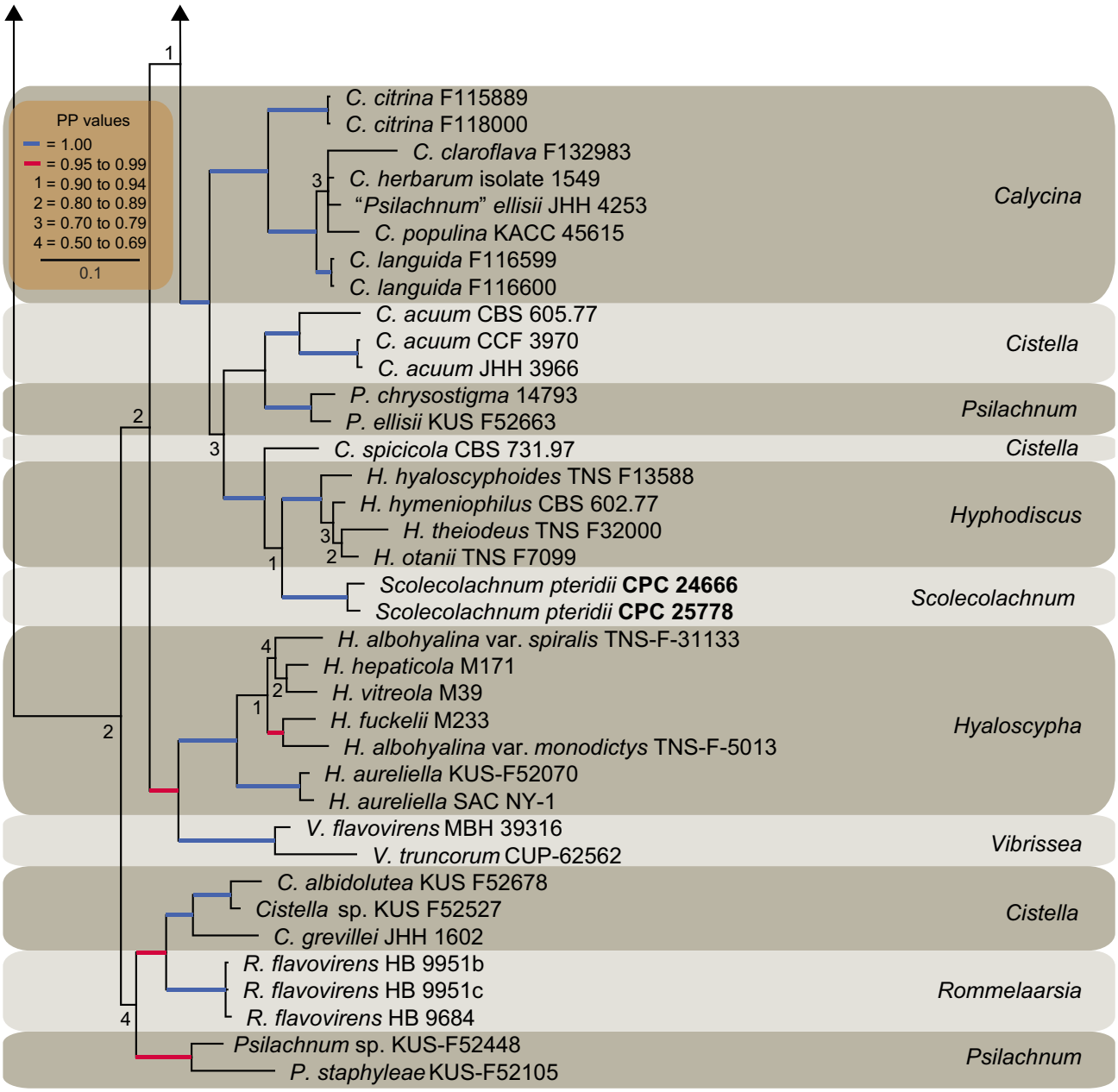

Fig. 3 (continued)

Holotype: Brazil, Rio de Janeiro, Nova Friburgo, Macaé de Cima, on fronds of *Cyathea delgadii*, both morphs, 29 Apr 2012, *R.W. Barreto* (VIC 42563).

Habitat/Distribution: Known from *C. delgadii* and *C. atrovirens* in the states of Minas Gerais, Paraná and Rio de Janeiro, Brazil.

Additional specimens examined: Brazil, Rio de Janeiro, Nova Friburgo, Macaé de Cima, on fronds of *C. delgadii*, 29 Apr 2012, *R.W. Barreto*, (VIC 42579) asexual morph; Paraná, Quatro Barras, on fronds of *C. atrovirens*, 1 Feb. 2012, *R.W. Barreto* (VIC 42574), sexual morph; Rio de Janeiro, Nova Friburgo, Mury, on fronds of *C. delgadii*, 29 Jul. 2012, *R.W. Barreto* (VIC 42584), asexual morph; Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, on fronds of *C. delgadii*, 23 Feb. 2014, *E. Guatimosim* (VIC 42460), asexual morph.

Notes: The genus *Bloxamia* includes seven species, and among them, only *Bl. foliicola* is known as a pathogen, causing disease on *Oxyspora paniculata* (Melastomataceae) in China (Liu and Zhang 1998). *Bloxamia foliicola* differs from

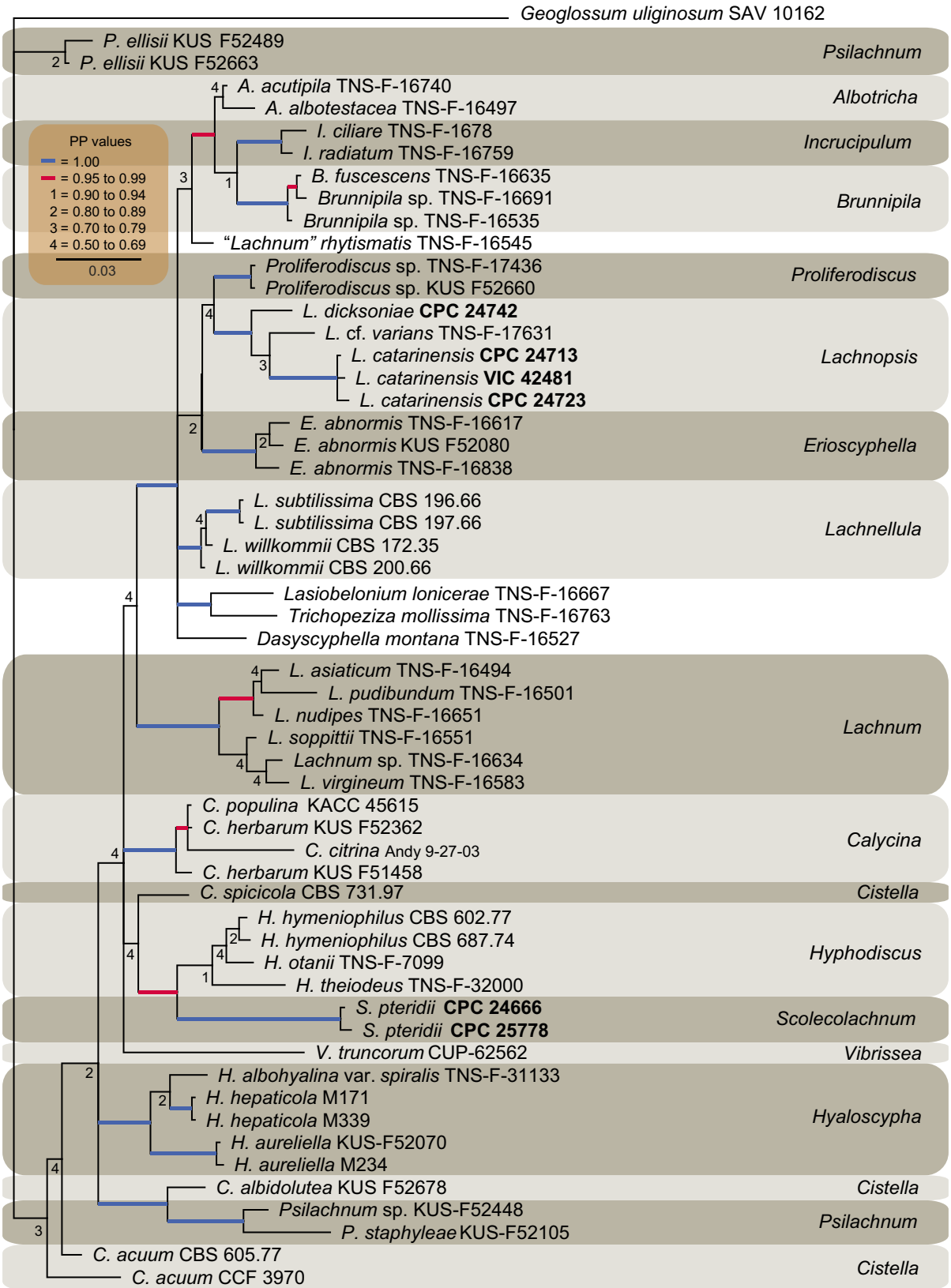

Fig. 4 Consensus phylogram (50 % majority rule) of lachnoid species, from a Bayesian analysis of the LSU sequence alignment. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend) and the *scale bar* indicates 0.03 expected changes per site. Isolates from Brazil are indicated in *bold*. The tree was rooted to *Geoglossum uliginosum* (isolate SAV 10162)

Fig. 5 *Bloxamia cyatheicola* (VIC 42563, holotype). **a** Colonized fronds of *Cyathea delgadii* showing yellowed colonized areas adaxially; **b**, **c** sporodochial conidiomata; **d**, **e** apothecia; **f** vertical section of conidioma; **g**, **h** conidiophores; **i** phialoconidia; **j** vertical section of apothecium; **k** asci; **l** ascospores (**f**, **g**, **k**, **l** in lactofuchsin; **h**–**j** in lactic acid). *Scale bars* (**f**, **j**) 100 μm, (**g**–**i**, **k**, **l**) 10 μm

Bl. cyatheicola by having its conidiophores organized in synnemata (Liu and Zhang 1998). The other species that have sporodochial conidiomata are not known from ferns (Table 2). Based on morphology, *Bl. cyatheicola* is rather similar to *Bl. cremea* recorded on dead wood from Argentina (Arambarri et al. 1992), and *Bl. truncata* recorded on decorticated wood of *Ulmus* sp. from England (Pirozynski and Morgan-Jones 1968). *Bloxamia cremea* has white to

Table 2 Morphological comparison of known *Bloxamia* species

Species	Substrate	Host	Country	Conidiomata		Conidigenous cells		Phialoconidia			Reference
				Type	Color	Feature	Size	Shape	Proliferation	Size	
Bl. bohemica	Rotting needles	*Pinus sylvestris*	Czechoslovakia	Sporodochial	Amber	Lageniform, pale brown	8–11 × 1.5–2 µm	Cylindrical	Catenate	3–5.5 × 1 µm	Minter and Holubová-Jechová (1981)
Bl. cremea	Rotting stems	Unknown	Argentina	Sporodochial	White to cream	Cylindrical, dark brown	24–26 × 2.5–3 µm	Cylindrical	Long and slimy chains	3–4 × 1–1.5 µm	Arambarri et al. (1992)
Bl. foliicola	Living leaves	*Oxyspora paniculata*	China	Synnematal	Brown	Cylindrical, brown	64–95 × 10–11 µm	Cubic, with truncate ends	Dry chains	6–9 × 5–8 µm	Liu and Zhang (1998)
Bl. hesterae	Submerged litter	*Schoenoplectus tabernaemontani*	Netherlands	Sporodochial	Opaque to black	Lageniform, black	14–24 × 2–3 µm	Oblong to clavate	Single or in slimy chains	5–6 × 2–3 µm	Spooren (2014)
Bl. nilagirica	Dead twigs	Unknown	India	Synnematal	Brown			Rectangular	Long and slimy chains	4–5 × 3–3.5 µm	Nag Raj and Kendrick (1975)
Bl. sanctae-insulae	Dead wood	Unknown	United Kingdom	Sporodochial	Brown to black	Lageniform, pale brown	10–14 × 1.5–2.5 µm	Globose with tiny hilum	Catenate	ca. 2 µm	Coppins and Minter (1980)
Bl. truncata	Decorticated wood	*Ulmus* sp.	England	Sporodochial	Black	Cylindrical to sub–cylindrical, pale brown	15–32 × 2–3 µm	Short cylindrical to oblong	Single or in easily dispersable chains	2–4 × 1.5–2.5 µm	Pirozynski and Morgan-Jones (1968)
Bl. cyatheicola	Living fronds	*Cyathea* spp.	Brazil	Sporodochial	Amber to black	Sub–cylindrical, light brown	17–41 × 1.5–3.5 µm	Cylindrical, truncate at both ends	Single or in easily dispersable chains	2.5–8 × 1–3 µm	This study

creamy conidiomata instead of amber-colored to black in *Bl. cyatheicola*, and dark brown, well-developed and larger conidiophores (24–26 × 2.5–3 μm) as compared with *Bl. cyatheicola* (17–41 × 1.5–3.5 μm). Additionally, phialoconidia in *Bl. cremea* are formed in long and slimy chains, whereas in *Bl. cyatheicola* these are short and easily fragmented (Arambarri et al. 1992). *Bloxamia truncata* differs from *Bl. cyatheicola* by having more or less cuboid phialoconidia, which are produced in endogenous chains (of up to six) within the conidiophore (Pirozynski and Morgan-Jones 1968; Minter and Holubová-Jechová 1981) instead of cylindrical phialoconidia as in *Bl. cyatheicola*. All attempts to isolate the fungus in culture were unsuccessful.

Erioscyphella Kirschst. Annales Mycologici 36: 384. 1938.

Notes: The genus *Erioscyphella* was reinstated to accommodate long-spored lachnoid tropical taxa (Haines and Dumont 1984) that cluster rather remotely from other genera of Hyaloscyphaceae, often having a yellow hymenium due to the presence of carotenoids, absence of croziers, hairs with partly light brown wall, never capitate at the apex, and often distantly septate (Perić and Baral 2014). Based on a Neighbor-joining analysis of the ITS region of selected species of Hyaloscyphaceae, Perić and Baral (2014) formally proposed the new combinations of *E. abnormis*, *E. brasiliensis*, *E. lunata*, *E. sclerotii*, and additionally, concluded that *Lachnum euterpes* S. A. Cantrell & J. H. Haines and *Lachnum lushanense* Zhuang & Wang, should also be considered as members of *Erioscyphella*. In the present ITS phylogenetic analysis (Fig. 3, part 1), we have expanded *Erioscyphella* by including the two aforementioned species (*L. euterpes* and *L. lushanense*) and other phylogenetically related isolates.

Erioscyphella euterpes (S.A. Cantrell & J.H. Haines) Guatimosim, R.W. Barreto & Crous, **comb. nov.**

Basionym: *Lachnum euterpes* S.A. Cantrell & J.H. Haines, Mycological Research 101: 1081. 1997.

MycoBank: MB817303

Description and Illustration — Cantrell and Haines (1997).

Holotype: Puerto Rico, Caribbean National Forest, Luquillo Experimental Forest, El Yunque, on fronds of *Prestoea montana* (= *Euterpe globosa*), 5 Jun 1970, *J. H. Haines* et al. (PR 30, NYS-f-4891, isotype PRM).

Specimen examined: Puerto Rico, Adjuntas, Guilarte Trail, on fronds of *Prestoea montana* (= *Euterpe globosa*), 3 Dec 1994, *S. A. Cantrell* (PR 147, GAM, epitype designated here, MBT 371982).

Notes: Perić and Baral (2014) recently indicated that *E. euterpes* was a likely member of the genus *Erioscyphella*. Unfortunately the only specimen of *E. euterpes* from which DNA is available (PR 147; Cantrell and Hanlin 1997) is not the holotype (PR-30; Cantrell and Haines 1997). However, this specimen is cited under the description of *L. euterpes* as being identical to the type (Cantrell and Haines 1997). On the present study, we have thus decided to designate this specimen as epitype for *E. euterpes*, and based on the phylogenetic inference, also propose a new combination.

Erioscyphella lushanensis (W.Y. Zhuang & Z. Wang) Guatimosim, R.W. Barreto & Crous, **comb. nov.**

Basionym: *Lachnum lushanense* W.Y. Zhuang & Z. Wang, Mycotaxon 66: 429. 1998.

MycoBank: MB817304

Description and Illustration — Zhuang and Wang (1998).

Holotype: China, Lushan Mountains, Jiangxi Province, on dead leaf sheath at stem base of an unknown grass (Gramineae), 18 Oct 1996, *W.Y. Zhuang* and *Z. Wang* (1462, HMAS 71903)

Specimen examined: China, Changjiang County, Hainan Province, on stems of *Miscanthus* sp. (Gramineae), 6 Dec 2000, *Z.H. Yu* and *W.Y. Zhuang* (3631, HMAS 81575, epitype designated here, MBT 372554).

Notes: As for *E. euterpes*, the only specimen of *E. lushanensis* from which DNA is available (HMAS 81575; Zhao and Zhuang 2011), it is not the holotype (HMAS 71903; Zhuang and Wang 1998), but this specimen shares the same characteristics of the type (Zhuang, personal communication). Therefore, we decided to designate an epitype for *E. lushanensis* and, based on the phylogenetic inference, propose this new combination.

Lachnopsis Guatimosim, R.W. Barreto & Crous, **gen. nov.**

MycoBank MB817300

Type species: *Lachnopsis catarinensis* Guatimosim, R.W. Barreto & Crous

Etymology: Resembling *Lachnum*, but phylogenetically distinct.

Ascomata apothecial, superficial, scattered or gregarious, usually stipitate, plane or concave, white or pigmented, clothed with hairs. *Hairs* cylindrical or tapering towards the apex, obtuse, straight or curved, sometimes apically clavate, thin- or thick-walled, multiseptate, hyaline or pigmented, granular throughout their length and sometimes also bearing resinous or crystalline matter. *Asci* 8-spored, without croziers, cylindrical or cylindric-clavate, apex conical, with euamyloid pore. *Ascospores* fusoid or filiform, rarely ellipsoid, 0- to multiseptate, hyaline smooth. *Paraphyses* lanceolate subcylindrical, sometimes with pointed apex, and frequently exceeding the asci in length. The genus *Lachnopsis* is only distinguishable from *Lachnum* based on DNA sequence data. ITS as well as LSU sequence data (and SSU, data not shown), can easily differentiate between these genera.

Notes: *Lachnopsis* is morphologically a typical species of *Lachnum* s.l., but it is phylogenetically distinct from that

genus. Presently, no asexual morphs are known, and the only distinguishing characters from *Lachnum* are to be found in its DNA sequences.

Lachnopsis catarinensis Guatimosim, R.W. Barreto & Crous, **sp. nov.** (Fig. 6).

MycoBank MB813047

Etymology: Name refers to the Brazilian state of Santa Catarina where the fungus was first found.

Frond blight irregular, starting as small necrotic areas and leading to necrosis of the pinnulae, affecting the apex of pinnulae. *Ascomata* apothecial, hypophyllous, scattered, discoid, 0.23–0.25 mm, opened, when wet, closed and narrowly campanulate, when dry, short-stipitate, stipe 52 × 48 μm, entirely white. *Receptacle* concolorous with the disc, densely clothed with hyaline hairs. *Ectal excipulum* of *textura prismatica*, composed of 8–10 × 4–5 μm large thin-walled cells, becoming intricate towards the base, hyaline, smooth. *Hairs* subulate or acerose, straight, 56–94 × 3–5 μm at the widest point, 3–4-septate, tapering toward obtuse apex, hyaline, thin-walled, densely roughened with hyaline, rod-shaped granules, non-amyloid. *Asci* unitunicate, clavate, straight or curved, short-pediculate, without croziers, 45–58 × 9–13 μm, 8-spored, with small euamyloid apical ring. *Ascospores* uniseriate, overlapping, subcylindrical or narrowly fusoid-acicular, straight to slightly curved, 32–46 × 1–2 μm, 3-septate, tapering towards each subacute end, guttulate, hyaline, smooth, germinating from both ends. *Paraphyses* cylindric-clavate, straight or slightly curved, unbranched, 55–60 μm long, 4–5 μm wide at the widest point, 3–4-septate, apex rounded, slightly longer than asci, hyaline, smooth. *Asexual morph*: not observed.

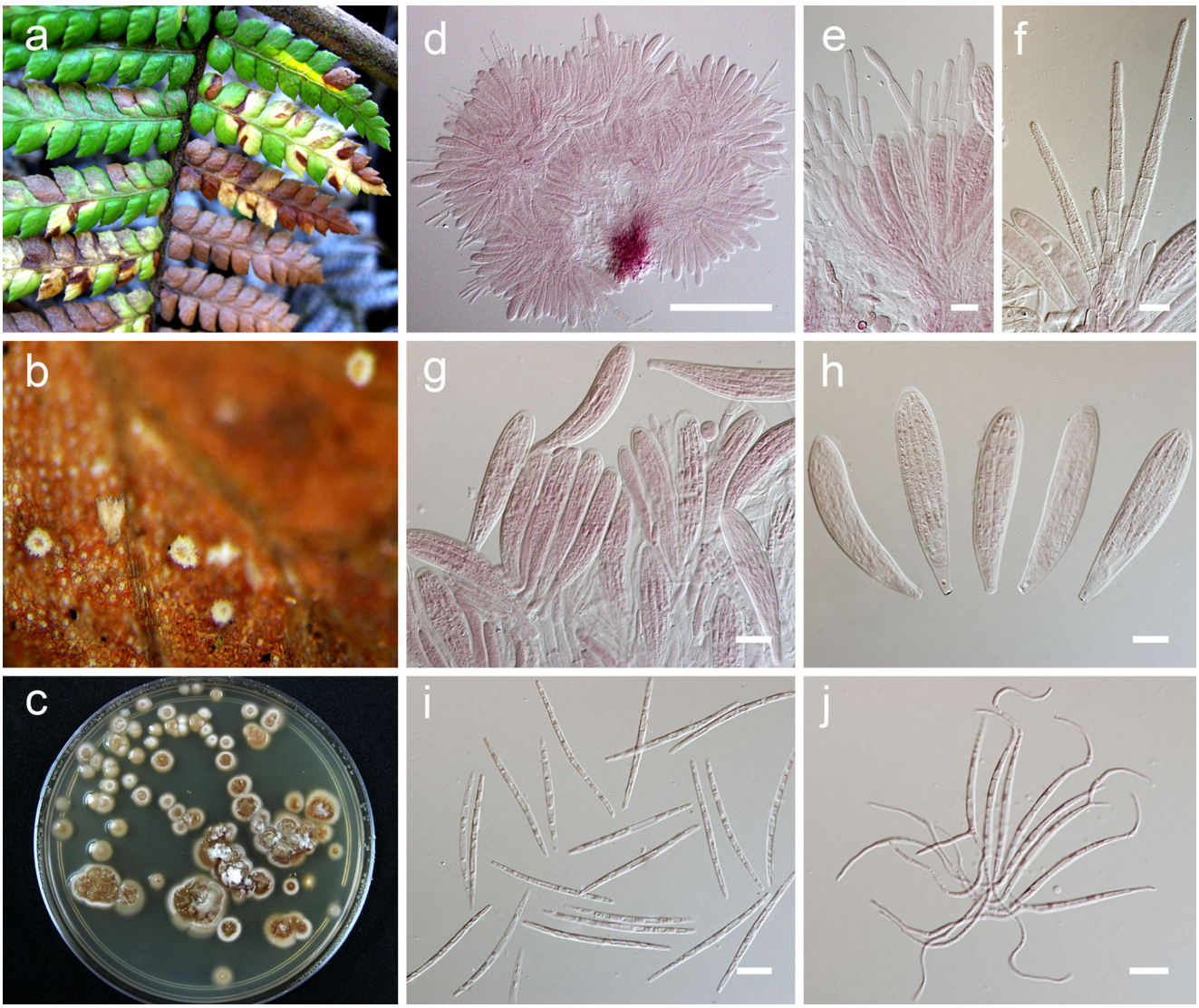

Fig. 6 *Lachnopsis catarinensis* (VIC 42507, holotype). **a**, **b** Frond blight on *Dicksonia sellowiana*; **b** apothecia; **c** colony on PDA; **d** squashed apothecium; **e** detail of paraphyses with obtuse apices, longer than asci; **f** roughened hairs, with hyaline rod-shaped granules; **g**, **h** asci; **i** ascospores; **j** ascospores germinating at both ends (**d**–**j** in lactofuchsin). *Scale bars* (**d**) 100 μm, (**e**–**j**) 10 μm

Culture characteristics: Colonies on PDA and PCA very slow-growing, 16 mm diam after 28 days; circular, dome-shaped, radially striate with lobate margins, centrally yeast-like, initially ochreous, passing to umber with age, aerial mycelium sparse to absent, salmon towards the periphery; reverse buff. Cultures sterile.

Holotype: Brazil, Santa Catarina, Urubici, roadside, on dead pinnulae of living fronds of *Dicksonia sellowiana*, 15 Apr. 2013, *E. Guatimosim* (VIC 42507, culture ex-type CPC 24723, COAD 2006).

Habitat/Distribution: Known from *Dicksonia sellowiana* in southern Brazil.

Additional specimens examined: Brazil, Santa Catarina, Luizinho, Highway to São José dos Ausentes, roadside, on fronds of *D. sellowiana*, 16 Apr. 2013, *E. Guatimosim* (VIC 42478, culture CPC 24713); *ibid*., (VIC 42481, cultures CPC 24714, COAD 2003).

Notes: Following the dichotomous key of Spooner (1987), *Lachnopsis catarinensis* does not fit into any previously described species. Based on the key of Haines and Dumont (1984), *Lachnopsis catarinensis* could be compared to *Lachnum cyphelloides* (Pat.) Haines and Dumont, a rare and poorly known species described on twigs and stems of dicotyledonous trees, which presents pale-colored discoid apothecia and long needle-shaped spores (as in *Lachnopsis catarinensis*), and distinctively covered with bright, white hairs ornamented with white to pale brown resinous or crystalline matters (Haines and Dumont 1984), absent in *Lachnopsis catarinensis*. According to the keys of Haines (1980, 1992), and Zhuang and Hyde (2001), *Lachnopsis catarinensis* is close to *Lachnum macrosporum* (Penz. & Sacc.) Haines – a well-known species distinguished from other species of *Lachnum* from tropical ferns, by its long fusiform spores – as in *Lachnopsis catarinensis* (Haines 1992). However, *Lachnum macrosporum* differs from *Lachnopsis catarinensis* by having cylindrical to narrowly ellipsoid asci, distinctly obclavate in the latter and aseptate or with one single medianly septate ascospores, 3-septate in the latter (Haines 1992). Additionally, *Lachnum macrosporum* is only known from an unidentified fern from Java and Guyana, without any information on living cultures derived from either collections (Haines 1980, 1992). Until a proper reassessment of *Lachnum macrosporum* has been made, with the generation of reliable ex-type cultures and DNA data, we prefer to maintain it as a separate taxon.

Lachnopsis dicksoniae Guatimosim, R.W. Barreto & Crous, **sp. nov.** (Fig. 7).

MycoBank MB817301

Etymology: Name refers to the tree fern host genus *Dicksonia*.

Frond blight irregular, starting as small pale to dark brown areas, becoming necrotic, where ascomata are formed, affecting random parts or entire pinnulae. *Ascomata* apothecial, hypophyllous, scattered, discoid,

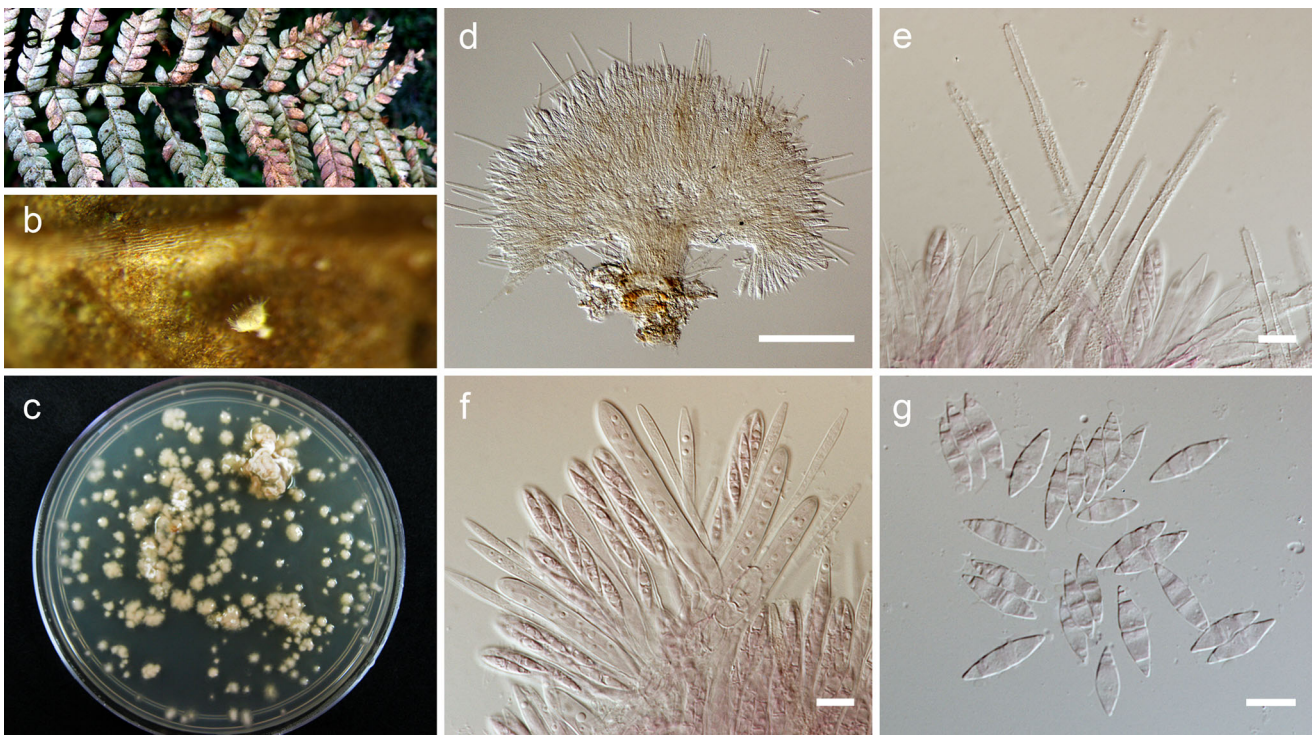

Fig. 7 *Lachnopsis dicksoniae* (VIC 44526). **a** Frond blight on *Dicksonia sellowiana*; **b** apothecium; **c** colony on PDA; **d** squashed apothecium; **e** roughened hairs, with hyaline rod-shaped granules; **f** asci intermixed with narrowly lanceolate or subcylindrical paraphyses; **g** ascospores (**d** in lactic acid; **e**–**g** in lactofuchsin). *Scale bars* (**d**) 100 μm, (**e**–**g**) 10 μm

0.18–0.26 mm, stipitate, stipe 40–315 × 35–290 μm, cream to ochre. *Receptacle* concolorous with the disc, densely clothed with hyaline hairs. *Ectal excipulum* of *textura prismatica*, composed of 9–11 × 3–5 μm large thin-walled cells, becoming intricate towards the base, pale brown, smooth. *Hairs* acerose, straight, 40–70 × 2.5–5 μm, 3–4-septate, gradually tapering toward the obtuse apex, hyaline, thin-walled, roughened with hyaline rod-shaped granules, more crowded towards the apex, non-amyloid. *Asci* unitunicate, cylindrical, straight, 52–61 × 6–8 μm, 8-spored, with a tapered base, without croziers, and subconical apex, with small euamyloid apical ring. *Ascospores* uniseriate, overlapping, fusiform, 13–19 × 4–6 μm, 1-septate, tapering towards acute ends, guttulate, hyaline, smooth, germination not seen. *Paraphyses* narrowly lanceolate or subcylindrical, straight, unbranched, 47–87 × 2–4.5 μm, 1-septate at the base, tapering at the apex, slightly longer than asci, hyaline, smooth. *Asexual morph*: not observed.

Culture characteristics: Colonies on PDA and PCA very slow-growing, 9 mm diam after 28 days; circular, dome-shaped, margin fimbriate, aerial mycelium centrally sparse to absent, velvety, white; reverse buff. Cultures sterile.

Holotype: Brazil, Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, Serra das Cabeças, atlantic rainforest, on fronds of *Dicksonia sellowiana*, 27 Apr. 2013, *P.B. Schwartsburd* (VIC 44526, culture ex-type CPC 24742, COAD 1429).

Notes: When the dichotomous keys of Haines (1980, 1992) are followed, *Lachnum pteridophylli* (Rodway) Spooner (as '*pteridophyllum*') appears as the closest option. Nevertheless, significant morphological differences for shapes and sizes of asci and ascospores indicate the distinction between *Lachnum pteridophyllli* and the fungus on *D. sellowiana*. The key of Spooner (1987) leads to *Lachnum pinnicola* Spooner – described from dead pinnae of *Dicksonia antarctica* from Australia. In this species, apothecia are also superficial, cupulate, stipitate, covered with white hairs, and its ascospores are hyaline, fusoid and have acute ends. Nevertheless, *Lachnum pinnicola* differs from *Lachnopsis dicksoniae* by having thinner (3.5–4 μm) and 3-septate ascospores (Spooner 1987), which are 4–6 μm, and only 1-septate in the latter. Although distribution on lachnoid fungi should not be considered as an important feature related to species boundaries (Haines 1992), the absence of living cultures or DNA data related to the Australian species prevents further considerations about whether or not *Lachnum pinnicola* should be placed in *Lachnopsis*.

Possible additional members of *Lachnopsis*

Lachnopsis cf. *varians*

Notes: *Lachnum varians* (Rehm) Spooner was described on dead stems of an unidentified fern from Brazil (Haines 1980; Spooner 1987). It represents the most common and widespread discomycete inhabiting decaying remains of tropical ferns and has been collected in localities in northern and western South America, Australia, Hawaii, Japan, New Guinea and New Zealand, on members of Cyatheaceae, Dicksoniaceae (including *Dicksonia*), and Gleicheniaceae (Haines 1980; Spooner 1987; Hosoya et al. 2010). Despite the large number of collections, only a single isolate from Japan (TNS-F-7631; Hosoya et al. 2010), has had its DNA assessed, and clearly represents an entirely different clade (Hosoya et al. 2010; Perić and Baral 2014). Based on both ITS and LSU phylogenies (Figs. 3 and 4), this isolate clusters within the newly proposed genus *Lachnopsis*. Nevertheless, at this stage TNS-F-7631 cannot be proposed as an epitype for *Lachnum varians* given the source of the specimen widely differing from that of the type material. A confirmation of the placement of *Lachnum varians* within *Lachnopsis* and the proposal of a new combination depends on recollecting suitable material to be designated as epitype for this taxon.

Lachnopsis cf. *pteridophylli*

Specimens examined: China, Yunnan, Xishuangbanna, on rotten herbaceous stems, 19 Oct. 1999, *W.Y. Zhuang* and *Z.H. Yu* (3155, HMAS 78572), marked as *L.* cf. *pteridophylli*. Puerto Rico, Guilarte Trail, Adjuntas, on fronds of an unidentified fern, 3 Dec. 1994, *S.A. Cantrell* (PR 148, GAM 18397).

Notes: *Lachnum pteridophylli* was originally described on a dead stipe of *Dicksonia antarctica* from Tasmania, but later it was widely collected on ferns from tropical areas like Australia, Colombia, Jamaica, Mexico, Panama, Peru, Puerto Rico, New Guinea, New Zealand, and Venezuela. It was found associated with different species of Cyatheaceae, Dicksoniaceae and Gleicheniaceae (Haines 1980; Spooner 1987). Two specimens of *Lachnum pteridophylli* had their DNA assessed, one from Puerto Rico (SAC PR148; Cantrell and Hanlin 1997), and the other from China (HMAS 78572; Zhao and Zhuang 2011), but the latter was marked as cf., suggesting that the authors were not confident about its identification. Perić and Baral (2014) have already demonstrated that these isolates are not related to each other. The Chinese material would be related to *Erioscyphella*, whereas the Puerto Rican material belongs to an entirely different clade.

Based on both ITS and LSU phylogenies (Figs. 3 and 4), the Puerto Rican isolate clusters within the newly proposed genus *Lachnopsis*. Haines (1980) and Spooner (1987) studied *Lachnum pteridophylli* and *Lachnum varians* extensively, and concluded that they are closely related, as reflected by the inferred phylogeny in the present study. Haines (1980) studied a large number of collections, including the holotype from Tasmania, and several specimens from a range of ferns from the Neotropics. Because SAC PR 148 cannot be considered as an epitype, given the different locality of this collection, it is

hereby maintained as possibly related to *Lachnum pteridophylli*, and based on the inferred phylogeny, placed within the new genus *Lachnopsis*.

Scolecolachnum Guatimosim, R.W. Barreto & Crous, **gen. nov.**

MycoBank MB817302

Type species: *Scolecolachnum pteridii* Guatimosim, R.W. Barreto & Crous

Etymology: Name refers to a combination of the shape of the ascospore - which is long and narrow – and the overall morphological similarity with fungi belonging to the genus *Lachnum*.

Ascomata apothecial, hypophyllous, scattered, discoid, sessile, campanulate, pale to cream or white. *Receptacle* concolorous with the disc. *Medullary excipulum* perpendicular to the host tissue, composed of hyaline *textura angularis*. *Ectal excipulum* of hyaline *textura epidermoidea*, becoming intricate toward the base. *Hairs* cylindrical, aseptate, hyaline, smooth. *Asci* unitunicate, sub-cylindrical, straight, short-pedicellate, 8-spored, without croziers, with small euamyloid apical ring. *Ascospores* parallel in a bundle, filiform or slightly clavate, straight, 0–3-septate, guttulate, hyaline, smooth. *Paraphyses* filiform, flexuous, unbranched, septate, as long as the asci, hyaline, smooth.

Notes: Although the fungus found on bracken (*Pteridium*) in Brazil is morphologically similar to *Lachnum*, attempts at determining its identity with the dichotomous keys of Haines (1980, 1992), Haines and Dumont (1984), Spooner (1987), and Zhuang and Hyde (2001) have shown that it does not fit in *Lachnum* or any other described genus. Additionally, it clearly differs morphologically from other genera of lachnoid fungi related to tropical ferns by having distinctly longer (>20 μm) ascospores which are clavate and aseptate when immature, becoming sub-cylindrical and septate at maturity, by its hairs which are cylindrical, aseptate, hyaline and smooth, and its paraphyses which are as long as the asci, hyaline and smooth. Phylogenetically (Figs. 3 and 4), the newly proposed genus is shown to be an entirely separated clade from *Lachnum* on both ITS and LSU analyses, having *Hyphodiscus* as its sister clade. The SSU phylogeny (data not shown) also support *Scolecolachnum* as a separate genus.

Scolecolachnum pteridii Guatimosim, R.W. Barreto & Crous, **sp. nov.** (Fig. 8).

MycoBank MB813048

Etymology: Name refers to *Pteridium*, the generic name of its host genus.

Frond spots amphigenous, irregular, starting as pale brown areas, becoming necrotic, affecting individual pinnulae. *Ascomata* apothecial, hypophyllous, scattered, discoid, 150–270 μm diam and 260–310 μm high, narrowly campanulate, with elevated margins – when wet, closing as insect egg-like bags – when dry, cream centrally and white at periphery, sessile. *Receptacle* concolorous with the disc. *Medullary excipulum* oriented perpendicularly to the host tissue, composed of hyaline *textura angularis*, cells 4–10 μm diam, thin-walled. *Ectal excipulum* of hyaline *textura epidermoidea*, cells 1–2.5 μm diam, thin-walled, becoming intricate toward the base. *Hairs* cylindrical, 13–16 × 5–6.5 μm, aseptate, hyaline, smooth, thin-walled, non-amyloid. *Asci* unitunicate, subcylindrical, straight, short-pedicellate, 54–100 × 11–18 μm, 8-spored, without croziers, apex conical to somewhat umbonate, slightly thickened, with a small euamyloid apical ring. *Ascospores* parallel in a bundle, filiform, initially somewhat clavate, becoming subcylindrical, straight, 44–57 × 2–3 μm, initially aseptate, becoming 3-septate, apex rounded, tapering toward a subacute base, guttulate, hyaline, smooth, germination not seen. *Paraphyses* filiform, flexuous, unbranched, up to 1 μm wide, septate, apex rounded, as long as the asci, hyaline, smooth. *Asexual morph*: not observed.

Culture characteristics: Colonies on PCA, slow-growing 11–12 × 15–23 mm after 30 day; circular, dome-shaped, margins entire, aerial mycelium dense, cottony, white to buff; reverse salmon. Cultures on PDA irregular, undulate, margins entire, aerial mycelium dense, cottony, centrally lavender-grey, ochreous in the outer region; reverse luteous. Cultures sterile.

Holotype: Brazil, Pernambuco, Taquaritinga do Norte, trilha do Mirante, Serra da Taquara, on fronds of *Pteridium arachnoideum*, 9 Jul. 2014, *D.J. Soares* (VIC 42921, cultures ex-type CPC 25778, COAD 1796).

Habitat/*Distribution*: Known from *P. arachnoideum* in the states of Pernambuco and Rio de Janeiro, Brazil.

Additional specimen examined: Brazil, Rio de Janeiro, Nova Friburgo, on fronds of *Pteridium arachnoideum*, 13 Jun. 2011, *R.W. Barreto* (VIC 42544, culture CPC 24666).

Notes: Based on both phylogenetic analyses (Figs. 3 and 4), *S. pteridii* has *Hyphodiscus* as its sister clade. *Scolecolachnum* and *Hyphodiscus*, however, are clearly morphologically distinct genera having different ascospore shape and size (subcylindrical, long and septate in the former, rather ellipsoid, small, and aseptate in all described species belonging to the latter; Zhuang 1988; Hosoya 2002), and hairs (smooth in the former and warted-tuberculate in the latter; Hosoya 2002). Additionally, the genus *Hyphodiscus* is known as having a gelatinous ectal excipulum (Hosoya 2002; Untereiner et al. 2006) a feature found to be absent in *Scolecolachnum*.

Zymochalara Guatimosim, R.W. Barreto & Crous, **gen. nov.**

MycoBank MB815563

Type species: *Zymochalara cyatheae* Guatimosim, R.W. Barreto & Crous

Etymology: indicating a combination of the yeast-like growth of colonies in pure culture, with a chalara-like morphology.

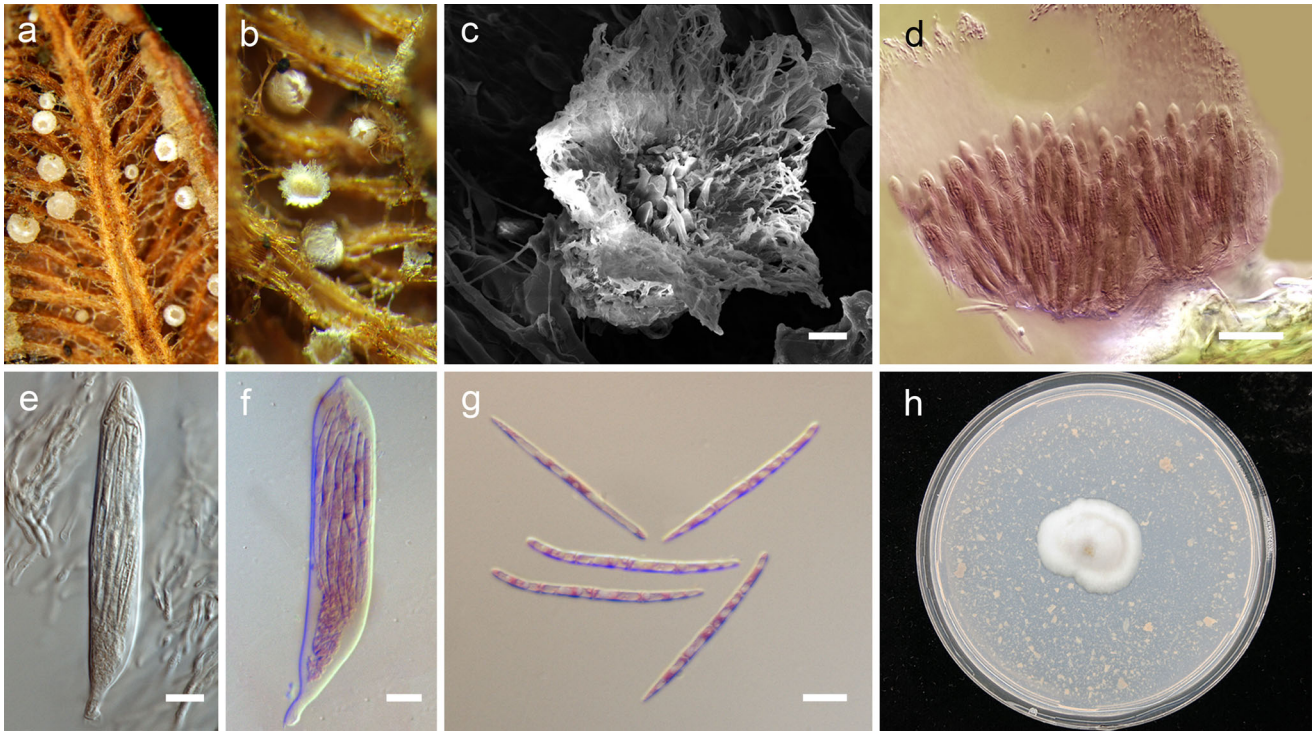

Fig. 8 *Scolecolachnum pteridii* (VIC 42921, holotype). **a**, **b** Hypophyllous apothecia (wet) on *Pteridium arachnoideum*; **c** SEM image of apothecium (note the smooth hairs, typical of the genus); **d** vertical section through apothecium; **e**, **f** asci; **g** ascospores; **h** colony on PDA (**d**, **f**, **g** in lactofuchsin; **e** in lactic acid). *Scale bars* (**c**) 20 μm, (**d**–**f**) 50 μm, (**g**) 10 μm

Conidiophores reduced to phialides. *Phialides* scattered, solitary, unbranched, lageniform, subulate or subcylindrical, aseptate, brown to cinnamon-brown, paler towards the apex, smooth; venter subcylindrical or ellipsoid, pedicellate or not; collarette cylindrical, transition from venter to collarette gradual. *Phialoconidia* endogenous, basipetal, extruded singly or in somewhat long and easily fragmenting chains, cylindrical, truncate at both ends, aseptate, hyaline, biguttulate, smooth. Yeast-like in culture.

Notes: *Zymochalara* is morphologically similar to *Chalara*, but distinct from fungi in that genus by producing a yeast-like colony in pure culture, instead of having filamentous growth as in *Chalara* (Nag Raj and Kendrick 1975). *Chalara* is known to be polyphyletic (Cai et al. 2009). Several genera have a chalara-like morphology (Coetsee et al. 2000; Paulin-Mahady et al. 2002; de Beer et al. 2014). Our phylogenetic analyses clearly place *Zymochalara* as a separate taxon with *Bloxamia cyatheicola* as sister clade (Figs. 1 and 2).

Zymochalara cyatheae Guatimosim, R.W. Barreto & Crous, **sp. nov.** (Fig. 9).

MycoBank MB815126

Etymology: Name refers to the tree fern host genus *Cyathea*.

Frond spots amphigenous, somewhat angular, starting as small necrotic areas along the margins of the pinnulae, increasing in size (up to 2.5–4 × 1.5–3 mm), coalescing and leading to blight of entire pinnulae. *Internal hyphae* not observed. *External hyphae* absent. *Stromata* absent. *Conidiophores* reduced to the conidiogenous cells. *Phialides* hypophyllous, scattered, solitary, erumpent through the cuticle, unbranched, subulate or subcylindrical, 32–50 μm long, 5–8.5 μm wide at the base, aseptate, brown to cinnamon-brown, becoming paler towards the apex, smooth; venter subcylindrical or ellipsoid, 12–26 × 3–7 μm; collarette cylindrical, 15–23 × 2–3.5 μm, transition from venter to collarette gradual. *Phialoconidia* endogenous, basipetal, extruded singly or in long and easily fragmenting chains, cylindrical, truncate at both ends, 6–10 × 1.5–3 μm, aseptate, hyaline, biguttulate, smooth.

Culture characteristics: Colonies on PDA slow growing, 2.2–3.5 cm diam after 30 day; circular, flat, centrally either yeast-like (in PCA), white with some central random tiny dots of aerial mycelium, dark mouse-grey, dry; or with felty aerial mycelium rosy-buff, becoming white and finally buff at periphery (on PDA); reverse either centrally hazel, passing to honey, passing to buff towards the periphery or equivalent to colors at surface. Cultures sterile in PDA, sporulating abundantly on PCA.

Holotype: Brazil, Rio de Janeiro, Nova Friburgo, Limeira, on fronds of *Cyathea delgadii*, 13 Jun. 2011, *R.W. Barreto* (VIC 42543, culture ex-type CPC 24665, COAD 1092).

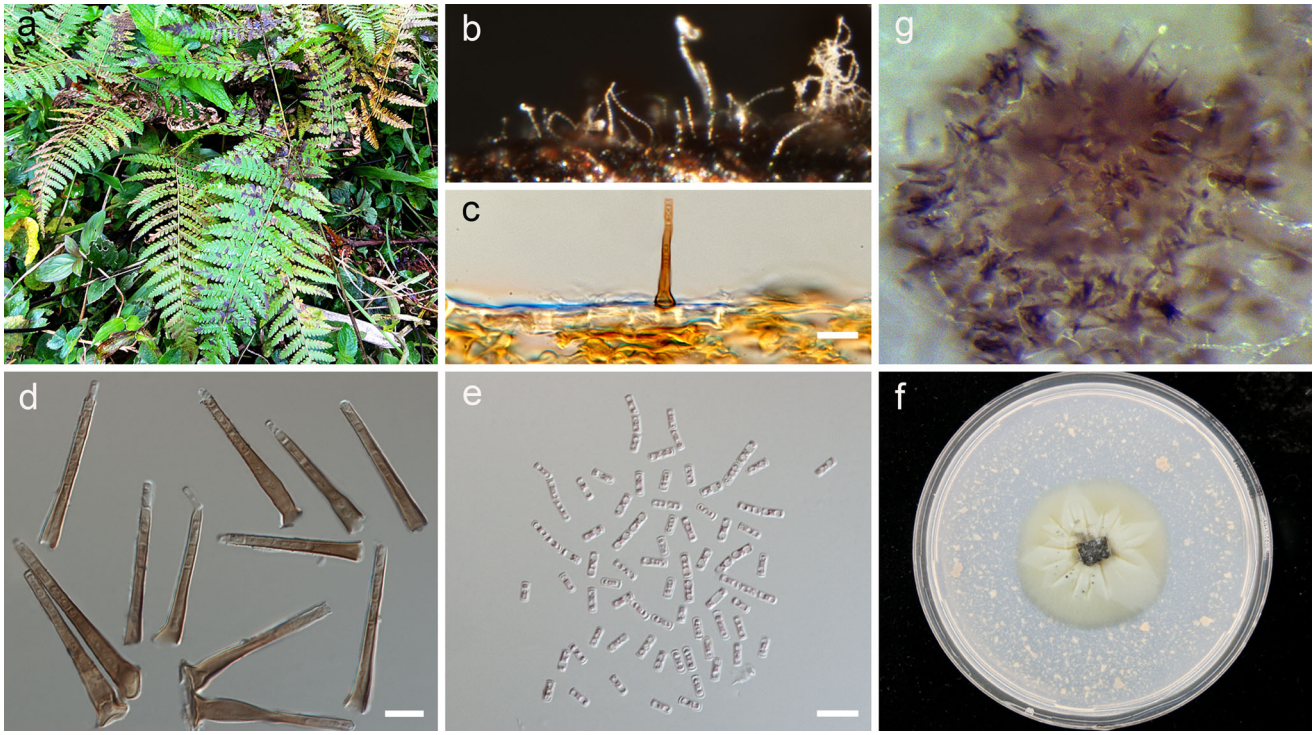

Fig. 9 *Zymochalara cyatheae* (VIC 42543, holotype). **a** Frond spots on *Cyathea delgadii*; **b**–**d** conidiophores; **e** phialoconidia; **f** colony on PDA; **g** close-up of sporulation on PCA (**c**, **d** in lactic acid; **e** in lactofuchsin). *Scale bars* (**c**–**e**) 10 μm

Habitat/*Distribution*: Known from *C. delgadii* in the states of Minas Gerais and Rio de Janeiro, Brazil.

Additional specimens examined: Brazil, Rio de Janeiro, Nova Friburgo, Macaé de Cima, on fronds of *C. delgadii*, 29 Apr. 2012, *R.W. Barreto* (VIC 42562, culture CPC 24690); Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, on fronds of *C. delgadii*, 23 Feb. 2014, *E. Guatimosim* (VIC 42518, culture CPC 24735); *ibid*. (VIC 42462, cultures CPC 24736, COAD 2013); Rio de Janeiro, Nova Friburgo, Macaé de Cima, on fronds of *C. delgadii*, 1 Jun. 2014, *R.W. Barreto* (culture CPC 25072, COAD 1758).

Note: See the notes for *Z. lygodii*.

Zymochalara lygodii Guatimosim, R.W. Barreto & Crous, **sp. nov.** (Fig. 10).

MycoBank MB813046

Etymology: Name refers to the host genus *Lygodium*.

Frond blight irregular, starting as small, vein-delimited, pale brown to cinnamon-brown spots, close to the main vein of the pinnulae and spreading towards the apex. At later stages, becoming dark, necrotic and distorting the pinnulae, sometimes causing necrosis of the entire pinnulae, affecting mostly the upper pinnulae. *Internal hyphae* not observed. *External hyphae* absent. *Stromata* absent. *Conidiophores* reduced to the conidiogenous cells. *Phialides* hypophyllous, scattered, solitary, erumpent through the cuticle, unbranched, lageniform, 29–38 μm long, 5.5–9 μm wide at the base, brown to cinnamon-brown, paler towards the apex, smooth; venter subcylindrical or ellipsoid, pedicellate, 13–16 × 5–6.5 μm; collarette cylindrical, 16–21 × 3–4 μm, transition from venter to collarette gradual. *Phialoconidia* endogenous, basipetal, extruded in easily fragmenting chains, cylindrical, truncate at the base, apex rounded, 6.5–12 × 1.5–3 μm, aseptate, hyaline, biguttulate, smooth.

Culture characteristics: Colonies on PDA slow-growing, 15 mm diam after 28 days; circular to irregular, convex with papillate surface, margin crenate, aerial mycelium velvety, leaden-black intermixed with umber, and white hyphal tufts, mouse-grey at periphery; reverse leaden-black. Colonies on PCA umbonate, radially striate with lobate margins, yeast-like, mostly rosy-buff and buff at periphery; reverse buff. Cultures sterile.

Holotype: Brazil, Minas Gerais, Viçosa, Cristais, on fronds of *Lygodium volubile*, 6 Mar. 2013, *E. Guatimosim* (VIC 42470, culture ex-type CPC 24710, COAD 2001).

Habitat/*Distribution*: Known from *L. volubile* in the states of Minas Gerais and Rio de Janeiro, Brazil.

Additional specimens examined: Brazil, Rio de Janeiro, Lumiar, on fronds of *L. volubile*, 2 May 2013, *R.W. Barreto* (VIC 42600, culture CPC 24699, COAD 1992).

Notes: The morphology of *Z. lygodii* is similar to that of *Chalara fungorum*, but differs from it by having phialides with wider bases (5.5–9 μm in the former and 3–6.5 μm in the latter), and larger phialoconidia (6.5–12 μm long in the former and up to 8 μm in the latter) (Nag Raj and Kendrick

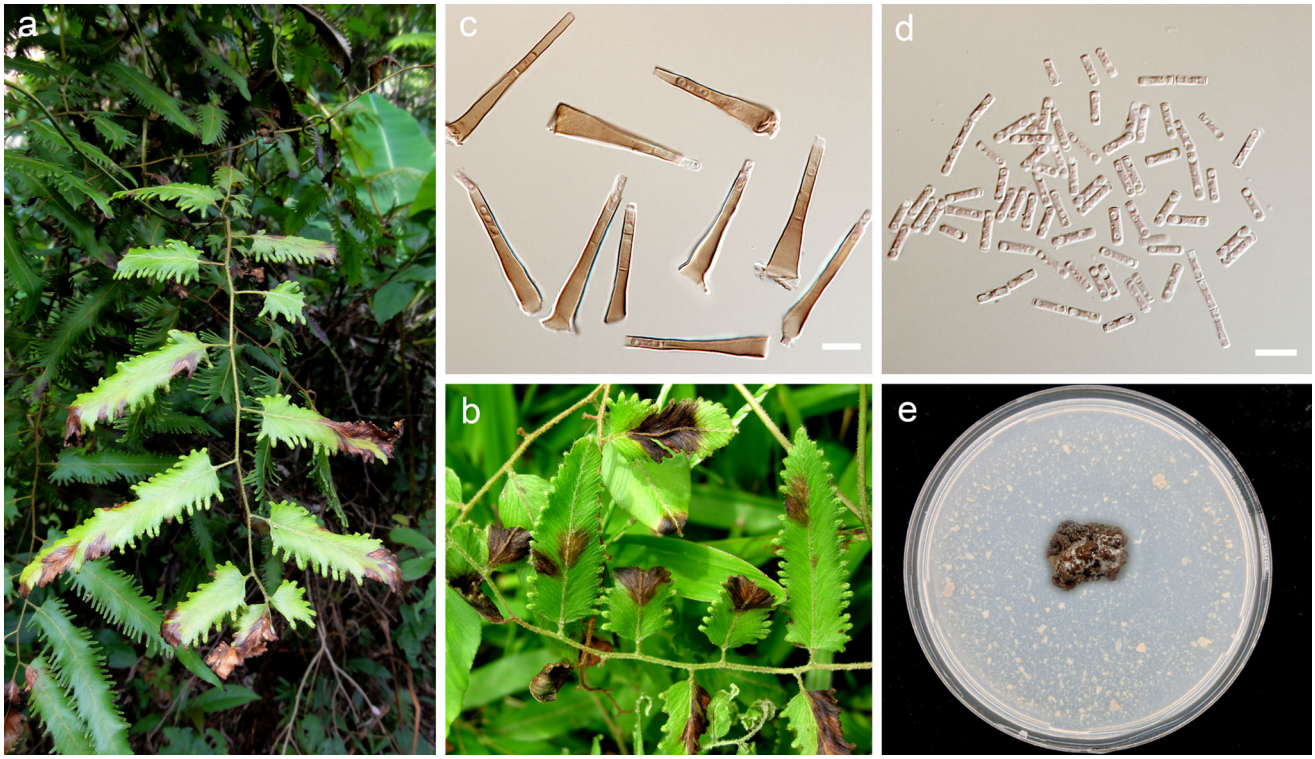

Fig. 10 *Zymochalara lygodii* (VIC 42470, holotype). **a**, **b** Frond blight on *Lygodium volubile*; **c** conidiophores; **d** phialoconidia; **e** colony on PDA (**d** in lactofuchsin; **c** in lactic acid). *Scale bars* (**c**, **d**) 10 μm

1975). Additionally, *C. fungorum* is only known attacking the following hosts: *Abies lasciocarpa*, *Fagus sylvatica*, *Ilex pernyl*, *Laurus nobilis*, *Pistacia lentiscus*, *Quercus ilex*, and *Rhododendron ponticum* in Canada, Italy and the United Kingdom (Nag Raj and Kendrick 1975; Farr and Rossman 2015). Conversely, *Z. lygodii* was found only on the Neotropical liana fern *L. volubile* in Brazil.

Besides having a different host, *Z. lygodii* differs from *Z. cyatheae* by having longer phialides (29–38 μm in the former and 32–50 μm in the latter). The Bayesian analyses generated in this study provide clear evidence that *Z. lygodii* is distinct from *Z. cyatheae* by having 15 bp of variable sites for the ITS locus (Fig. 1), and 10 bp of variable sites for LSU (Fig. 2).

Discussion

The present study aimed to determine the potential fungal diversity occurring on ferns in Brazil. Based on the results obtained here focusing on chalara-like and lachnoid fungi, three genera and six species were found to be new. Furthermore, two novel taxa occurred on an endangered host, *Dicksonia sellowiana*, and should therefore be considered as potentially endangered.

Of the species collected, one was found to represent a new species of *Bloxamia*. Berthet (1964) reported the development of *Bl. truncata* (type species of the genus) from cultures of single ascospore isolations of *Bisporella sulphurina*. Johnston (1998) also obtained evidence of the connection between these sexual and asexual morphs by recovering a *Bloxamia* asexual morph through isolation of *Bisporella discedens* from New Zealand in pure culture. However, the latter author did not propose a separate name for the asexual morph. The genus *Bisporella* is characterised by its bright yellow, sessile to substipitate apothecia, which generally occur on woody substrata in temperate regions. In vertical section, the internal anatomy of the apothecium is characterized by a gelatinised or subgelatinised ectal excipulum, with little or no differentiation of a medullary excipulum; asci 8-spored, 0-1-septate (Carpenter and Dumont 1978; Saccardo 1884). Over the years, this genus was treated as a repository of a large variety of fungi, having significant differences in morphology (e.g., 3-septate ascospores in *Bi. triseptata* and aseptate ascospores in *Bi. calycellinoides*, *Bi. iodocyanescens* and *Bi. oritis*). It includes 25 species (Kirk et al. 2008) and is likely to be a generic complex. This assumption is strengthened by the fact that *Bi. resinicola* has an asexual morph residing in *Eustilbum* (Baranyay and Funk 1969; Seifert and Carpenter 1987), completely different from *Bloxamia*. In addition, a recently published phylogeny has shown that some of the species

recognised as members of *Bisporella* (namely *Bi. citrina*, *Bi. claroflava*, *Bi. drosodes*, *Bi. lactea*, and *Bi. scolochloae*) are in fact members of *Calycina*, once they grouped with its type species *C. herbarum* (Baral et al. 2013). This conclusion, however, did not result in synonymizing the whole genus *Bisporella*, from which its type species has never been studied with molecular tools in the evolutionary context. For the clarification of the true evolutionary relationships within *Bisporella* it is necessary to recollect and epitypify its type species, *Bi. monilifera*, and generate DNA data.

Except for *Bl. foliicola*, all species of *Bloxamia* were described from dead wood, or from rotting plant material (Table 2), suggesting a saprobic life style. Nevertheless, *Bl. cyatheicola* was only found on living fronds either seemingly causing frond spots on *Cyathea* spp. or sporulating without any obvious symptoms on the host tissue. It may be either a weak pathogen or a specialized hemibiotrophic endophyte. Based solely on this ecological evidence (pathogen instead of wood-inhabiting), this is considered by us as insufficient to justify proposing a separate genus to accommodate *Bl. cyatheicola*.

Thus far, despite the fact that the new species from Brazil had both sexual and asexual morphs, we decided to describe it in *Bloxamia*, since this genus is morphologically better circumscribed and older than *Bisporella* (Nag Raj and Kendrick 1975).

The genus *Lachnum* is widely distributed and characterized by having small, discoid apothecia covered by numerous subcylindrical, septate and granular hairs (Haines and Dumont 1984). The genus includes about 250 species (Kirk et al. 2008). Most of them are not known from molecular data but it has already been shown that the genus is polyphyletic (Han et al. 2014). The present phylogenetic survey (Fig. 3), agrees with Zhao and Zhuang (2011), who demonstrated that the ITS locus is reliable for delimiting species boundaries within *Lachnum*, having only *L. rhytismatis* (strains TNS-F-16544 and TNS-F-16545) grouping in a different clade. The present study, in consonance with Perić and Baral (2014), treats *Lachnum* as a genus-complex, from which, based on the phylogenetic inference, different genera can be separated like *Erioscyphella*, *Lachnopsis* and *Scolecolachnum*.

The topology of both ITS and LSU trees (Figs. 1, 2) suggests that the genus *Zymochalara* (including *Z. lygodii* and *Z. cyatheae*) is related to *Chalara*, but significantly distant from all the species included in this study, having *Bl. cyatheicola* as sister clade.

Only three species of *Chalara* are known from ferns, namely *C. crassipes* causing frond spots on *Pteridium aquilinum* in Germany, *Ch. parvispora* on *Cyathea medullaris* from New Zealand, and *Ch. pteridina* on *P. aquilinum* from Austria, Australia, England, Germany, Poland, and the United Kingdom (Nag Raj and Kendrick 1975; Farr and Rossman 2015). Although DNA information available for these taxa is limited to LSU sequences for *C. crassipes* and *C. parvispora* (Cai et al. 2009), it is clear that data from this locus alone are sufficient to separate *C. crassipes* and *C. parvispora* from both *Z. cyatheae* and *Z. lygodii* (Fig. 2).

Four specimens of fungi were collected on the endangered tree fern *D. sellowiana* during our surveys. These were found to represent two novel species within the new genus *Lachnopsis*. Interestingly, these species were not found on any other taxa of tree ferns collected during this study; often occurring in the same habitat. This suggests that these two fungal species are host-specific. Further studies are needed to confirm this conjecture and to demonstrate that these two species found exclusively on *D. sellowiana* are not capable of colonizing other substrates, confirming the hypothesized risk of co-extinction. These considerations follow the line of previously published work conducted in Brazil focused on the foliar mycobiota of endangered Brazilian plant species. Previous publications covered the leaf mycobiota of the endangered tree species *Coussapoa floccosa* (Cecropiaceae) and *Dimorphandra wilsonii* (Fabaceae). Unique fungi were collected on these two endemic trees highlighting the need to preserve endangered plant species from a mycological as well as a botanical viewpoint (Rocha et al. 2010; Silva et al. 2016). The addition of *L. catarinensis* and *L. dicksoniae* to the list of potentially endangered Brazilian microfungi raises the total of species with such status to 11, all of which are recently described as new to science. It is expected that further evidence of complete dependency on endangered plant-hosts will translate into them being added to the IUCN Red List of Threatened Species, as well as to their inclusion in the Brazilian list of endangered species.

The present work contributes towards a better understanding of fungi on tropical ferns as well as of the assemblages of lachnoid, and chalara-like genera within the Hyaloscyphaceae *sensu lato*. The large proportion of taxonomic novelties obtained from the survey in Brazil, as reflected in the present study and that of Guatimosim et al. (2016), confirmed tropical ferns as a rich and poorly investigated fungal niche, deserving further attention by mycologists.

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