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Molecular data resolve a new order of Arthoniomycetes sister to the primarily lichenized Arthoniales and composed of black yeasts, lichenicolous and rock-inhabiting species

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Abstract Lichenicolous fungi belonging to the anamorph-typified genus *Phaeosporobolus* and to the teleomorph-typified genus *Lichenostigma* were isolated in pure culture or sequenced directly, with nuLSU and mtSSU sequences obtained. Phylogenetic analyses place the species of *Phaeosporobolus* in a strongly supported clade with the generic type of *Lichenostigma* (*L. maureri*), the genus *Phaeococcomyces* and several melanized rock-inhabiting isolates. This strongly supported nonlichenized lineage is sister to the primarily lichenized Arthoniales in the Arthoniomycetes and is here described as the Lichenostigmatales. The new order is characterized by cells multiplying by budding, either representing black yeasts, or species in which conidiomata and ascomata are entirely made of an organised agglomeration of spherical yeast-like cells. This way of life is not only very different from all other Arthoniomycetes that exist only in the mycelial stage, but ascomata and conidiomata representing a dense and organised agglomeration of yeast cells might be unique amongst fungi. A further difference with the Arthoniales is the absence of paraphysoids. Phylogenetic results suggest that *Phaeosporobolus usneae* is the asexual stage of *Lichenostigma maureri*. Most species of *Phaeosporobolus* are transferred to the genus *Lichenostigma* except *P. trypethelii*, for which the new genus *Etayoa* is described. The genus *Diederimyces* is reduced into synonymy with *Lichenostigma*. Several other members of *Lichenostigma* are placed in the Dothideomycetes and are intermixed with *Lichenothelia* species.

Keywords Anamorphic fungi · Arthoniomycetes · Dothideomycetes · Lichenostigmatales · Phaeococcomycetaceae · Yeasts

Introduction

Lichenicolous fungi are lichen-associated organisms that live mainly as parasites or commensals. Around 1800 species have been described throughout the Ascomycota and Basidiomycota (Lawrey and Diederich 2003; http://www.lichenicolous.net), and it is estimated that 3000–5000 species will eventually be described. About 20 % of described lichenicolous ascomycetes are known only in the asexual stage (anamorphs), most of uncertain phylogenetic position. Molecular data are especially needed to assess the phylogenetic position of these organisms and to make connections between sexual (teleomorphs) and asexual stages that are often described in different genera.

We began our study to investigate the phylogenetic placement and hypothesized relationship between the teleomorph-typified lichenicolous species *Lichenostigma maureri* and the anamorph-

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typified lichenicolous species *Phaeosporobolus usneae*. *Lichenostigma maureri* grows on *Usnea* and various other macrolichens and forms superficial rounded ascomata containing bitunicate, subglobose asci and 1-septate, dark brown ascospores (Hafellner 1982). *Phaeosporobolus usneae* grows on various macrolichens (mainly Parmeliaceae such as *Bryoria*, *Evernia*, *Letharia*, *Usnea*, but also *Ramalina*) and forms tiny, brownish, superficial, stromatic conidiomata containing dark brown, subglobose or ellipsoid, multicellular conidia (Hawksworth and Hafellner 1986). An anamorph-teleomorph relationship between the two was suggested by the remarkable similarity of the ascomata of *L. maureri* to the conidiomata of *P. usneae* (Hawksworth and Hafellner 1986; Berger and Brackel 2011), and the fact that they sometimes grow intermixed (Berger and Brackel 2011). The fruit bodies of the two taxa are so similar that a microscopical examination of squash preparations is required for an accurate identification (Diederich 2004). *Lichenostigma maureri* was supposed to produce only ascomata whereas *P. usneae* only conidiomata.

The phylogenetic position of these fungi has been discussed but never firmly resolved. *Lichenostigma* has been thought by some authors (Hafellner 1982; Hafellner and Calatayud 1999) to belong to the Arthoniales based on ascus type, but other authors consider it a member of the Dothideomycetes close to *Lichenothelia* (Eriksson and Hawksworth 1986: 137; Navarro-Rosinés and Hafellner 1996; Muggia et al. 2012). *Phaeosporobolus* was originally suggested to be most closely allied to the lichenicolous genus *Sclerococcum* (Hawksworth and Hafellner 1986), but Etayo (1995) has more recently suggested that it is the asexual stage of the lichenicolous genus *Diederimyces*.

Analysis of sequences obtained from *L. maureri* and *P. usneae* yielded one expected result—that the two were conspecific, but also an unexpected one—that *L. maureri* is in a clade sister to the Arthoniomycetes along with several rock-inhabiting fungi (RIF) and does not group with the other sequences of Lichenotheliaceae placed in the Dothideomycetes. The existence of this clade had been recognized earlier by Ruibal et al. (2008, 2009) but its composition was far from certain. Many of the RIF in this clade were originally identified in Ruibal et al. (2008) as *Phaeococcomyces* species or "phaeococcomyces-like", but these labels were dropped in the subsequent paper (Ruibal et al. 2009). Nevertheless, our results now indicated a possible unrecognized link between *Lichenostigma* and *Phaeococcomyces*.

Given these initial results, we expanded our objectives to include a larger number of specimens of *Lichenostigma* and *Phaeosporobolus* in a two-locus molecular phylogenetic study designed to determine the composition of the new clade in Arthoniomycetes, and provide formal recognition of the clade. Moreover, this study aims to test the monophyly of the two subgenera of *Lichenostigma*.

Materials and methods

Morphological study

Herbarium specimens are deposited in BR, KRAM, MSC, NY and UBC, and in the private collections of P. Diederich, J. Etayo and K. Kalb. Dry herbarium specimens were examined and measured under a binocular microscope Leica MZ 7.5 (magnification up to 50×). Macroscopic photographs were done using a Canon 40D camera with Nikon BD Plan 10 or Nikon ELWD 40 microscope objectives, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Entire unsectioned ascomata or conidiomata were studied in water, either without or with pressure on the coverslip. Microscopic photographs of stromata in water were prepared using a Zeiss Photomikroskop III or Leica DMLB microscope, a Leica EC3 camera and Helicon Focus. Measurements based on statistical data are indicated as $(\text{minimum}–)\overline{X}-\sigma_x–\overline{X}+\sigma_x(-\text{maximum})$, followed by the number of measurements (N). Unless otherwise indicated, iodine reactions were done using 1 % IKI (1 g I_2, 2 g KI, 100 ml water), without (I) or with (K/I) pre-treatment with 10 % KOH, without or with pre-treatment with commercial bleach (C).

Isolation of fungal cultures

Ascomata, conidiomata or hyphae of freshly collected material of *Lichenostigma* or *Phaeosporobolus* were washed in sterilized water and transferred to Malt-Yeast Extract agar following Yoshimura et al. (2002). The cultures were kept at room temperature in the laboratory of the National Botanic Garden of Belgium and exposed to a natural day light regime. No culture chambers were used to test whether different light or temperature conditions could improve the growth rate. All the strains were slow growing and therefore 3 to 5 months were required in order to obtain sufficient material for DNA extraction.

Molecular techniques

Genomic DNA was isolated from mycobiont cultures following the procedures cited by Ertz et al. (2011). When cultures failed, 2 to 4 ascomata or conidiomata of freshly collected specimens were added to a tube containing the PCR reaction mixture and amplified directly as explained in Lawrey et al. (2007: 780). Because *Lichenostigma maureri* and *Phaeosporobolus usneae* often grow intermixed, we carefully checked under the microscope each ascoma or conidioma by crushing them in a drop of sterile water before adding the material to the PCR tube. We amplified and sequenced a fragment of about 1.2 kb of the nuLSU using primers LIC15R, LR3R, LR3, LR7, and LR6 (Miadlikowska et al. 2002; Vilgalys and Hester 1990). Primers for amplification and sequencing of the mtSSU rDNA were mrSSU1 and mrSSU3R (Zoller et al. 1999).

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). The yield of the PCRs was verified by running the products on a 1 % agarose gel using ethidium bromide. Both strands were sequenced by Macrogen®. Sequences were edited and overlapping fragments were assembled in larger consensus sequences using Sequencher v4.6 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were subjected to BLAST searches to verify their closest relatives and to detect potential contaminations.

Taxon selection and phylogenetic analyses

We obtained 51 new sequences for this study from Canada, the Canary Islands (Gomera, Tenerife), Croatia, Florida, France (including Corsica), Luxembourg, South Africa and Switzerland. For the phylogenetic analyses, 126 sequences were retrieved from GenBank in addition to the newly generated sequences. We selected members representing all major clades in the Arthoniomycetes and Dothideomycetes and as far as possible having both nuLSU and mtSSU sequences (Table 1). These data were selected mainly from Diederich et al. (2012b), Ertz and Tehler (2011), Lawrey et al. (2011), Muggia et al. (2012) and Ruibal et al. (2009). More taxa were retrieved from GenBank based on BLAST searches of our newly generated sequences. Three outgroup species were chosen to represent the Eurotiomycetes (*Caliciopsis pinea* and *Capronia munkii*) and the Leotiomycetes (*Lachnum virgineum*). The sequences of taxa listed in Table 1 were aligned manually using MacClade 4.05 (Maddison and Maddison 2002). Ambiguous regions and introns representing a total of 3054 bp (1282 bp for nuLSU and 1772 bp for mtssu; mainly due to long introns) were delimited manually and excluded from the analyses.

Because it was not possible to complete the nuLSU and mtSSU sequences for the same set of 97 samples, analyses for topological incongruence among loci were carried out on the same data sets for each gene (i.e., 80 nuLSU and 80 mtSSU sequences; Table 1). Analyses were carried out using 1000 replicates of Neighbor-Joining bootstrapping (NJ-bs) with distance measure estimated by maximum likelihood under a six-parameter (GTR) best-fit evolutionary model for nucleotide substitution (Cunningham et al. 1998; Liò and Goldman 1998; Yang et al. 1994) using PAUP* 4.0b10 (Swofford 2002). Best-fit evolutionary models were estimated for all NJ analyses using Akaike Information Criterion (AIC) as implemented in Modeltest v. 3.06 (Posada and Crandall 1998). The TIM+I+G model was selected for the nuLSU data set while the GTR+I+G model was selected for the mtSSU data set. All topological bipartitions with NJ-bs values≥70 % were compared for the two loci. A conflict was assumed to be significant if two different relationships (one being monophyletic and the other being non-monophyletic) for the same set of taxa were both supported with bootstrap values≥70 % (Mason-Gamer and Kellogg 1996). Based on this criterion, no conflict was detected and therefore the nuLSU and mtSSU data sets were concatenated. The combined two-loci data set consisted of 97 taxa and 1803 unambiguously aligned sites, 1249 for nuLSU and 554 for mtSSU.

Bayesian analyses were carried out on the two-loci data set using the Metropolis-coupled Markov chain Monte Carlo method (MCMCMC) in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) on the CIPRES Web Portal (Miller et al. 2010). Analyses were run under the selected models for each gene partition using a gamma-distributed rate parameter and a proportion of invariable sites. Two parallel MCMCMC runs were performed each using four independent chains and 30 million generations, sampling trees every 1000th generation. TRACER v.1.5 (Rambaut and Drummond 2007) was used to ensure that stationarity was reached by plotting the log-likelihood values of the sample points against generation time. Posterior probabilities (PP) were determined by calculating a majority-rule consensus tree generated from the 45002 post-burnin trees of the 60002 trees sampled by the two MCMCMC runs using the sumt option of MrBayes. A Maximum Likelihood (ML) analysis was performed on the two-loci data set using GARLI (Zwickl 2006, v.0.951 for OSX) with default settings, and a single most likely tree was produced (−lnL=25100.4677). One thousand bootstrap pseudoreplicates were used to calculate a majority rule consensus tree in PAUP* to assess the Maximum Likelihood bootstrap values (ML-bs). In addition, the aligned sequences were subjected to a maximum likelihood (ML) search using RAxML 7.2.6 (Stamatakis et al. 2005; Stamatakis 2006) on the Cipres Web Portal, with parametric bootstrapping using 1000 replicates under the GTRGAMMA model. The Bayesian tree did not contradict the ML trees topology for the strongly supported branches and hence only the majority rule consensus tree of the ML analysis using GARLI is shown with the posterior probabilities of the Bayesian analysis added below the internal branches and the bootstrap support values of the RAxML analysis added above the internal branches. ML-bs≥70 % and PP≥95 % were considered to be significant. Phylogenetic trees were visualized using FigTree v1.3.1 (Rambaut 2012).

Results

The phylogenetic analyses resolved the Dothideomyceta (Arthoniomycetes and Dothideomycetes) as a strongly supported group, but the Dothideomycetes does not form a sister group to the Arthoniomycetes as shown in other studies using a wide sampling in the Arthoniomycetes and Dothideomycetes (Ruibal et al. 2009; Muggia et al. 2012, Fig. 1). Some main groups of Dothideomycetes are strongly supported by the three analyses, such as the Dothideales, the Capnodiales, the

Table 1 Specimens and DNA sequences used in this study, with their respective voucher information. GenBank accession numbers (in bold) refer to sequences (51) generated by this project. All other sequences (126 GenBank identification numbers) were obtained directly from GenBank

Name	Order	Family	Voucher information	nuLSU	mtSSU
Acrospermum compressum	Acrospermales	Acrospermaceae	M151; J. Vesterholt s.n. (TUR)	EU940084	EU940237
Alyxoria ochrocheila	Arthoniales	‘Lecanographaceae’	Ertz 7519 (BR)	EU704100	EU704072
Alyxoria varia	Arthoniales	‘Lecanographaceae’	Ertz 7570 (BR)	EU704103	EU704075
Arthonia didyma	Arthoniales	Arthoniaceae	Ertz 7587 (BR)	EU704083	EU704047
Arthonia dispersa	Arthoniales	Arthoniaceae	UPSC2583	AY571381	AY571383
Arthopyrenia salicis	Pleosporales	Arthopyreniaceae	CBS 368.94	AY538339	AY538345
Botryosphaeria dothidea	Botryosphaeriales	Botryosphaeriaceae	CBS 115476 (AFTOL-ID 946)	DQ678051	FJ190612
Botryosphaeria ribis	Botryosphaeriales	Botryosphaeriaceae	CBS 115475 (AFTOL-ID 1232)	DQ678053	AF271148
Briancoppinsia cytospora	Arthoniales	Arthoniaceae	Ertz 15244 (BR)	JF830770	JF830772
Caliciopsis pinea	Coryneliales	Coryneliaceae	AFTOL-ID 1869	DQ678097	FJ190653
Capnodium coffeae	Capnodiales	Capnodiaceae	CBS 147.52 (AFTOL-ID 939)	DQ247800	FJ190609
Capronia munkii	Chaetothyriales	Herpotrichiellaceae	AFTOL-ID 656	EF413604	FJ225723
Chiodecton natalense	Arthoniales	Roccellaceae	Ertz 6576 (BR)	EU704085	EU704051
Chrysothrix caesia	Arthoniales	Chrysotrichaceae	AFTOL-ID 775	FJ469668	FJ469671
Chrysothrix chrysophthalma	Arthoniales	Chrysotrichaceae	Ertz 10927 (S)	HQ454519	**KF176966**
Coniosporium apollinis	Unknown	Unknown	CBS 109860	GU250899	GU250910
Coniosporium uncinatum	Unknown	Unknown	CBS 100212	GU250902	GU250913
Cryomyces antarcticus	Unknown	Unknown	CCFEE 536	GU250365	GU250411
Cryptothecia candida	Arthoniales	Arthoniaceae	Ertz 9260 (BR)	HQ454520	EU704052
Cystocoleus ebeneus	Capnodiales	Unknown	L161 Hafellner & Muggia (GZU)	EU048578	EU048584
Dendrographa leucophaea	Arthoniales	Roccellaceae	Sparrius 7999 (BR)	AY548810	AY548811
Dimidiographa longissima	Arthoniales	Roccellographaceae	Ertz 9155 (BR)	EU704097	EU704069
Dothidea insculpta	Dothideales	Dothideaceae	CBS 189.58 (AFTOL-ID 359/921)	DQ247802	FJ190602
Dothideomycetes sp. AN13	Unknown	Unknown	AN13	GU250928	N/A
Enterographa crassa	Arthoniales	Roccellaceae	Ertz 5041 (BR)	EU704088	EU704056
Enterographa zonata	Arthoniales	Roccellaceae	Vigneron 104 (BR)	EU704109	EU704081
Erythrodecton granulatum	Arthoniales	Roccellaceae	Ertz 9908 (BR)	EU704090	EU704058
Etayoa trypethelii	Lichenostigmatales	Phaeococcomycetaceae	Common 9200-G	**KF176940**	**KF176967**
Etayoa trypethelii	Lichenostigmatales	Phaeococcomycetaceae	Common 9215-P	**KF176941**	**KF176968**
Etayoa trypethelii	Lichenostigmatales	Phaeococcomycetaceae	Common 9434-K	**KF176942**	**KF176969**
Etayoa trypethelii	Lichenostigmatales	Phaeococcomycetaceae	Mukherjee s.n. (BR)	**KF176943**	**KF176970**
Farlowiella carmichaeliana	Unknown	Unknown	CBS 206.36 (AFTOL-ID 1787)	AY541492	AY571387
Friedmanniomyces endolithicus	Capnodiales	Teratosphaeriaceae	CCFEE 5180	GU250367	GU250415
Gloniopsis praelonga	Hysteriales	Hysteriaceae	CBS 112415	FJ161173	N/A
Hortaea werneckii	Capnodiales	Teratosphaeriaceae	CBS 708.76	GU301818	GU561844
Hysteropatella clavispora	Patellariales	Patellariaceae	CBS 247.34 (AFTOL-ID 1254)	AY541493	AY571388
Kirschsteiniothelia aethiops	Unknown	Unknown	CBS 109.53 (AFTOL-ID 925)	AY016361	FJ190604
Lachnum virgineum	Helotiales	Hyaloscyphaceae	AFTOL-ID 49	AY544646	AY544745
Laurera megasperma	Trypetheliales	Trypetheliaceae	Ertz 9725 (BR) (AFTOL-ID 2094)	FJ267702	GU561847
Lecanactis abietina	Arthoniales	Roccellaceae	Ertz 5068 (BR) (AFTOL-ID 305)	AY548812	AY548813
Lichenoconium erodens	Unknown	Unknown	JL363-09	HQ174267	HQ174266
Lichenostigma alpinum	Lichenostigmatales	Phaeococcomycetaceae	Diederich 17379	**KF176944**	**KF176971**
Lichenostigma alpinum	Lichenostigmatales	Phaeococcomycetaceae	Ertz 17522 (BR)	**KF176945**	**KF176972**
Lichenostigma alpinum	Lichenostigmatales	Phaeococcomycetaceae	Ertz 17519 (BR)	**KF176946**	**KF176973**
Lichenostigma chlaroterae	Lichenostigmatales	Phaeococcomycetaceae	Diederich 17329	**KF176947**	**KF176974**
Lichenostigma chlaroterae	Lichenostigmatales	Phaeococcomycetaceae	Neuberg	**KF176948**	**KF176975**
Lichenostigma chlaroterae	Lichenostigmatales	Phaeococcomycetaceae	Goward 12-30	N/A	**KF176976**
Lichenostigma chlaroterae	Lichenostigmatales	Phaeococcomycetaceae	Goward 12-42	**KF176949**	**KF176977**
“*Lichenostigma*” cf. *elongatum*	Unknown	Lichenotheliaceae	Ertz 15255 (BR)	**KF176950**	N/A
Lichenostigma maureri asexual stage	Lichenostigmatales	Phaeococcomycetaceae	Diederich 17306	**KF176951**	**KF176978**

Table 1 (continued)

Name	Order	Family	Voucher information	nuLSU	mtSSU
Lichenostigma maureri asexual stage	Lichenostigmatales	Phaeococcomycetaceae	Diederich 17337	**KF176952**	N/A
Lichenostigma maureri sexual stage	Lichenostigmatales	Phaeococcomycetaceae	Diederich 17326	**KF176953**	**KF176979**
Lichenostigma maureri sexual stage	Lichenostigmatales	Phaeococcomycetaceae	Diederich 17337	**KF176954**	**KF176980**
Lichenostigma sp.	Lichenostigmatales	Phaeococcomycetaceae	Diederich 17240	**KF176955**	**KF176981**
“*Lichenostigma*” sp.	Unknown	Lichenotheliaceae	Ertz 16122 (BR)	**KF176956**	**KF176982**
“*Lichenostigma*” sp.	Unknown	Lichenotheliaceae	Ertz 16340 (BR)	**KF176957**	**KF176983**
“*Lichenostigma*” sp.	Unknown	Lichenotheliaceae	Ertz 16455 (BR)	**KF176958**	**KF176984**
“*Lichenostigma*” sp.	Unknown	Lichenotheliaceae	Ertz 17457 (BR)	**KF176959**	**KF176985**
“*Lichenostigma*” sp.	Unknown	Lichenotheliaceae	Ertz 17540 (BR)	**KF176960**	**KF176986**
Lichenostigma sp.	Lichenostigmatales	Phaeococcomycetaceae	Ertz 17591 (BR)	**KF176961**	**KF176987**
Lichenothelia cf. *calcarea* 1	Unknown	Lichenotheliaceae	L1324; Muggia L.	KC015062	N/A
Lichenothelia cf. *calcarea* 2	Unknown	Lichenotheliaceae	L1708; Knudsen K. 12672	KC015065	N/A
Lichenothelia convexa	Unknown	Lichenotheliaceae	Diederich P. 17491	**KF176962**	**KF176988**
Lichenothelia L985	Unknown	Lichenotheliaceae	L985; Perlmutter G. 2621	KC015075	N/A
Lichenothelia rugosa	Unknown	Lichenotheliaceae	Diederich 17310	**KF176963**	**KF176989**
Lichenothelia rugosa	Unknown	Lichenotheliaceae	Ertz 16065 (BR)	**KF176964**	N/A
Lophium mytilinum	Mytilinidiales	Mytilinidiaceae	CBS 269.34 (AFTOL-ID 1609)	DQ678081	N/A
Mycosphaerella punctiformis	Capnodiales	Mycosphaerellaceae	CBS 113265 (AFTOL-ID 942)	DQ470968	FJ190611
Myriangium duriaei	Myriangiales	Myriangiaceae	CBS 260.36 (AFTOL-ID 1304)	DQ678059	AY571389
Opegrapha vermicellifera	Arthoniales	Opegraphaceae	Ertz 7562 (BR)	EU704105	EU704077
Opegrapha vulgata	Arthoniales	Opegraphaceae	Ertz 7564 (BR)	EU704108	EU704080
Paralecanographa grumulosa	Arthoniales	Opegraphaceae	Ertz 13565 (BR)	HQ454555	N/A
Phaeococcomyces eucalypti	Lichenostigmatales	Phaeococcomycetaceae	CPC:17606	KC005791	N/A
Phaeococcomyces nigricans	Lichenostigmatales	Phaeococcomycetaceae	CBS 652.76	AF361048	N/A
Phaeococcomyces cf. *nigricans*	Lichenostigmatales	Phaeococcomycetaceae	MZ107	AJ276065	N/A
Phaeotrichum benjaminii	Unknown	Phaeotrichaceae	CBS 541.72	AY004340	AY538349
Phoma cladoniicola	Pleosporales	Phaeosphaeriaceae	CBS 128025	JQ238625	JQ238624
Phyllobathelium anomalum	Unknown	Strigulaceae	Lücking s.n. (F)	GU327722	GU327698
Preussia terricola	Pleosporales	Sporormiaceae	DAOM 230091 (AFTOL-ID 282)	AY544686	AY544754
Racodium rupestre	Capnodiales	Unknown	L346 - Hafellner & Muggia (GZU)	EU048583	EU048588
Reichlingia leopoldii	Arthoniales	Arthoniaceae	Ertz 13293 (BR)	HQ454581	JF830773
Roccella fuciformis	Arthoniales	Roccellaceae	Diederich 15572 (AFTOL-ID 126)	AY584654	EU704082
rock isolate CCFEE 5284	Lichenostigmatales	Phaeococcomycetaceae	CCFEE 5284	GU250373	N/A
rock isolate D007_09	Lichenostigmatales	Phaeococcomycetaceae	D007_09	GU250402	N/A
rock isolate TRN 213	Lichenostigmatales	Phaeococcomycetaceae	TRN 213	N/A	GU324040
rock isolate TRN 452	Lichenostigmatales	Phaeococcomycetaceae	TRN 452	GU323985	GU324048
rock isolate TRN 456	Lichenostigmatales	Phaeococcomycetaceae	TRN 456	GU323986	GU324049
rock isolate TRN 529	Lichenostigmatales	Phaeococcomycetaceae	TRN 529	GU323987	GU324050
Schismatomma pericleum	Arthoniales	Roccellaceae	Tehler 7701 (S)	AF279408	AY571390
Sydowia polyspora	Dothideales	Dothioraceae s.l.	CBS 116.29 (AFTOL-ID 1300)	DQ678058	FJ190631
Trypethelium nitidiusculum	Trypetheliales	Trypetheliaceae	Ertz 9716 (BR) (AFTOL-ID 2099)	FJ267701	GU561848
Tubeufia cerea	Unknown	Tubeufiaceae	CBS 254.75 (AFTOL-ID 1316)	DQ470982	FJ190634
Tylophoron hibernicum	Arthoniales	Arthoniaceae	Ertz 11546 (BR)	JF295083	JF830778
Tyrannosorus pinicola	Unknown	Unknown	CBS 124.88 (AFTOL-ID 1235)	DQ470974	FJ190620
Venturia chlorospora	Unknown	Venturiaceae	Kruys 502 (UPS)	DQ384101	DQ384084
Xanthoriicola physciae	Capnodiales	Teratosphaeriaceae	Diederich 16713	**KF176965**	**KF176990**
Zwackhia viridis	Arthoniales	‘Lecanographaceae’	Ertz 7619 (BR)	EU704106	EU704078

Botryosphaeriales, the Pleosporales and the Trypetheliales, but the relationships among them are not supported. Most of the Lichenotheliaceae cluster together, but the clade is not well supported. Two *Lichenothelia* specimens (*Lichenothelia* cf.

Fig. 1 Phylogenetic relationships among 94 samples within Dothideomyceta (with three outgroup taxa) based on a combined data set of nuLSU and mtSSU sequences that resulted from a maximum likelihood analysis using Garli. Internal branches with maximum likelihood bootstrap values≥70 obtained from a Garli analysis are considered strongly supported and represented by *thicker lines*. Posterior probabilities≥0.95 resulting from a bayesian analysis are shown below internal branches and bootstrap support values from a RAxML analysis are shown above the internal branches. Lichenized taxa are in *green* and lichenicolous fungi in *red* (*P. grumulosa* might have both live styles). Collecting numbers of the authors following the species names act as specimen and sequence identifiers. Countries and host lichens are indicated for the lichenicolous Lichenostigmatales

calcarea 2 and *Lichenothelia* L985) are placed in a different lineage as sister to 'Dothideomycetes AN13', but this placement is not supported either. In the combined nuLSU and nuSSU tree by Muggia et al. (2012), the *Lichenothelia* clade also lacks significant support.

The Arthoniomycetes are strongly supported by the three analyses and are divided in two main groups corresponding to the Lichenostigmatales and to the Arthoniales. However, the sister relationship of the Chrysothricaceae with the rest of the Arthoniales is not supported. As a consequence, the phylogenetic position of this family is uncertain within the Arthoniomycetes. The relationships within the Arthoniales are strongly supported and are congruent with the well-supported lineages in Ertz and Tehler (2011). The Lichenostigmatales are divided in two main lineages, a first well-supported clade including the new genus *Etayoa* with some unidentified rock isolates and a second poorly supported clade divided itself in a well-supported clade including the genus *Phaeococcomyces* (with the generic type *Phaeococcomyces nigricans*) and another well-supported clade corresponding to *Lichenostigma* s. str.

Discussion

This is the first molecular phylogenetic study that includes members of the genera *Lichenostigma* and *Phaeosporobolus*. The genus *Lichenostigma* has been considered a member of the Lichenotheliaceae Henssen (Eriksson and Hawksworth 1986: 137), which otherwise includes only the genus *Lichenothelia*. The Lichenotheliaceae were considered to belong to the Arthoniales by some authors (e.g., Fernández-Brime et al. 2010, Ihlen 2004, Valadbeigi and Brackel 2011), but they have also been placed in the Dothideomycetes (e.g., Lumbsch and Huhndorf 2010). Based on molecular data, Muggia et al. (2012) recently suggested that the Lichenotheliaceae (*Lichenostigma* not included) were part of the Dothideomycetes and not of the Arthoniales. Our phylogenetic tree confirms the results of Muggia et al. (2012), as most described *Lichenothelia* and *Lichenostigma* species form a clade within the Dothideomycetes. However, in our phylogenetic tree, the generic type *Lichenostigma maureri* does not belong to this clade but occupies an isolated position in the Arthoniomycetes. Moreover, all species sequenced here and previously accepted in the genus *Phaeosporobolus* are part of the Arthoniomycetes in a lineage with *Lichenostigma maureri*, black yeasts (*Phaeococcomyces*) and several unidentified rock inhabiting fungi.

This well-supported clade is sister to the Arthoniales and we are recognizing it here as a new order in the Arthoniomycetes, the Lichenostigmatales. Previous phylogenetic studies (Ruibal et al. 2008, 2009) hinted at the existence of this group, but its composition and placement were somewhat equivocal. Ruibal et al. (2008) identified several of the rock isolates in the group as *Phaeococcomyces* spp. or phaeococcomyces-like species, but most isolates have not been named. Ruibal et al. (2009) suggested the entire group may represent an example of an early diverging Dothideomycetes lineage, supporting the hypothesis that rock surfaces were important substrates for ancient fungal lineages, as appears to the be case for lichens in *Verrucariales* (Gueidan et al. 2008). Given its position sister to the lichenized Arthoniales, the Lichenostigmatales, which thus far appears to be nonlichenized, may represent a possible transitional group between the Arthoniomycetes and the Dothideomycetes, but one showing clear affinities with the Arthoniomycetes.

The position of *Lichenostigma maureri* within the Arthoniomycetes is supported by the ascus type. Calatayud and Barreno (2003) observed a K/I+ blue ring in the lower part of the apical dome (near the ocular chamber) of the asci when studying material of *Lichenostigma maureri*. We observed that parts of the ascus tholus (not only a ring) may become blue (Fig. 2b). This reaction, not mentioned in the original description of the species (Hafellner 1982), supports the placement of the generic type of *Lichenostigma* close to the Arthoniales. Moreover, they observed that the outer wall of some young hyaline ascospores of *L. maureri* reacted K/I+ blue (confirmed by us, see Fig. 2b), as in the gelatinous sheath of certain *Arthonia* species, a diagnostic character also supporting the close relationship with the Arthoniales.

However, the Lichenostigmatales appears to be morphologically quite distinct from the Arthoniales. Our detailed studies of *Lichenostigma maureri* and the related *Phaeosporobolus* species revealed that the cells forming the conidiomata and ascomata of these taxa are spherical and multiply by budding (Figs. 2d, 3f and 7b). Only three specialized cells types of the conidiomata and ascomata are not spherical: the asci, the ascospores and the conidiogenous cells. The ascospores are the only one of these having real septa and that could form a mycelial stage, although this is rarely observed (see Taxonomy section, Fig. 4j–l). Germination of conidia is also exclusively by budding. This way of life is very different from all Arthoniales species that exist only in the mycelial stage (=multiplication of cells by septa), never in the yeast stage. Moreover, to our knowledge, ascomata and conidiomata representing a dense and organised agglomeration of yeast cells is unique amongst fungi. The order also includes black yeasts of the genus *Phaeococcomyces* that exclusively reproduce by budding but do not agglomerate. A further difference with the Arthoniales is the absence of paraphysoids in the ascomata of *Lichenostigma maureri*. Some groups within the Dothideomycetes (e.g., Cookellaceae) are similar to *Lichenostigma* s. str. by having small ascomata with scattered globose-subglobose asci dispersed in stromatic tissue lacking pseudoparaphyses. However, the Cookellaceae differ from the Lichenostigmatales by having a different ecology (parasitic on leaves, possibly fungicolous), asci lacking an ocular chamber, muriform ascospores and non-spherical stromatic cells that probably do not multiply by budding. They were placed by von Arx (1963) in the Myriangiales but are considered now of uncertain order in Dothideomycetes (Lumbsch and Huhndorf 2010). Molecular data are needed here to confirm their placement in the Myriangiales.

Interestingly, *Lichenostigma maureri* and *Phaeosporobolus usneae* are closely related in our phylogenetic tree. Along with the morphological similarities of the fruit bodies, our results strongly suggest that *Lichenostigma maureri* is the sexual stage of *Phaeosporobolus usneae*. As *Lichenostigma maureri* is the

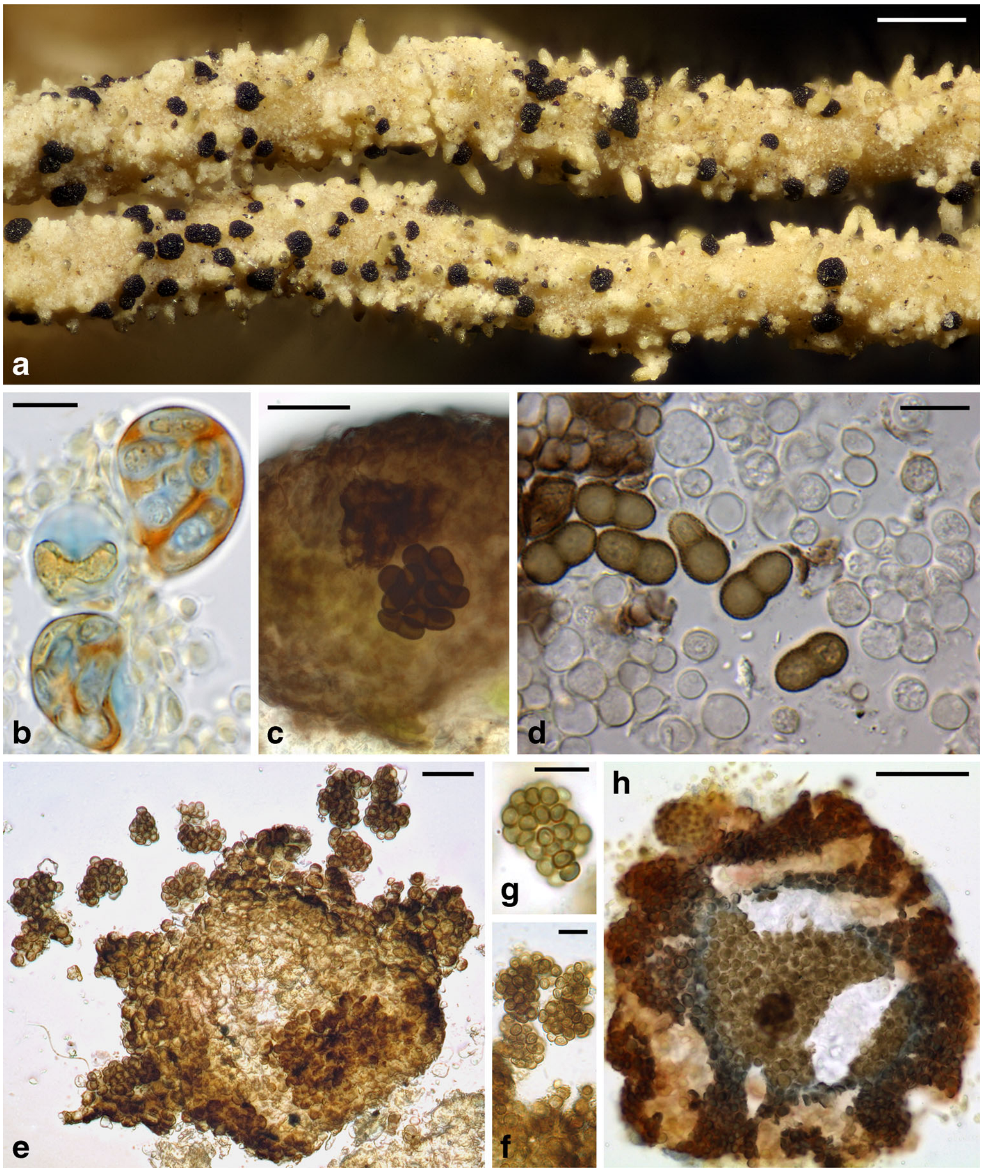

Fig. 2 *Lichenostigma maureri*. **a** Ascomata on *Usnea hirta* (Diederich 6642). **b** Young asci with hyaline ascospores in 0.15 % IKI, after pre-treatment with C and K), showing blue reaction of ascus wall and of ascospore wall (Miller s.n.). **c** Ascoma with mature ascospores in water (Diederich 17326). **d** Stromatic cells and verruculose ascospores in squash preparation in water (Van Cappellen s.n.). **e** Conidioma with conidia in water (Diederich 6633). **f** Magnification of e. **g** Conidium in water (Diederich 17334). **h** K/I-reaction of ascoma (shortly bluish; inner part did not yet react) (Diederich 17334). Scale bars: a=200 μm, b–c, e=20 μm, d,f–g=10 μm, h=50 μm

generic type of *Lichenostigma* and *Phaeosporobolus usneae* the generic type of *Phaeosporobolus*, both genera are consequently considered as synonyms, and most *Phaeosporobolus* species are here newly combined in the genus *Lichenostigma*. Because *Phaeosporobolus trypethelii* clusters with rock isolates in a strongly supported clade sister to the *Phaeococcomyces*+ *Lichenostigma*, it is consequently accommodated in the new genus *Etayoa* (see Taxonomy section).

Our phylogenetic tree clearly shows that the genus *Lichenostigma* as it is currently recognized is polyphyletic, with several described species placed in the *Lichenothelia* clade as defined by Muggia et al. (2012). The genus *Lichenostigma* is divided in two subgenera: the subgenus *Lichenostigma* characterized by rounded, cushion-like ascomata and by the absence of visible vegetative hyphae or strands on the surface of the host lichens, and the subgenus *Lichenogramma* Nav.-Ros. & Hafellner characterized by oval to elongate or irregularly shaped ascomata interconnected by superficial black hyphae or hyphal strands (Calatayud et al. 2004, 2002; Navarro-Rosinés and Hafellner 1996). In our analysis, all species of *Lichenostigma* subgen. *Lichenogramma* (type *L. elongatum*) are part of the Lichenotheliaceae s. str. in the Dothideomycetes. Surprisingly, *Lichenostigma rugosum*, supposed to belong to the subgenus *Lichenostigma* (type *L. maureri*) clusters with the species of the subgenus *Lichenogramma* far away from *Lichenostigma maureri*. *L. rugosum* does not form the typical superficial pigmented hyphal strands as do species of subgen. *Lichenogramma*. A careful study of the ascomatal structure showed that stromatic cells in *L. rugosum* divide by septa (a character confirmed in cultures of *L. rugosum*), whereas those of *L. maureri* divide by budding (no cultures of *L. maureri* available). We conclude that the main diagnostic

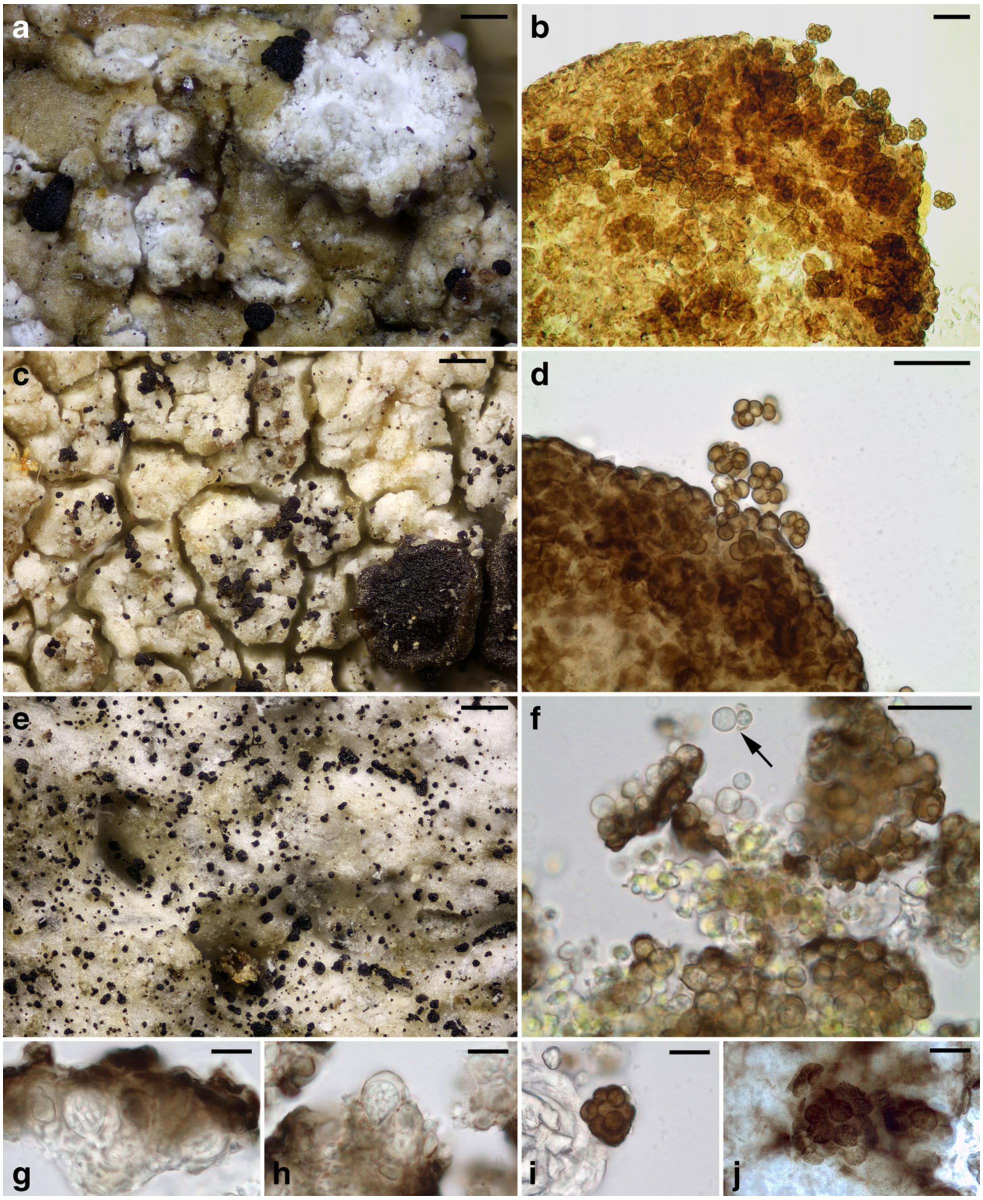

Fig. 3 **a–b** *Lichenostigma alpinum* (Diederich 7878). **a** Conidiomata on *Pertusaria amara*. **b** Conidioma with conidia in water. **c–d** *Diederimyces fuscideae* (Etayo 12083, isosyntype). **c** Conidiomata on *Fuscidea cyathoides*. **d** Conidioma and conidia in water. **e, g–j** *Phaeosporobolus minutus* (isotype). **e** Conidiomata on *Coccotrema cucurbitula*. **g–h** Asci with hyaline ascospores in water. **i-j** Conidia in water. **f** *Lichenostigma* cf. *alpinum* on *Phaeographis*, stromatic cells in squash preparation in water, showing cells multiplying by budding (*arrow*) (Ertz 17638). Scale bars: a, c, e=200 μm, b, d, f=20 μm, g–j=10 μm

character to distinguish between *Lichenostigma* s.str. and the species of *Lichenostigma* belonging to the Lichenotheliaceae is the way cells divide, a character never highlighted before. Based on this character, we combine *Lichenostigma rugosum* in *Lichenothelia* even though more data are probably needed to decide if the *Lichenothelia* clade represents one or several genera. Henssen (1987) reported cells that divide by budding in several *Lichenothelia* species, but these were only occasionally observed for macroconidia or some absorption hyphae when cells from other parts, including those from ascomatal structure, divide by septa.

It must be noted that *Lichenostigma* subgenus *Lichenogramma*, originally comprising only *L. elongatum*, now includes most of the described *Lichenostigma* species. This species assemblage is quite heterogeneous because it includes species having plurihyphal (stromatic) superficial strands (most of the species, including *L. elongatum*) and others having single hyphal strands (= formed by a single row of cells; e.g., *L. cosmopolites*, *L. epipolinum*, *L. semiimmersum*). It also includes species having submuriform ascospores (e.g., *L. amplum*, *L. diploiciae*).

Lichenicolous fungi make up over 13 % (215 species) of the Arthoniomycetes and over 1 % (266 species) of the Dothideomycetes, and they appear to have evolved independently in many different lineages in each class. In our phylogenetic tree, lichenicolous species were newly added to both the Arthoniomycetes (Lichenostigmatales) and to the Dothideomycetes (the *Lichenothelia* clade). As shown in our phylogenetic tree, lichenicolous fungi are also represented in the Dothideomycetes by *Phoma* s. lat. species (in our tree

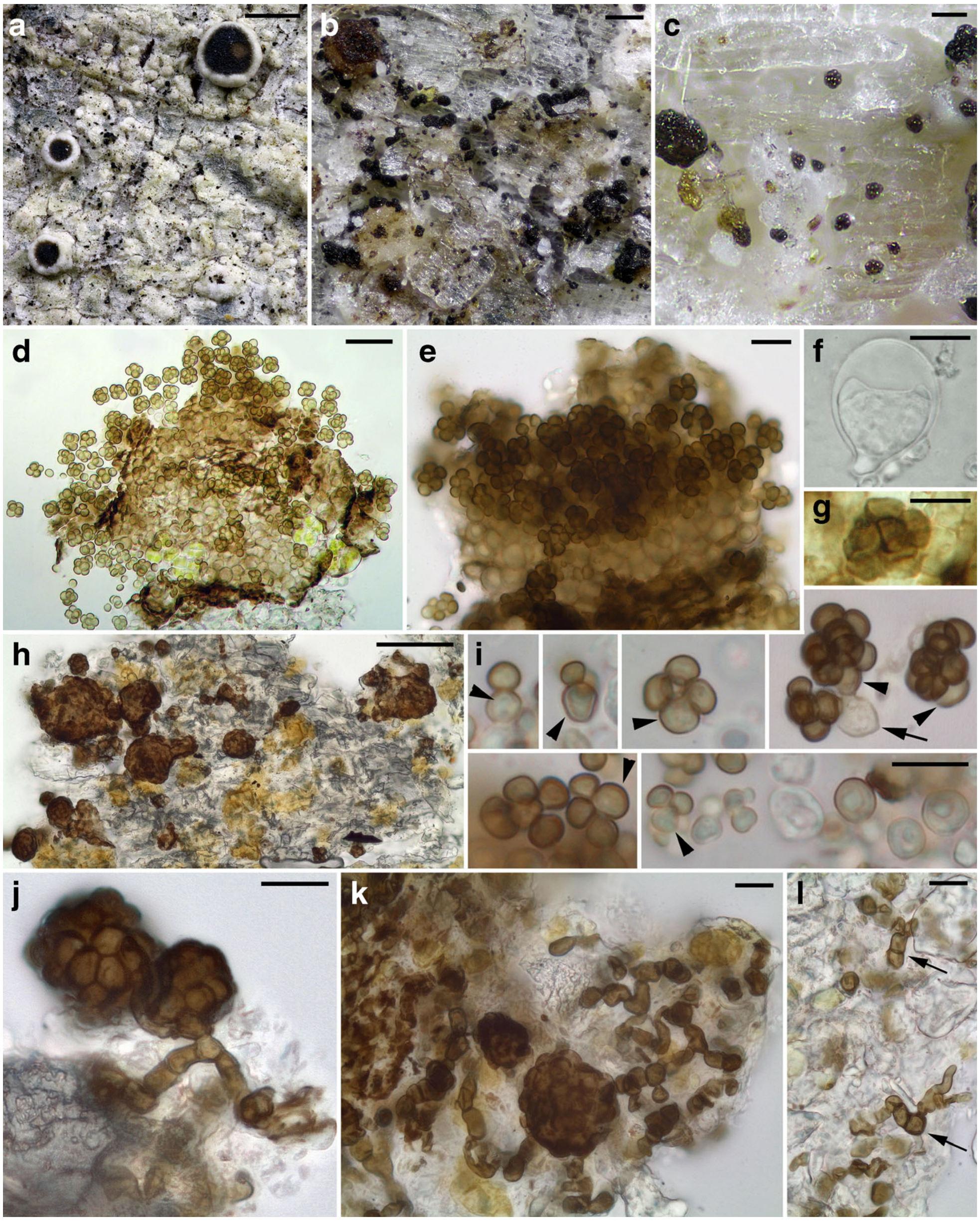

Fig. 4 *Lichenostigma chlaroterae* (**a**–**c**, **e**–**g**, **i**: Diederich 17267; **h**, **j**–**l**: Diederich 17270; **d** Neuberg; all in water). **a**–**b** Conidiomata on *Lecanora* cf. *chlaroterae*. **c** Conidioma and single conidia deposited on the thallus of *Lecanora* cf. *chlaroterae*. **d**–**e** Conidiomata with conidia. **f** Young ascus. **g** Ascus with mature ascospores. **h** Development of conidiomata from conidia. **i** Development of conidia from initial conidiogenous cells (*arrow heads*) (note the upper right is still fixed to a cell of the stroma: *arrow*). **j** Mycelium developing in a conidioma. **k** Mycelium with young conidiomata. **l** Mycelium originating from two ascospores deposited on the host thallus. Scale bars: a=500 μm, b=100 μm, c–d=20 μm, e–g, i–l=10 μm, h=50 μm

represented by *Phoma cladoniicola*) as part of the Pleosporales (Lawrey et al. 2012), by *Xanthoriicola physciae* in the Teratosphaeriaceae (Capnodiales) (Ruibal et al. 2011) and by the genus *Lichenoconium* (Lawrey et al. 2011) that will be described in the family Lichenoconiaceae (in prep.). The '*Lichenostigma*' species belonging to the *Lichenothelia* clade are intermixed with *Lichenothelia* species in our phylogenetic tree, suggesting that transitions between rock inhabiting fungi and the lichenicolous habit possibly occurred several times in the evolution of closely related species. Some species may even have both life habits as suggested by Henssen (1987) for *Lichenothelia* species (i.e., *Lichenothelia convexa*, *L. patagonica*, *L. tenuissima*; one species, *L. solitaria*, is also facultatively fungicolous) and by Knudsen and Kocourkova (2010) for *Lichenostigma saxicola*.

In the Arthoniales, a group of mainly lichenized species, the lichenicolous life habit has also evolved independently several times (Ertz and Tehler 2011; Diederich et al. 2012b), a conclusion supported in our phylogenetic tree by *Briancoppinsia cytospora* (Arthoniaceae) and *Paralecanographa grumulosa* (Opegraphaceae). The latter species starts as a lichenicolous fungus on different hosts belonging to the Arthoniales (e.g., *Dirina*, *Roccella*), sometimes killing the host lichen, and eventually develops its own thallus suggesting that the transition between the lichenicolous and lichenized habit might be easy (Egea et al. 1993; Ertz and Tehler 2011). Many other lichenicolous taxa are expected to belong to the Dothideomycetes (e.g., *Echinothecium*, *Sphaerellothecium*) or to the Arthoniales (e.g., many species of *Arthonia* s.l. and *Opegrapha* s.l.), and these will need to be included in future phylogenetic studies to better understand the evolution of the life habits in these groups.

Taxonomy

Lichenostigmatales Ertz, Diederich & Lawrey **ord**. **nov**.

MycoBank MB 804670

Type: *Lichenostigma* Hafellner

Mycelium extremely rare, brown, superficial, smooth-walled. *Ascomata* and *conidiomata* absent or present, and then almost indistinguishable, stromatic, lichenicolous, dark brown to blackish, subspherical to elongate, internally I– or I+ reddish or blue, K/I– or K/I+ blue; *stromatic cells* subspherical, thick-walled, multiplying by budding, exposed cells dark brown, verrucose, internal cells hyaline to pale brown. *Ascomata* without hamathecial filaments; *asci* developing between stromatic cells, subspherical to shortly ellipsoid, wall apically thickened when young, often with an ocular chamber, wall I– or I+ reddish and K/I– or K/I+ pale blue, ascoplasm I and K/I+ orange, part of the tholus may be I+ reddish, K/I+ blue; *ascospores* hyaline when young, outer wall occasionally K/I+ blue, hyaline or dark brown when old, 1-septate, ellipsoid or elongate, with roundish or pointed apices. *Conidiomata* macroscopically indistinguishable from ascomata; *conidiophores* absent; *conidiogenous cells* pale to medium brown, developing from spherical stromatic cells, shortly subcylindrical to almost ellipsoid, with an indistinctly truncate base, polyblastic, seceding with conidia; *conidia* multicellular, composed of the conidiogenous cell and the conidial cells, ellipsoid, brown, smooth, but sometimes with a verrucose to echinulate ornamentation when over-mature, dry, single, secession schizolytic; *conidial cells* subspherical to ellipsoid, c. 3–5 µm diam. *Yeast stage* of dark brown, aseptate, thick-walled cells covered by mucus and reproducing by multilateral budding.

Our phylogenetic analyses show that the new order belongs to the *Arthoniomycetes* and is sister to the *Arthoniales*. The inclusion in the *Arthoniomycetes* is supported by the ascus type and the amyloid reactions of hymenium and asci (see 'Discussion'). It differs from the *Arthoniales* by the yeast-like cells multiplying by budding, either agglomerated in dense stromata (ascomata devoid of hamathecial filaments or conidiomata) or not (black yeasts), and the extreme rarity of a mycelial stage.

The new order includes the single family *Phaeococcomycetaceae*. As *Lichenostigma* species are likely to be predominant in the order, we decided to typify the new order on the genus *Lichenostigma* because the name Lichenostigmatales will be more informative to most mycologists than a name typified on the genus *Phaeococcomyces*.

Phaeococcomycetaceae McGinnis & Schell

MycoBank MB81139

In McGinnis, Schell & Carson, *Sabouraudia* **23**: 183 (1985). Type: *Phaeococcomyces* de Hoog

The family *Phaeococcomycetaceae* initially included 'fungi that occur as solitary 1- or 2-celled cells that reproduce by budding' (McGinnis et al. 1985), with presence of melanin in the fungal cell wall, and included the genera *Phaeoannellomyces* McGinnis & Schell and *Phaeococcomyces* de Hoog. Following our phylogenetic analyses, the family is here emended to include the black yeast genus *Phaeococcomyces*, two genera producing ascomata and conidiomata, *Etayoa* and *Lichenostigma*, and one additional unnamed clade with undescribed rock-inhabiting fungi known only from cultures.

Phaeococcomyces de Hoog

MycoBank MB9294

Taxon **28**: 348 (1979). Type species: *Cryptococcus nigricans* M.A. Rich & A.M. Stern

Syn.: *Phaeococcus* de Hoog, *Stud*. *Mycol*. **15**: 122 (1977); non *Phaeococcus* Borzi, Atti Congr. Int. Genova 1892: 463 (1892) (Algae). *Nigrococcus* E.K. Novák & Zsolt, *Acta bot*. *Hung*. **7**: 101 (1961); non *Nigrococcus* Castell. & Chalm., Man. Trop. Med.: 932 (1919) (Bacteria).

The genus *Phaeococcomyces* was originally described for the single species *P. nigricans* (de Hoog 1979). Later, more species were added, but some of these, such as *Phaeococcomyces catenatus* (de Hoog & Herm.-Nijh.) de Hoog, were eventually shown to be phylogenetically not related to the generic type, but instead belong to the *Chaetothyriales* (Ruibal et al. 2008; Tsuneda et al. 2011). Currently, *Phaeococcomyces eucalypti* is the only additional species that is known to be congeneric with *P. nigricans*, based on molecular data.

Phaeococcomyces nigricans (M.A. Rich & A.M. Stern) de Hoog

MycoBank MB319531

Taxon **28**: 348 (1979). Basionym: *Cryptococcus nigricans* M.A. Rich & A.M. Stern, *Mycopath*. *Mycol*. *appl*. **9**: 191 (1958). *Nigrococcus nigricans* (M.A. Rich & A.M. Stern) E.K. Novák & Zsolt, *Acta bot*. *Hung*. **7**: 142 (1961) (nom. inval., Art. 33.4). *Melanocryptococcus nigricans* (M.A. Rich & A.M. Stern) Della Torre & Cif., in Della Torre, *Atti lst*. *Bot*. '*Giovanni Briosi*' **21**: 9 (1964). *Phaeococcus nigricans* (M.A. Rich & A.M. Stern) de Hoog, *Studies in Mycology* **15**: 125 (1977). Type: Isolated from paint on storage tank, United States; ex-type culture CBS 652.76–neotype, derived from original isolate (McGinnis et al. 1985).

For a description, discussion and illustrations, see Rich and Stern (1958).

Phaeococcomyces eucalypti Crous & R. G. Shivas
MycoBank MB801769

Persoonia **29**: 159 (2012). Type: Australia, Queensland, Anderson Park Botanic Garden, Townsville, 19°17′28.5″ S, 146°47′13.5″ E, on leaf litter of *Eucalyptus* sp., together with ascomata of *Thyriopsis sphaerospora*, 5 Aug. 2009, P.W. Crous (CBS H-21091– holotype), ex-type cultures CPC 17606=CBS 132526, ITS sequence GenBank KC005769, LSU sequence GenBank KC005791.

For a description, discussion and illustrations, see Crous et al. (2012).

Lichenostigma Hafellner
MycoBank MB2854

Herzogia **6**: 301 (1982). Type: *Lichenostigma maureri* Hafellner

Diederimyces Etayo, *Nova Hedwigia* **61**: 190 (1995) (not validly published, as type species not validly published, ICN: Art. 38.5). Type: *Diederimyces fuscideae* Etayo

Phaeosporobolus D. Hawksw. & Hafellner, *Nova Hedwigia* **43**: 525 (1986). Type: *Phaeosporobolus usneae* D. Hawksw. & Hafellner

Mycelium extremely rare, brown, superficial, smooth-walled. *Stromata* dispersed over the host thallus, dark brown to blackish, subspherical when young, later often becoming elongate, frequently slightly or distinctly centrally depressed, sometimes resembling lirellae when old, 10–160 μm diam., internally I– or I+ reddish or blue, K/I– or K/I+ pale blue; *stromatic cells* subspherical, thick-walled, multiplying by budding, exposed cells dark brown, often with a verrucose or mosaic-like ornamentation, internal cells hyaline to pale brown. *Ascomata* present or absent, without hamathecial filaments; *asci* developing between stromatic cells, (4–)8-spored, subspherical to shortly ellipsoid, wall apically very thick, often with a distinct ocular chamber, wall I– or I+ reddish, K/I– or K/I+ pale blue, ascoplasm I and K/I+ orange, part of the tholus may be I+ reddish, K/I+ blue; *ascospores* hyaline when young, outer wall occasionally K/I+ blue, hyaline or dark brown when old, 1-septate, ellipsoid or elongate, with roundish or attenuated and pointed apices. *Conidiomata* frequent, often intermixed with ascomata from which they are macroscopically indistinguishable, but sometimes smaller; *conidiophores* absent; *conidiogenous cells* pale to medium brown, developing from spherical stromatic cells, shortly subcylindrical to almost ellipsoid, with an indistinctly truncate base, c. 4.5–6.5 μm long and 3.5–5.5 μm diam., polyblastic, seceding with conidia; *conidia* multicellular, composed of the conidiogenous cell and the conidial cells, ellipsoid, brown, smooth, but sometimes with a verrucose to echinulate ornamentation when over-mature, dry, single, secession schizolytic; *conidial cells* subspherical to ellipsoid, c. 3–5 μm diam.

Notes:

1. Ascomata and conidiomata are entirely composed of globose cells that reproduce by budding (e.g., Fig. 3f). The only septa that can be observed within an ascoma are those of the 2-celled ascospores. Thus, this represents an interesting case in which ascomata and conidiomata are entirely made of a compact agglomeration of yeast-like cells.
2. Mature conidia deposited on a natural substratum do not germinate. Instead, individual cells grow in diameter, more cells are added by budding, and the outer wall of the external cells rapidly obtains a typical dark brown, verrucose ornamentation (Figs. 3j, 4h and 5f). They either develop into an ascoma or a morphologically almost identical conidioma. When young, developing conidiomata reach a size somewhat superior to the size of the conidia, e.g., 20 μm diam., then start producing conidia in the interior of the stroma. From some of the globose cells inside the conidioma, specialized, subcylindrical conidiogenous cells develop (these and the asci and ascospores are the only cells within stromata that are not globose), and a more or less larger number of subspherical conidial cells are produced around this conidiogenous cell. When mature, the entire complex—i.e., the original conidiogenous cell together with the surrounding conidial cells—are released together and act as a single multi-celled conidium (Figs. 2-e–f, 3b, 4d and 5a–b). In conidia with a large number of cells, the conidiogenous cell is often difficult to see.
3. Mature ascospores deposited on a natural substratum usually do not germinate either, but also produce new cells by budding, developing into multicellular young stromata. On rare occasions, ascospores may germinate, producing a brown mycelium from which new stromata are produced. This filamentous stage is extremely rare in *Lichenostigma* and has been observed by us in one specimen of *Lichenostigma chlaroterae* from Canada (Diederich 17270) (Fig. 4j–l). Sterile mycelia of other fungi often overgrow the surface of host thalli and these should not be confused with a mycelium of *Lichenostigma*.
4. Conidia are typically smooth-walled. However, in some specimens, conidia with an echinulate ornamentation have been observed (Fig. 3j). Brackel (2011) observed such material on *Fuscidea stiriaca* and considered it as an undescribed species that was, however, left unnamed. We have observed similar specimens with echinulate conidia in *Lichenostigma alpinum* (e.g., Diederich 7878, on *Pertusaria amara*), in *L.* cf. *alpinum* (Diederich 7988b, on *Fuscidea lightfootii*) and in *L. chlaroterae* (Diederich 15608, on *Lecanora symmicta*). Careful examination of this material has shown that conidia are smooth until maturity, but when overmature, individual cells may become larger and develop an outer layer with such an echinulate ornamentation. Therefore, this character has probably no diagnostic value.

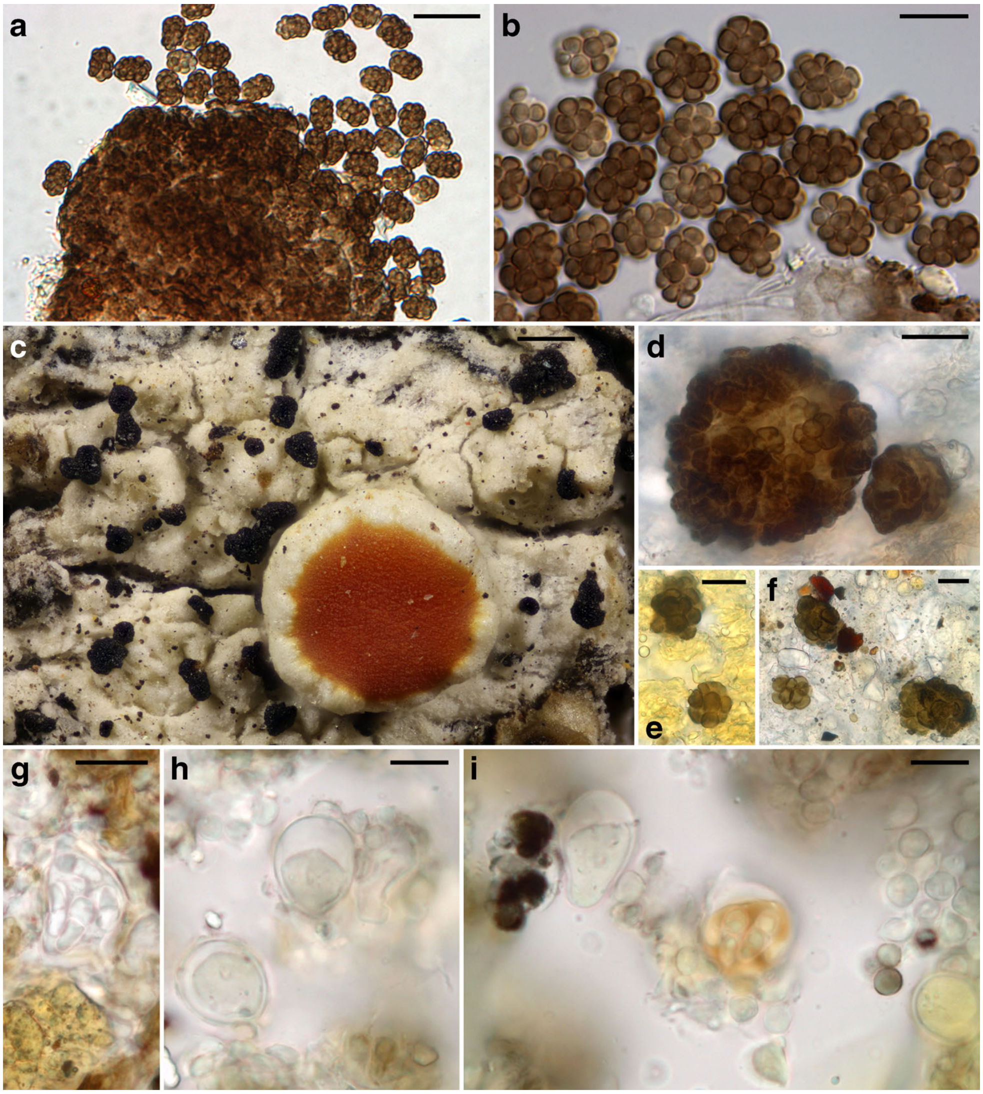

Fig. 5 **a**–**b** *Lichenostigma fellhanerae* (holotype; in water). **a** Conidioma with conidia. **b** Conidia. **c**–**i** *Lichenostigma hyalosporum* (holotype). **c** Ascomata on thallus of *Haematomma eremaeum*. **d** Conidioma containing several conidia in water. **e**–**f** Conidia (the lower right is developing in a young conidioma). **g**–**i** Asci, in g and i with ascospores (**e**–**i**: in diluted K/I). Scale bars: a=20 µm, b=10 µm, c=200 µm, d–i=10 µm

5. Young, developing ascomata and conidiomata are typically roundish, with an irregular opening to release ascospores and conidia. Older fructifications in some species tend to become elongate, with a depressed centre and they consequently often resemble lirellae. Their size appears to depend largely on the age of the population and does not represent a character that allows distinguishing species.

Lichenostigma maureri Hafellner (Fig. 2)
MycoBank MB109054

Herzogia **6**: 301 (1982). Type: Austria, Steiermark, Grazer Bergland, auf Fichten an der Nordseite des Schöckels, 1400 m, auf *Usnea florida*, 24 Nov. 1974, W. Maurer (GZU–holotype; UPS, hb. Hafellner 8701–isotypes, non vid.).

Phaeosporobolus usneae D. Hawksw. & Hafellner, *Nova Hedwigia* **43**: 526 (1986). Type: Austria, Ostalpen, Gurktaler Alpen, Steiermark, Frauenalm S von Murau, subalpiner Fichten-Lärchenwald ober der Murauer Hütte, 1700 m, auf *Usnea*, 24 May 1981, J. Hafellner 9106 (IMI 281394–holotype; GZU–isotype, non vid.).

Mycelium unknown. *Stromata* (30–)50–100(−120) µm diam.; *external cells* with a verrucose ornamentation. *Ascomata* frequent; *asci* (4–)8-spored, 20–25×13–18 µm; *ascospores* ellipsoid, 1-septate, when young hyaline and smooth, soon becoming dark brown and verruculose, 9–12×4.5–6 µm. *Conidiomata* frequent, often intermixed with ascomata from which they are macroscopically indistinguishable; *conidia* subspherical to ellipsoid, (9–)14.3–22.5(−24)×(8–)10.5–16.0(−17) µm, composed of (8–)14–36(−45) cells [in optical section (6–)10–21(−23) cells], cells (3–)3.6–4.6(−6) µm diam.

For a detailed description and illustrations, see Hafellner (1982) and Hawksworth and Hafellner (1986).

This species is particularly common in montane coniferous forests, growing mainly on fruticose lichens (*Bryoria*, *Evernia*, *Letharia*, *Pseudevernia*, *Ramalina*, *Usnea*, etc.), and is known from all continents (Kocourková 2000).

Notes:

1. Hawksworth and Hafellner (1986) described the conidiomata of *Ph. usneae* as being "surrounded by a pellicle-like layer of brown hyphae 2.5–3 μm wide forming a layer 4–7 μm thick". Alstrup and Hawksworth (1990) described the new *Ph. alpinus* as differing notably by "the absence of a pellicle-like outer hyphal layer over the stroma". In the many specimens examined by us, we never observed such a pellicle-like layer, and we only observed spherical, never hyphal cells forming the stromata. Berger and Brackel (2011) similarly explained that in 'some' specimens of *Ph. usneae* examined, the outer hyphal layer was missing, and they wondered if that layer might appear only at a later stage of development, resulting from collapsing of the previously spherical outer cells.
2. Following Hawksworth and Hafellner (1986), conidia in *Ph. usneae* are composed of 6–12 or more globose cells of 4–6 μm diam. Alstrup and Hawksworth (1990) distinguished the new *Ph. alpinus* partly on the basis of conidia composed of 10–15(−20) much smaller cells, 3–4 μm diam. Berger and Brackel (2011) confirmed this information for *Ph. alpinus*, but slightly modified the data on *Ph. usneae* in that conidia are composed of 6–12(−16) cells of (3–)4–6 μm. Our study of many specimens has shown that conidia in *Ph. usneae* are typically larger than those of *Ph. alpinus*, with a larger number of cells (and not the contrary as stated by previous authors), but that the diameter of individual cells is statistically more or less identical in both species.
3. In rich populations, both morphs often seem to occur together. However, this has rarely been documented, probably as most collectors just studied one or two stromata, and depending on the morph observed called the specimen either *L. maureri* or *Ph. usneae*. When many stromata from the same host thallus are examined, a mixture of both morphs is often observed.
4. Many lowland specimens examined by us (e.g., all specimens from Belgium and Luxembourg) only presented the asexual stage. More studies are needed to identify whether these represent a distinct species that is rarely or never accompanied by a sexual stage, or if the sexual stage of *L. maureri* has a more restricted area of distribution, being mainly present in montane or boreal environments, while in more temperate environments, only the asexual stage develops.
5. Iodine reactions present in ascomata and conidiomata are difficult to observe because of the dark pigmentation. It was found that bleaching (with C) until the stromata became colourless made the reactions much easier to observe. Both reddish and blue reactions were seen in many stromata. Sequential pre-treatment with C and K, however, proved to be too harsh, often resulting in removal of the reactive material. After pre-treatment with K only, blue colouration is increased and reddish staining was not seen. It is the gelatinous material between cells which reacts, most strongly on the outer surface of the cells. In 1 % IKI, cytoplasmic staining is often strong, making the observation of reddish or pale blue reactions difficult. The reactions were best seen after C, in 0.15 % IKI. Reddish cell wall staining visible in 0.15 % IKI is lost when transferred to Melzer's reagent. This type of reddish reaction with iodine was termed hemiamyloid by Baral (1987), and the blue staining seen without K pre-treatment termed euamyloid. These observations are also compatible with the amylomycan type reaction described by Common (1991). The reddish colouration of the ascoplasm, and to a lesser extent other cells of the stromata, is not converted to a blue staining form by pre-treatment with K, so would be called dextrinoid in the terminology of Baral.

Selected specimens examined: **France**: *Pyrénées-Orientales*: NW of Mont-Louis, on *Pinus*, on *Usnea hirta*, 30 July 1985, Diederich 6642 (hb. Diederich); SE of Eyne, on *Pinus*, on *Usnea*, 31 July 1985, Diederich 6633 (hb. Diederich). **Switzerland**: *Valais*: S of Nendaz, 2 km SSW of Siviez, 150 m W of téléphérique station Tortin, 46°06′49″ N, 7°18′03″ E, 2145 m, on *Larix*, on *Letharia vulpina*, 26 July 2012, Diederich 17306 (hb. Diederich); 3 km S of Haute-Nendaz, road to Siviez, between main road and Planchouet, 46°09′07″ N, 7°18′59″ E, 1500 m, on *Larix*, on *Usnea*, 21 July 2012, Diederich 17326 (hb. Diederich); Aletschwald, NNW of Riederalp, 46°23′03″ N, 8°01′13″ E, 2050 m, on *Larix*, on *Usnea*, Diederich 17337 (hb. Diederich); SE of Les Haudères, forêt de Tauge, 46°04′42″ N, 7°31′09″ E, 1500 m, on *Larix*, on *Usnea*, 23 July 2012, Diederich 17334 (hb. Diederich); Saint-Luc, sentier des Grands Pras, 1650 m, on *Larix*, on *Usnea*, 5 Feb. 2013, Van Cappellen s.n. (BR). **U.S.A.**: California, Siskiyou Co., 6 mi. west of Gazelle, 4000 ft., 41°31′ N, 122°31′ W, 8 Oct. 2010, Miller s.n., on *Letharia vulpina* (MSC).

Lichenostigma alpinum (R. Sant., Alstrup & D. Hawksw.) Ertz & Diederich **comb. nov.** (Figs. 3 and 7c–d)

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Basionym: *Phaeosporobolus alpinus* R. Sant., Alstrup & D. Hawksw., in Alstrup & Hawksworth, *Meddelelser om Grønland*, *Bioscience* **31**: 51 (1990). Type: Greenland, Disko, Qutdligssat, 1 km SW of churchyard, 70°05′N, 53°01′W, 100 m, on *Ochrolechia frigida*, 27 July 1950, Gelting 13333 (UPS–holotype, non vid.) [in Alstrup and Hawksworth (1990, caption of fig. 28), another specimen, Christiansen 5507, was erroneously said to be the holotype].

Syn. (?): *Diederimyces fuscideae* Etayo, *Nova Hedwigia* **61**: 190 (1995). Type: Spain, Alava, Sierra de Entzia, Opacua pass, *Fagus*-wood north-oriented, on *Fuscidea cyathoides*, 995 m, 6 April 1994, Etayo 12083!, 12059 (MA-Lich–

syntype, hb. Etayo!–isosyntype) (not validly published, as two different specimens were designated as the holotype, ICN: Art. 40.2).

Syn. (?): *Diederimyces microsporus* Etayo, in Etayo & Sancho, *Bibl. Lichenol.* **98**: 84 (2008). Type: Chile, Navarino, bosquete de lengas achaparrado antes del Cerro Bandera, sobre *Pertusaria microcarpa* corticícolas, 54°57′34″S, 67°37′46″W, 550 m, 15 Jan. 2005, J. Etayo 22265, A. Gómez-Bolea & U. Søchting (MAF–holotype; UMAG, hb. Etayo!–isotypes) (not validly published, as generic name not validly published).

Syn. (?): *Phaeosporobolus minutus* Etayo, in Etayo & Sancho, *Bibl. Lichenol.* **98**: 164 (2008). Type: Chile, Navarino, bosquete de lengas achaparrado antes del Cerro Bandera, sobre *Pertusaria microcarpa* y *Coccotrema cucurbitula* corticícolas, 54°57′34″S, 67°37′46″W, 550 m, 15 Jan. 2005, J. Etayo 22265, A. Gómez-Bolea & U. Søchting (MAF–holotype; UMAG, hb. Etayo!–isotypes).

Mycelium unknown. *External cells of stromata* medium to dark reddish brown, with a verrucose to sometimes indistinctly mosaic-like ornamentation. *Sexual stage* not certainly identified, as two kinds of ascomata have been found associated with a *L. alpinum*-like asexual stage (see below). *Conidiomata* frequent, c. (20–)25–100(−150) μm diam.; *conidia* subspherical to ellipsoid, (10–)11.2–13.6(−15)×(7–)8.5–10.6(−12) μm, composed of (4–)8–17(−22) smooth, rarely verrucose cells [in optical section (4–)6–10(−12) cells], cells (2.9–)3.5–4.9(−5.8) μm diam.

'*Diederimyces fuscideae*' sexual stage: *Ascomata* rare, intermixed with conidiomata, 150–300 μm diam.; *asci* 8-spored, 31–39×13–17 μm; *ascospores* hyaline, 1-septate, with attenuated and pointed ends, bent spirally, 20–27×3–4 μm (description from Etayo 1995).

'*Diederimyces microsporus*' sexual stage: *Ascomata* extremely rare, intermixed with conidiomata, 70–100 μm diam.; *asci* 8-spored, 14–17×9–12 μm; *ascospores* hyaline, 1-septate, ellipsoid, with rounded ends, 6.5–7×2–2.5 μm (description from Etayo and Sancho 2008).

For a detailed description and illustrations, see Alstrup and Hawksworth (1990), Etayo (1995) and Etayo and Sancho (2008).

This species is rather common on terricolous and corticolous species of *Ochrolechia*, *Pertusaria* and *Varicellaria*, and has also been reported on a variety of other hosts, including *Fuscidea* and *Coccotrema*. Following Kocourková (2000), the species is known from Europe, Asia, North and South America and Antarctica, and additional hosts include *Caloplaca*, *Lecanora* (possible confusion with *L. chlaroterae*), *Physcia* and *Sphaerophorus*. Berger and Brackel (2011) added *Anaptychia*, *Baeomyces*, *Buellia*, *Cladonia*, *Flavocetraria*, *Hypogymnia*, *Maronea*, *Megaspora*, *Melanohalea*, *Parmelia*, *Physconia*, *Platismatia*, *Rhizoplaca*, *Stereocaulon*, *Umbilicaria*. However, as the taxonomic value of morphological characters allowing the distinction of *Phaeosporobolus* species was often misunderstood, most of these records should be revised before being accepted. Furthermore, the existence of further, undescribed species within the *L. alpinum* complex is likely. We have additionally studied material on *Graphis pulverulenta*, *Ropalospora viridis* and *Fuscidea lightfootii* that is morphologically indistinguishable from *L. alpinum*.

Notes:

1. *L. alpinum* is widespread and rather common on species of *Ochrolechia*, *Pertusaria* and *Varicellaria*. Owing to difficulties in separating the species from the asexual stage of *L. maureri* (i.e., *Ph. usneae*) by morphological characters, many authors based the identification of their specimens mainly on a hypothetic host-specificity.
2. The distinction between this species and *Ph. usneae* (i.e., *Lichenostigma maureri*) has always been problematic. In the original account of *Ph. alpinus* (Alstrup and Hawksworth 1990), both species were considered to differ by several characters, but the discussion was rather confusing as the authors mixed characters of both species when characterizing *Ph. alpinus* (first sentence after 'Notes'). Following these authors, *Ph. usneae* is distinguished by conidiomata covered by a pellicle-like layer (a character not confirmed by us, see notes under *L. maureri*), larger conidia (15–25 μm diam.), ellipsoid rather than subglobose conidia (not confirmed by us: length/breadth ratio 1.2–1.4 in *Ph. alpinus* and 1.2–1.6 in *Ph. usneae*), conidia adhering in chains (not observed by us), and much larger individual cells of conidia (4–6 μm, versus 3–4 μm in *Ph. alpinus*; a difference not confirmed by us, see notes under *L. maureri*). Furthermore cells in the central parts of the stroma would be more pigmented in one of the two species, but it is not possible to understand which species is meant (this character has not been confirmed by us). Following our observations, the main characters that allow distinguishing both species in the asexual stage are the conidial dimensions and the number of cells per conidium.
3. Morphologically similar specimens have been reported from many other host genera (see above). Specimens from *Fuscidea lightfootii* and *Phaeographis smithii* included in our phylogenetic analyses appear not to be conspecific with material on *Pertusaria* or *Fuscidea cyathoides*, suggesting that several cryptic species might be hidden in the *L. alpinum* species complex. The inclusion of more specimens and the use of a more variable locus such as ITS would be needed to solve species circumscription here.
4. The conidiomatal diameter is very variable within this species and probably reflects the maturity of a population. For example, in specimen Ertz 17522, conidiomata are (20–)26–40(−50) μm diam. (*N*=38), whilst in specimen Kalb 15574, they are (50–)59–103(−150) μm diam. (*N*=39) [in the case of elongate conidiomata, the largest diameter has been measured]. Both specimens are from the same host, *Pertusaria albescens*.

5. Etayo (1995) considered *Diederimyces fuscideae* (on *Fuscidea cyathoides*), characterized by narrow, elongate, hyaline, apically attenuated ascospores with pointed ends, spirally bent in the asci, to be the sexual stage of *Ph. alpinus* (described from *Ochrolechia* and *Pertusaria* species) (Fig. 3-c–d). As both have been described from different host genera, they might represent two distinct, morphologically similar species. However, two arguments speak in favour of a synonymy: (1) a specimen on *F. cyathoides* included in our phylogenetic analysis groups with specimens on *Pertusaria* (Fig. 1); (2) Alstrup and Hawksworth (1990) have observed and illustrated in their fig. 29 a fertile specimen on *Ochrolechia frigida* with hyaline ascospores strongly resembling those of the type of *D. fuscideae*, depicted by Etayo (1995). We have studied an isosyntype (Etayo 12083), but were unable to find any ascomata. However, we examined several conidiomata with conidia of variable size, mostly intermediate between those of *L. alpinum* and *L. chlaroterae*, probably representing young, developing conidia of *L. alpinum*.
6. Etayo and Sancho (2008) described a second species of *Diederimyces*, *D. microsporus*, from southern South America on corticolous *Pertusaria microcarpa* (Fig. 3e, g–j). That species has narrowly ellipsoid, hyaline ascospores, and ascomata are intermixed with an asexual *Phaeosporobolus* stage that the authors described as the new *Ph. minutus*. This asexual stage was characterized by particularly small conidia, 6.5–10.5×5–8 μm, composed of (3–)4–5(−8) smooth-walled cells, formed in small conidiomata 20–60 μm diam., and grew on *Pertusaria* and *Coccotrema* species. We have examined many conidiomata in an isotype and were surprised to find much larger conidia, 15–19×11–15 μm, composed of 13–18 cells (in optical section of 8–10 cells), and individual cells were relatively large, mainly 4.5–6 μm diam. All morphological characters were those of *Ph. alpinus*, of which *Ph. minutus* is possibly a synonym.
7. As some populations of *Ph. alpinus* s. lat. on *Fuscidea cyathoides* are intermixed with ascomata of *Diederimyces fuscideae*, and others on *Pertusaria microcarpa* [i.e., *Ph. minutus*] are intermixed with ascomata of *D. microsporus*, the main question is to know which of these two *Diederimyces* species represents the sexual stage of *Ph. alpinus*. To answer this question, more populations of *Ph. alpinus* need to be sequenced, including those on unusual hosts, and material of both *Diederimyces* species needs to be sequenced. If possible, material collected close to the type localities of *Ph. alpinus* and both *Diederimyces* species should be included in a phylogenetic analysis. For the time being, we consider that *Ph. alpinus* probably represents an assemblage of several species that cannot be distinguished by morphological characters in the asexual stage, and that it is not possible to identify with certainty the sexual stage of *Ph. alpinus* s. str. Therefore, we provisionally include both *Diederimyces* names and *Ph. minutus* under the synonymy of *Lichenostigma alpinum*, pending further studies.
8. The new species *Diederimyces fuscideae* was not validly published, as two different specimens (Etayo 12083 and Etayo 12059) were designated as the holotype (ICN: Art. 40.2). Consequently the new generic name *Diederimyces*, typified on *D. fuscideae* is not valid (ICN: Art. 38.5) and the new *D. microsporus* is not validly published as well.
9. The ICN, Art. 57.2, says that widely used teleomorph-typified names cannot be displaced by earlier anamorph-typified names. However, as the names *Diederimyces fuscideae* and *D. microsporus* have not been widely used – both species have never been reported after the original description, as the anamorph-typified name *Ph. alpinus* is widely used, as further the names *D. fuscideae* and *D. microsporus* are not validly published (see above), and as the anamorph-teleomorph relationship has not yet been solved in this species complex, the anamorph-typified name *Ph. alpinus* has to be used for this taxon.

Selected specimens examined: **Croatia**: Plitvice, Plitvice Lakes National Park, west-shore of Lake Kozjak, 44°52′48″ N, 15°36′48″ E, on *Fraxinus*, on *Pertusaria albescens*, 16 Aug. 2012, Diederich 17379 (hb. Diederich). **France**: *Finistère*: SE de Commana, Monts d'Arrée, 48°24′05″ N, 3°55′35″ W, on saxicolous *Fuscidea cyathoides*, 18 July 2012, Ertz 17519 (BR); centre du village de Huelgoat, 48°21′49″ N, 3°44′56″ W, on *Liriodendron*, on *Pertusaria albescens*, 18 July 2012, Ertz 17522 (BR). **Luxembourg**: S of Doncols, ruisseau de Sonlez, 49°57′ N, 5°50′ E, on *Carpinus*, on *Pertusaria amara*, 22 March 1987, Diederich 7878 (hb. Diederich). **U.S.A.**: *Michigan*: Chippewa Co., Tahquamenon Falls State Park, on M-123, 0.2 miles from boundary with Luce Co., swampy area near road, on corticolous *Pertusaria*, 22 July 1980, Common 4443 L (MSC).

Selected specimens of *Lichenostigma alpinum* s. lat.: **France**: *Ille-et-Vilaine*: Paimpont, près de Tréhorenteuc, Val Sans Retour, 48°00′01″ N, 2°17′04″ W, on *Carpinus*, on *Phaeographis smithii*, 23 July 2012, Ertz 17591 (BR); Paimpont, au nord de l'étang de Paimpont, 48°01′26″ N, 2°10′22″ W, on *Ilex*, on *Phaeographis*, 22 July 2012, Ertz 17638 (BR). **Luxembourg**: Lellingen, verson droit du Lellgerbaach, op Baerel, 49°59′22″ N, 6°01′48″ E, on *Sorbus*, on *Fuscidea lightfootii*, 3 Sept. 2011, Diederich 17240 (hb. Diederich).

Lichenostigma chlaroterae (F. Berger & Brackel) Ertz & Diederich **comb. nov.** (Figs. 4 and 7a–b)

MycoBank MB 804672.

Basionym: *Phaeosporobolus chlaroterae* F.Berger & Brackel, *Herzogia* **24**: 351 (2011). Type: Austria, Oberösterreich, Donautal, Engelhartszell, Oberranna, MTB 7548/2, 290 m, on

Malus domestica, on *Lecanora chlarotera*, 9 Jan. 1999, Berger 12950 (LI–holotype; hb. Berger–isotype, non vid.).

Mycelium extremely rare, brown, superficial, smooth-walled, originating from the germination of ascospores. *Stromata* variable in diameter, in young populations frequently less than 20 μm diam., in older populations up to 80 μm diam. or more; *external cells* with a verrucose or granulose ornamentation. *Ascomata* extremely rare, mixed with conidiomata from which they are macroscopically indistinguishable; *asci* c. 28×22 μm (1 ascus measured); *ascospores* ellipsoid, 1-septate, brown, c. 9–9.5×4–4.5 μm (2 spores measured inside ascus). *Conidiomata* frequent; *conidia* subspherical to ellipsoid, (5.5–)7.1–10.2(−13)×(5–)6.3–8.8(−11) μm, composed of (3–)4–9(−16) cells that are smooth, rarely with an echinulate ornamentation when overmature [in optical section (3–)4–6(−8) cells] (*N*=57), cells (2.5–)3.3–4.5(−6) μm diam. (*N*=207).

For a detailed description and illustrations, see Berger and Brackel (2011).

This is a rather common species, widespread in Europe, here newly reported from North America (Canada, U.S.A.) and probably cosmopolitan, but poorly recorded as only recently described. It mainly develops over corticolous *Lecanora* species, but has also been reported from other hosts, such as *Buellia griseovirens* (Diederich et al. 2012a). We have also examined specimens on *Fuscidea lightfootii* and *Graphis pulverulenta* with similar morphological characters.

Notes:

1. Following the literature, the asexual stage of this species can hardly be distinguished from *Ph. minutus*. Berger and Brackel (2011, Table 1) gave a number of characters that should allowing distinguishing both species. Following these authors, conidia of *L. chlaroterae* are composed of 5–7(−9) cells of 3–3.5(−5) μm diam., whilst those of *Ph. minutus* of (3–)4–5(−8) cells of 3–5 μm. Conidiomata were said to be 60–80(−110) μm in *L. chlaroterae*, much larger than those of *Ph. minutus* (20–60 μm), but this represents a lapsus in Table 1 published by these authors, as conidiomata of *L. chlaroterae* were said to be 20–80(−110) in the species description on p. 351, a result confirmed by us. A re-examination of an isotype of *Ph. minutus* revealed much larger conidia with more cells, similar to those of *L. alpinum*, and therefore no confusion between *L. chlaroterae* and *Ph. minutus* should occur.
2. In a Canadian specimen on corticolous *Lecanora* cf. *chlarotera* (Diederich 17267) we observed slightly larger conidia, (8.5–)8.8–10.6(−11.5)×(6.5–)6.9–8.4(−9) μm, composed of (7–)7–14(−16) cells [in optical section (5–)5–7(−8) cells] (*N*=7), cells (3–)3.3–4.0(−4.5) μm diam. (*N*=34), morphologically intermediate between *L. chlaroterae* and *L. alpinum*. The study of more conidiomata in the same specimen revealed some with exclusively very small conidia with few cells. Molecular data clearly placed this population with other specimens of *L. chlaroterae* on *Lecanora*. This suggests that the infraspecific variability in *L. chlaroterae* is rather large, and overlap of morphological characters with other species, especially *L. alpinum*, is not rare, making the identification of some specimens particularly difficult.
3. This Canadian collection is the only hitherto known specimen of *L. chlaroterae* in which a sexual stage could be found. This might be explained by a particularly favourable climate in British Colombia, resulting not only in the production of ascomata, but also in conidia becoming larger than in Central European material. Unfortunately, amongst the numerous stromata examined, only two represented the sexual stage, and all but one asci were immature, without ascospores (Fig. 4f–g). Additional material collected on the same trees 3 years later, and also specimens collected a few kilometres from this locality exclusively presented the asexual stage.
4. The frequency of *L. chlaroterae* on corticolous *Lecanora* species, the entire absence of the species on other lichens growing on the same trees, and the unexpected occurrence on *Buellia griseovirens* (three specimens published by Diederich et al. 2012a) and also on specimens of *Fuscidea lightfootii* and *Graphis pulverulenta* suggests that either several cryptic species are involved or that the species has very narrow ecological affinities that are found in several non-related lichen species or genera. Brackel (2011) also reported a specimen with small conidiomata and small, few-celled conidia from *Diplotomma alboatrum* (as *Ph.* aff. *minutus*). To answer this question, more specimens on unusual hosts will need to be sequenced, if possible using other genetical markers, such as ITS.

Selected specimens examined: **France**: *Pas-de-Calais*: Au S de Boulogne-sur-Mer, forêt domaniale d'Hardelot, on *Fuscidea lightfootii*, 12 Aug. 2000, Diederich 14369b (hb. Diederich). *Pyrénées-Atlantiques*: Au S de Pau, à l'E de Bielle, à 0.5 km à l'E du col de Marie-Blanque, on *Graphis pulverulenta*, 28 July 1990, Diederich 9301 (hb. Diederich). **Luxembourg**: Weiswampach, near lake, on *Alnus*, on *Lecanora argentata*, 5 Febr. 2012, Neuberg (hb. Diederich). Blaschette, Bëddelbësch, on *Carpinus*, on *Buellia griseovirens*, 30 Oct. 1983, Diederich 3943 (hb. Diederich). **Switzerland**: Valais: SE of Les Haudères, forêt de Tauge, 46°04′42″ N, 7°31′09″ E, 1500 m, on *Sorbus*, on *L. chlarotera*, 23 July 2012, Diederich 17329 (hb. Diederich). **Canada**: *British Columbia*: Wells Gray Provincial Park, Clearwater valley, 26 km N of Clearwater, Edgewood Blue (near house of T. Goward), 51°52′9″ N, 120°01′18″ W, on *Alnus*, on *L. chlarotera* s. lat., 24 July 2008, Diederich 17267 (hb. Diederich); ibid., 14 April 2012, Goward 12–30 (UBC), ibid., 9 May 2012, Goward 12–42 (UBC); ibid., Kingfishers Wood Cottage, 51°51′23″ N, 120°00′52″ W, on *Alnus*, on *Lecanora*, 22 July 2008, Diederich 17270 (hb. Diederich). **U.S.A.**: *Michigan*: Chippewa Co., Tahquamenon Falls State Park, on M-123, 0.2 miles from boundary with Luce Co., swampy area near road, on corticolous *Lecanora*, 22 July 1980, Common 4443E (MSC). *South Carolina*: locality unknown, on corticolous *Lecanora*, 28 Dec. 1975, Common 3658 N (MSC).

Lichenostigma fellhanerae (R.C. Harris & Lendemer) Ertz & Diederich **comb. nov.** (Fig. 5a–b)

MycoBank MB 804673

Basionym: *Phaeosporobolus fellhanerae* R.C. Harris & Lendemer, *Opuscula Philolichenum* **6**: 173 (2009). Type: U.S.A. North Carolina, Haywood Co., Great Smoky Mountains National Park, 3 miles southeast of Waterville, along Baxter Creek Trail, south of Big Creek Campsite, Mount Sterling Ridge, lower slopes of Mount Sterling, Cove Creek Quad., rich cove forest (*Aesculus hippocastanum*, *Acer saccharum*, *Liriodendron tulipifera*, *Betula lenta*, *Halesia carolina*) on north-facing slope with narrow rocky ravine of Baxter creek, on *Fellhanera granulosa* on rock, 28 Oct. 2006, J.C. Lendemer 8087 & E. Tripp (NY–holotype!).

Mycelium unknown. *External cells of stromata* medium to dark reddish brown, with a verrucose to sometimes indistinctly mosaic-like ornamentation. *Sexual stage* unknown. *Conidiomata* (20–)40–80 μm diam., I–, K/I+ pale blue; *conidia* produced in large numbers (typically more than 100 per conidioma), ellipsoid, very regular in form, (8–)8.7–11.0(−12.5)×(6.5-)6.8–7.7(−8) μm (*N*=18), composed of (16–)18–23(−27) smooth cells [in optical section 9–12 cells], cells (2.5–)2.6–3.2(−3.5) μm diam. (*N*=18).

For a description and illustrations, see Harris and Lendemer (2009).

The species is hitherto known only from the type specimen from the U.S.A. (North Carolina) on the thallus of saxicolous *Fellhanera granulosa*.

Note: This species is unusual by producing large numbers of very regularly formed, ellipsoid conidia. These conidia are rather compact, with relatively small cells, contrasting with the more loose conidia with larger cells in other *Lichenostigma* species, and we wondered if the species might belong to the new genus *Etayoa* described below. As no molecular data of this species are available (the species is just known from the type collection), a final answer to this question cannot be given now.

Lichenostigma hyalosporum Kalb & Hafellner (Fig. 5c–i)

MycoBank MB 413399

In Kalb et al., *Bibl. Lichenol.* **59**: 208 (1995) (as *L. hyalospora*). Type: Australia, Western Australia, 'Dryandra Forest', wenige km NW von Narrogin, 32°45′ S, 116°56′ E, 300 m, on *Haematomma eremaeum*, 17 Aug. 1994, K. & A. Kalb 27456 (hb. Kalb–holotype!; GZU [hb. Hafellner]–isotype).

Mycelium unknown. *External cells of stromata* with a dark reddish brown, mosaic-like ornamentation. *Ascomata* roundish to sometimes elongate, 60–160 μm diam., I– and K/I–. *Asci* 8-spored, 15–25×13–18 μm, when ascospores fully mature, 25–30×18–22 μm; *ascospores* hyaline, 1-septate, ellipsoid, 9–15×3.5–8 μm. *Conidiomata* rare, intermixed with ascomata and often smaller, 20–40 μm diam.; *conidia* subspherical, c. 10–15×8–13 μm, composed of c. 13 smooth cells [in optical section 8–9 cells] (two conidia observed), cells 4–6 μm diam.

For a detailed description and illustrations, see Kalb et al. (1995).

The species was known from three Western Australian specimens on *Haematomma eremaeum*. We have studied two further specimens, one from the type locality, overgrowing other species of corticolous crustose lichens, and one from the same area on corticolous species of *Protoparmelia* and *Hafellia*. All localities are from regions with a temperate climate.

Notes:

1. This was the only known *Lichenostigma* s. lat. species with hyaline ascospores. Following the detailed description and the high quality photographs given in the original account (Kalb et al. 1995), and after re-examination of the holotype, we can affirm that this species belongs to *Lichenostigma* s. str., although no sequences are available. Stromata are entirely composed of globose cells multiplying by budding, and typical *Phaeosporobolus*-type conidia, previously unknown in this species, have been observed.
2. Ascospores were given in the original description as 12–15×6.5–8 μm. We have examined one ascus with 8 hyaline ascospores measuring just 9–11×3.5–4.2 μm. The variability is thus larger than initially believed. A more accurate statistical analysis of ascospores needs additional material.

Additional specimens examined: Australia: Western Australia: Same locality and date as type, on several species of corticolous crustose lichens, Kalb 27616 (hb. Kalb). Ca. 30 km W von Hyden und ca. 30 km E von Kondinin, 32°26′ S, 118°30′ E, 300 m, on corticolous Protoparmelia and Hafellia spp., 18 Aug. 1994, Kalb 27617 (hb. Kalb).

Etayoa Diederich & Ertz gen. nov.

MycoBank MB 804674

Type: *Phaeosporobolus trypethelii* Flakus & Kukwa

Mycelium unknown. *Stromata* dispersed over the host thallus, dark brown to blackish, subspherical when young, later often becoming elongate, frequently slightly or distinctly centrally depressed, sometimes resembling lirellae when old; *stromatic cells* subspherical, thick-walled, multiplying by budding, exposed cells dark brown, with a mosaic-like ornamentation, internal cells hyaline to pale or medium brown. *Ascomata* present or absent, without hamathecial filaments, internally I+ and K/I+ blue; *asci* developing between stromatic cells, 8-spored, subspherical to shortly ellipsoid, wall apically very thick, often with a distinct ocular chamber, wall I– and K/I–, ascoplasm I and K/I+ orange; *ascospores* hyaline, 1-septate, ellipsoid, with roundish apices. *Conidiomata* frequent, not intermixed with ascomata from which they are macroscopically indistinguishable, but sometimes smaller, I– or I+ pale blue, K/I– or K/I+ pale blue; *conidiophores* absent; *conidiogenous cells* pale to medium brown, developing from spherical stromatic cells, shortly subcylindrical to almost ellipsoid, with an indistinctly truncate base, polyblastic, seceding with conidia; *conidia* multicellular, composed of the conidiogenous cell and the conidial cells, subspherical, brown, verrucose, dry, single, secession schizolytic; *conidial cells* subspherical to ellipsoid.

Notes:

1. Molecular data suggest that *Ph. trypethelii* does not belong to *Lichenostigma* s. str., but to a distinct, new genus. Such a new genus would be morphologically extremely similar to *Lichenostigma*, and we wondered how it could be justified on a morphological basis. The main differences are (1) almost spherical conidia in *Ph. trypethelii* (they are more ellipsoid in *Lichenostigma* s. str.); (2) more compact conidia in *Ph. trypethelii* (compared to the more loose conidia in *Lichenostigma* s. str., the only exception being *L. fellhanerae*, only provisionally placed in *Lichenostigma*, as no molecular data are available) with slightly smaller cells (in those species of *Lichenostigma* s. str. with large conidia, individual cells are slightly larger); and (3) external cells of stromata with a distinct, dark greyish brown mosaic-like ornamentation (in *Lichenostigma* s.str., the ornamentation is often verrucose, sometimes mosaic-like, but then slightly paler and more reddish brown).
2. The new genus is dedicated to our excellent friend Javier Etayo (Pamplona, Spain). Javier is one of the most productive recent explorers of lichenicolous fungi, working

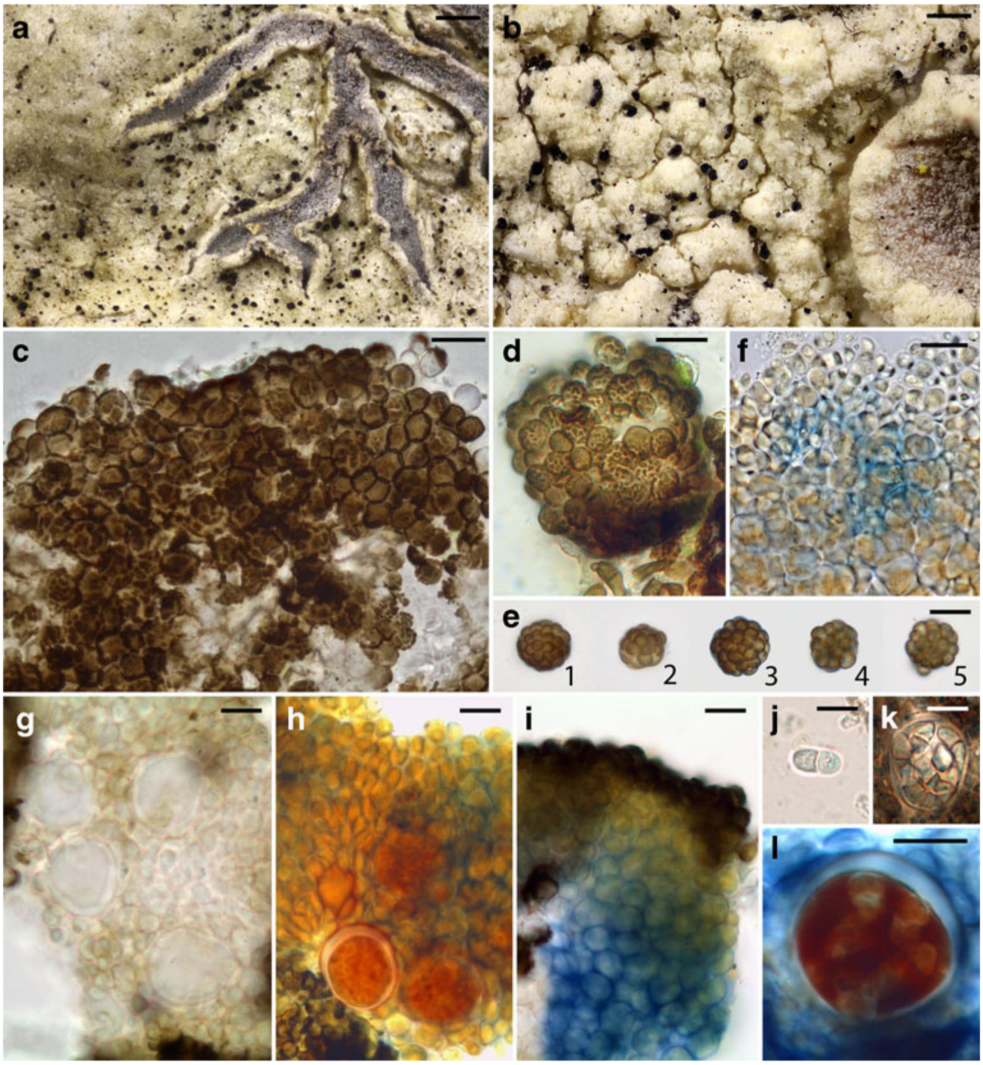

Fig. 6 *Etayoa trypethelii*. **a** Conidiomata on *Phaeographis major* (Common 9200G). **b** Ascomata on *Lecanora caesiorubella* (Buck 29314). **c** Conidioma showing mosaic-like ornamentation of exposed cells, in water (Common 9481D). **d** Young conidioma showing mosaic-like ornamentation, in water (Common 9215P). **e** Conidia in water (1: Common 9434 K, 2: Common 9200G, 3: Harris 39452A, 4–5: holotype). **f** Conidiostroma in 0.15 % IKI, after pre-treatment with C and K (Common 9200G). **g** Immature asci in water. **h** Immature asci in K/I (stroma at first entirely K/I+ blue, then becoming orange). **i** Ascostroma K/I+ blue (**g**–**i** Buck 29314). **j**–**k** Ascus and ascospores in water. **l** Ascus with ascospores in K/I (after pre-treatment with C) (**j**–**l** Diederich 11495). Scale bars: a–b=200 µm, c–l=10 µm

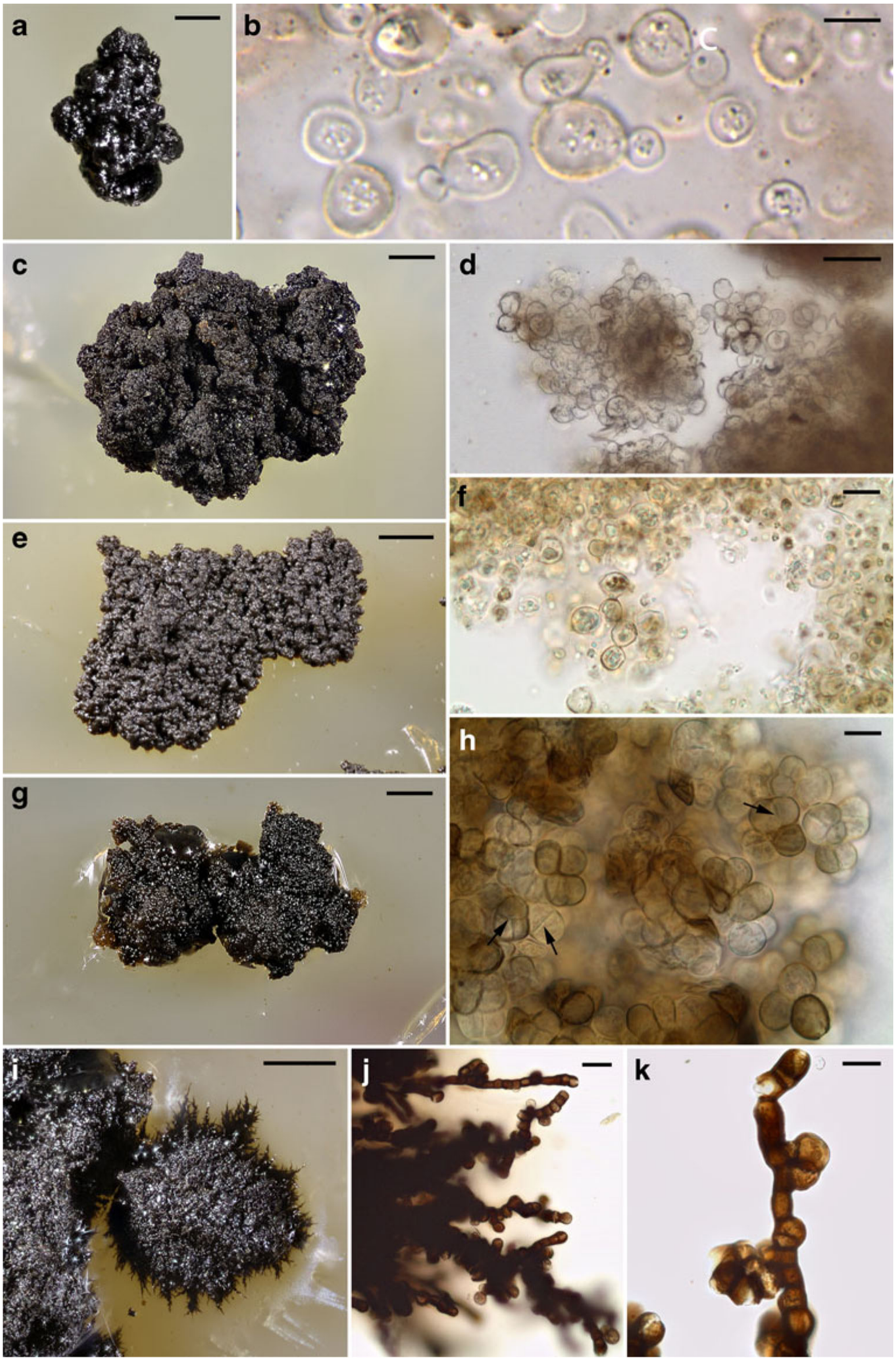

Fig. 7 Cultures of *Lichenostigma*, *Etayoa* and *Lichenothelia* species. **a**–**b** *Lichenostigma chlaroterae* (Neuberg). **c**–**d** *Lichenostigma* cf. *alpinum* on *Fuscidea lightfootii* (Diederich 17240). **e**–**f** *Etayoa trypethelii* (Common 9215P). **g**–**h** *Lichenothelia rugosa* (Ertz 16065). **i**–**k** '*Lichenostigma*' cf. *elongatum* (Ertz 15255). Scale bars: a, g, i=0.5 mm, b=5 μm, c=2 mm, d, j=20 μm, e=1 mm, f, h, k=10 μm

especially in South America, and he is the author of several taxa now included in *Lichenostigma* s. str.

Etayoa trypethelii (Flakus & Kukwa) Diederich & Ertz comb. nov. (Figs. 6 and 7e–f)

MycoBank MB 804695

Basionym: *Phaeosporobolus trypethelii* Flakus & Kukwa, *Lichenologist* **44**: 472 (2012). Type: Bolivia, Dept. Beni, Prov. Ballivian, near Reyes village, 14°18′10″S, 67°18′49″W, 192 m, savannah vegetation, on thallus of *Trypethelium ochroleucum*, 29 Nov. 2004, A. Flakus 3724 (KRAM–holotype!; LPB, hb. Flakus–isotypes).

External cells of stromata with a dark brown mosaic-like ornamentation. *Ascomata* provisionally referred to this species (30–)38–84(−160) µm diam. (N=56) (Buck 29314), I+ and K/I+ blue; *asci* 8-spored, c. 25×20 µm; *ascospores* hyaline, 1-septate, ellipsoid, c. 8.5–13×4.5–6 µm (very few asci and ascospores observed). *Conidiomata* (20–)31–69(−140) µm diam. (N=316, from 6 specimens including the holotype), I– or pale blue, K/I– or K/I+ pale blue; *conidia* subspherical, (10.5–)11.8–14.5(−16)×(10–)11.1–14.0(−15) µm, length/width ratio 1.0–1.1, composed of (16–)20–31(−36) verrucose cells [in optical section (11–)12–17(−19) cells] (N=19), cells (2.5–)3.0–4.0(−4.5) µm diam. (N=94).

For a detailed description and illustrations, see Flakus and Kukwa (2012).

The asexual stage was described from Bolivian material on *Trypethelium ochroleucum*. We have examined additional specimens from the U.S.A. (Florida), where it appears to be rather common on the thallus of Graphidaceae, viz. *Dyplolabia afzelii*, *Fissurina columbina*, *F. mexicana*, *Graphis caesiella*, *G. cupei*, *G. lucifica*, *Graphis* sp., *Ocellularia americana*, *Phaeographis major*, *P. inconspicua*, *P. schizoloma*, and *Phaeographis* sp., but also on *Bathelium carolinianum*, *Laurera megasperma* and *Trypethelium ochroleucum*, from Papua New Guinea on *Pertusaria ramulifera*, and from South Africa on *Graphis*. Material representing the sexual stage that we provisionally refer to the same species has been collected in the U.S.A. (Florida) on *Lecanora caesiorubella* subsp. *glaucomodes* and in Papua New Guinea on *Graphis* and *Pertusaria ramulifera*. All known specimens from both morphs have been collected in subtropical or tropical regions on corticolous lichens with a crustose thallus. The species does not visibly damage the host thallus.

Notes:

1. For many years we have studied an apparently undescribed *Phaeosporobolus* species that is rather common in Florida. Comparison with the type of the recently described *Ph. trypethelii* confirmed that the Florida material belongs to that species, and at the same time that the species is not host-specific (many Florida specimens are on Graphidaceae, whilst the Bolivian type is on *Trypethelium*). Instead, this species appears to be confined to subtropical to tropical environments on corticolous lichens with a crustose thallus. Field observations in Florida have shown that the species is rather specialized concerning host choice, and that some species tend to be highly colonized, whereas nearby thalli of related species may have little or no colonization. Further recent collections from South Africa on *Graphis* and from Papua New Guinea on *Pertusaria* confirm that the species is widespread in tropical habitats and that it prefers Graphidaceae hosts.
2. A specimen from Florida on corticolous *Lecanora caesiorubella* is macroscopically identical to *Ph. trypethelii* (Fig. 6b) and microscopically indistinguishable, except for the presence of asci instead of conidia. The anatomy of stromata, including the colour and ornamentation of the external cells are typically those of *Ph. trypethelii*. It most probably belongs to the same species, and thus represents the sexual stage of *Ph. trypethelii*. As the specimen is too old (collected in 1996), unfortunately no DNA sequences could be obtained. Two specimens that we collected in Papua New Guinea in 1992, one on corticolous Graphidaceae, the other on corticolous *Pertusaria ramulifera*, both appear to represent the same species, but all our efforts to obtain sequences from them also failed. On the latter host, *Pertusaria ramulifera*, we also collected the asexual stage.
3. The three specimens representing the sexual stage were compared with the type of *Lichenostigma hyalosporum* and found to represent a distinct species. *L. hyalosporum* differs by ascomata not becoming blue in I after K and by the presence of typical *Phaeosporobolus*-type conidia, much looser than the compact conidia of *E. trypethelii*.
4. The conidiomatal size is rather variable within this species and does not represent a taxonomically useful character. For example, in specimen Common 9200G, conidiomata measure (20–)30–45(−55) µm diam. (N=58), in Harris 39452A they are (25–)31–55(−80) µm diam. (N=46), whilst in Common 9481D they measure (55–)61–100(−140) µm (N=52) [in case of elongate conidiomata, the largest diameter has been measured]. After bleaching and observation in 0.15 % IKI, a pale to moderately strong I+ blue reaction was often seen in conidiomata. Distinct hemiamyloid staining was not seen.

Additional specimens examined: **Asexual stage**: **Papua New Guinea**: *Madang Prov.*: Huon Peninsula, Finisterre range, Yupna valley, Teptep village, trail in NNW and deep valley in N direction, 5.95° S, 146.55° E, 2500 m, on corticolous *Pertusaria ramulifera*, 30 July 1992, Diederich 10815, 12415 (hb. Diederich). **South Africa**: Mngazana Estuary, on *Graphis*, 2013, Mukherjee (BR). **U.S.A.**: *Florida*: Collier Co., Fakahatchee Strand State Preserve, trail north of Boardwalk, 25°56.51′ N, 81°28.16′ W, on *Graphis caesiella*, 11 Nov. 2011, Common 9434 K (hb. Diederich); Everglades City, near Everglades City Motel on Fla. 29, on Sabal palmetto leaf bases and on fallen tree, on *Bathelium carolinianum*, 29 April 1997, Common 7347L (MSC). Hernando Co., SR 50, between CR 595 N and CR 595S, about 4 miles east of Gulf of Mexico, wet hardwood forest, on *Ocellularia americana*, 31 Dec. 1975, Common 3737Q (MSC). Hillsborough Co., Hillsborough River State Park, 28°08.601′ N, 82°13.786′ W, on *Dyplolabia afzelii* and *Phaeographis major*, 3 Sept. 2011, Common 9200G (MSC, hb. Diederich); ibid., on *Laurera megasperma*, Dec. 1990, Common 4821 (MSC); ibid., trail from Parking area 2, 28°08.94′ N, 82°13.61′ W, on *P. major* and *P. schizoloma*, 2 Sept. 2011, Common 9215P (hb. Diederich); ibid., Florida Trail, 28°08.90′ N, 82°14.01′ W, on *Graphis* sp., *P. major* and *P. inconspicua*, 26 Oct 2011, Common 9244E (hb. Diederich); ibid., 28°08.94′ N, 82°14.10′ W, on *Phaeographis* sp., 15 Sept. 2011, Common 9481D (hb. Diederich); along CR. 581, 3.2 miles S of junction with I-75, hardwood and cypress area along stream, SE of bridge, on *Fissurina mexicana*, 26 April 1996, Common 6895A (MSC). Marion Co., Ocala National Forest, along Co. Rd. 316, c. 0.5 mi E of Oklawaha River bridge at Eureka, 0.2 mi W of Forest Serv. Rd. 67, 29°22′ N, 81°43′ W, *Quercus*-Ericaceae scrub, on *Lyonia*, on *F. columbina*, 29 Dec. 1995, Buck 28630 (NY). Pasco Co., Withlacoochee State Forest, on Clay Sink Rd., about 1 mile from W boundary of forest, on *Trypethelium*, 31 Dec. 1975, Common C3701P (MSC). Sarasota Co., Myakka River State Park, at first crossing of river from south entrance to park on north bank of river, on *Trypethelium ochroleucum*, 1 Jan. 1976, Common 3755D (MSC); ibid., along river near S entrance to park, on *Graphis cupei* and *G. lucifica*, 27 April 1997, Common 7216G (MSC). Taylor Co., Big Bend Wildlife Management Area, Tide Swamp Unit, along C. R. 361, 4.4 mi NW of bridge in Steinhatchee, 29°43′ N, 83°26′ W, planted *Pinus clausa* forest, on *P. clausa*, on *G. lucifica*, 3 Dec. 1996, Harris 39452A (NY).

Sexual stage of *E.* cf. *trypethelii*: **Papua New Guinea**: *Eastern Highlands Prov.*: Mount Gahavisuka Provincial Park, 11 km N of Goroka, along trail to lookout, little disturbed mossy mountain forest, 6°01′ S, 145°25′ E, 2400 m, on corticolous *Pertusaria ramulifera*, 3 Aug. 1992, Diederich 10630 (hb. Diederich). *Madang Prov.*: Near Bogia, long road Bogia-Josephstaal, near Tanggu church, 4°27′ S, 144°56′ E, 330 m, on crown and trunk of felled tree among gardens, on Graphidaceae, 25 July 1992, Diederich 11495 (hb. Diederich). **U.S.A.**: *Florida*: Seminole Co., along Willingham Road, 0.5 mi N of Co. Rd. 419 at Econlockhatchee River, on W border of Flying Seminole Ranch, 28°40′ N, 81°10′ W, oak-pine-palmetto scrub, on *Lecanora caesiorubella* subsp. *glaucomodes*, 10 Jan. 1996, Buck 29314 (NY).

Key to the species of Lichenostigmatales

1 Colonies of dark brown, aseptate, thick-walled yeast cells covered by mucus and reproducing by multilateral budding; mycelium formation, ascomata and conidiomata unknown.............. [***Phaeococcomyces***] 2

1 Colonies of stromatic ascomata or conidiomata, on rare occasions arising from an initial, brown mycelium... 3

2(1) Colonies jet black from the beginning; cells at first hyaline or subhyaline, soon becoming dark olivaceous brown, at first thin-, later usually thick-walled, finally often forming an irregular, verrucose capsule (granular pigment), globose to broadly ellipsoidal, 4–6.5×4–5 μm diam., each giving rise to similarly shaped, slightly smaller secondary cells from 1 to 3 loci; often the cells remain attached, forming short chains of compact clumps; isolated from paint of storage tank in the U.S.A..***P. nigricans***

2(1) Colonies iron-grey; cells globose, verruculose, 4–8 μm diam., remaining attached to one another, at colony margin producing ellipsoid to globose, hyaline, finely verruculose conidia-like cells covered by mucus, 3–5×2.5–5 μm; isolated from *Eucalyptus* leaves in South Africa .... ***P. eucalypti***

3(1) Ascomata I+ and K/I+ intensively blue (but conidiomata I+ pale blue); external cells of stromata (ascomata or conidiomata) with a dark brown, mosaic-like ornamentation; conidia regularly spherical, length/width ratio 1.0–1.1, (10.5–)11.8–14.5(−16)×(10–)11.1–14.0(−15) μm diam., rather compact, composed of 20–31 cells [in optical section 12–17 cells] mainly 3–4 μm diam., surface verrucose; ascospores hyaline, ellipsoid; colonies either representing the sexual or the asexual stage, never mixed; on crustose, corticolous lichens in subtropical to tropical environments.........................***Etayoa trypethelii***

3(1) Ascomata and conidiomata I- and K/I-, or I+ reddish or blue and K/I+ blue; external cells of stromata medium to dark brown, with a verrucose, rarely mosaic-like ornamentation; conidia ellipsoid [rarely irregular in form], length/width ratio 1.0–1.6 [if less than 1.2, then conidia composed of less than 16 cells], more loose, composed of cells that are often slightly larger, 3.5–4.5 μm diam. [if cells 2.5–3.5 μm diam., then conidia either composed of less than 8 cells or regularly ellipsoid], surface smooth, becoming echinulate when overmature; ascospores hyaline or brown; colonies mostly representing the asexual stage, more rarely the sexual stage and then both stages frequently mixed; in temperate to subalpine or boreal environments................................. [***Lichenostigma***] 4

4(3) Conidia produced in a very high number, typically more than 100 per conidioma, regularly ellipsoid, rather compact, (8–)8.7–11.0(−12.5)×(6.5-)6.8–7.7(−8) μm, composed of (16–)18–23(−27) smooth cells [in optical section 9–12 cells], cells 2.5–3.2(−3.5) μm diam.; ascomata unknown; on *Fellhanera granulosa*.. ***L. fellhanerae***

4(3) Conidia usually produced in a smaller number, often more irregular in form, looser, cells larger, 3–5 μm diam..5

5(4) External cells of stromata with a mosaic-like ornamentation; ascomata usually present; ascospores hyaline, ellipsoid, 1-septate, 9–15×3.5–8 μm; on crustose corticolous lichens… .... ***L. hyalosporum***

5(4) External cells of stromata with a verrucose, rarely mosaic-like ornamentation; ascomata either present and ascospores dark brown; or ascomata extremely rare, with hyaline ascospores that are much smaller, 6.5–7×2–2.5 μm, or elongate, attenuated and pointed at both ends, spirally bent, 20–27×3–4 μm; or ascomata absent 6

6(5) Conidia 6–10×5.5–9 μm, composed of 4–9 cells [in optical section 4–5(−8) cells]; sexual stage extremely rare; ascospores brown; on corticolous *Lecanora* and other corticolous crustose lichens.........***L. chlaroterae***

6(5) Conidia larger, at least 10×8.5 μm, composed of at least 8 cells; ascospores hyaline or brown..................7

7(6) Conidia mostly 14–23×10–16 μm, composed of 14–35 cells [in optical section 10–21 cells]; frequently accompanied by a sexual stage; ascospores dark brown, ellipsoid, 9–12×4.5–6 μm; asci 20–25×13–18 μm; mainly on epiphytic fruticose macrolichens..................................***L. maureri***

7(6) Conidia mostly 10–15×8.5–11 μm, composed of 8–17 cells [in optical section 6–10 cells]; sexual stage extremely rare; ascospores hyaline; mainly on terricolous, saxicolous or corticolous *Ochrolechia*, *Pertusaria*, *Varicellaria* and *Fuscidea*, also on other crustose lichens ....[***L. alpinum*** s. lat.] 8

8(7) Ascospores elongate, attenuated and pointed at both ends, spirally bent, 20–27×3–4 μm; asci 31–39×13–17 μm; on *Fuscidea cyathoides* and *Ochrolechia frigida* ....***L. alpinum*** s. lat. p. p. (‘*Diederimyces fuscideae*’)

8(7) Ascospores ellipsoid, 6.5–7×2–2.5 μm; asci 14–17×9–12 μm; on *Pertusaria* and *Coccotrema* ....***L. alpinum*** s. lat. p. p. (‘*Diederimyces microsporus*’)

A new combination for *Lichenostigma rugosum*

Lichenothelia rugosa (G. Thor) Ertz & Diederich **comb. nov.** (Fig. 7g–h)

MycoBank MB 804675

Basionym: *Lichenostigma rugosum* G. Thor, *Lichenologist* **17** (3): 269 (1985) [as *L. rugosa*]. Type: Libya, Cyrenaica, 62 km SSW of Al Marj, 25 km SSE of Al Abyar, 32°00′ N, 20°65′ E, on *Diploschistes steppicus*, 26 March 1983, G. Thor 3458 (S–holotype; BM, GZU, UPS–isotypes, non vid.).

For a detailed description and illustrations, see Thor (1985).

Notes:

1. This species was originally distinguished from the very similar *Lichenostigma maureri* by several characters, such as usually aggregated, black ascomata (compared to brown to black, non-aggregate ascomata), and larger and darker ascospores with deep irregular fissures (ascospores of *L. maureri* are verrucose) (Thor 1985). Our phylogenetic results show, however, that these two species are not related. They differ entirely by the structure of the ascostroma, as stromatic cells in *L. rugosum* divide by septa, whilst those of *L. maureri* by budding. We have confirmed this character in cultures of *L. rugosum* (Fig. 7h).
2. Phylogenetically, *Lichenostigma rugosum* groups with species of *Lichenothelia* (Lichenotheliaceae). As the species clearly does not belong to *Lichenostigma*, we herewith combine it in *Lichenothelia*. Sequences from several other *Lichenostigma* species belonging to subgen. *Lichenogramma* Nav.-Ros. & Hafellner also belong to this group, but no other transfers in *Lichenothelia* are formally proposed here, as that subgenus was not the main object of the present study. These species include *Lichenostigma elongatum*, a species that also multiplies by septa (Fig. 7i–k), and not by budding as in *Lichenostigma* s.str.

Selected specimens of *Lichenothelia rugosa* examined: **France**: *Ardennes*: Chooz, Rochers de Petit-Chooz sur la rive droite de la Meuse, 200 m, affleurement de roches de l’Emsien supérieur, sur *Diploschistes scruposus*, 20 Febr. 2011, Ertz 16065 (BR). **Switzerland**: *Valais*: S of Nendaz, 2 km SSW of Siviez, 46.11176° N, 7.30436° E, on *Diploschistes scruposus*, 26 July 2012, Diederich 17310 (hb. Diederich).

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