The Lichenologist **46**(1): 115–128 (2014) © British Lichen Society, 2014
doi:10.1017/S0024282913000741

Molecular phylogeny resolves a taxonomic misunderstanding and places *Geisleria* close to *Absconditella* s. str. (*Ostropales*: *Stictidaceae*)

André APTROOT, Sittiporn PARNMEN, Robert LÜCKING, Elisabeth BALOCH, Patricia JUNGBLUTH, Marcela E. S. CÁCERES and H. Thorsten LUMBSCH

Abstract: The phylogenetic position of the genus *Geisleria* and its type species *G. sychnogonioides* was reconstructed using sequence data of the mitochondrial small subunit (mtSSU), the nuclear large subunit rDNA (nuLSU) and the first subunit of the RNA polymerase (*RPB1*). The species, previously classified in *Verrucariaceae* (Eurotiomycetes) and *Strigulaceae* (Dothideomycetes), is sister to the type of the genus *Absconditella*, *A. sphagnorum*, and nested within the genera *Absconditella* and *Cryptodiscus* combined (which also includes the lichenized *Bryophagus*). At first glance it appears to be a further example of parallel evolution of perithecioid ascomata within *Stictidaceae* (Lecanoromycetes: *Ostropales*), besides *Ostropa* and *Robergea*, adding to the growing list of perithecioid forms nested within apothecioid lineages in Ostropomycetidae, and specifically *Ostropales*, with other examples known from *Graphidaceae* (several genera), *Gyalectaceae* (*Belonia*), and *Porinaceae*. However, revision of type material collected by Nitschke revealed that the species actually develops typical apothecia with a narrowly exposed disc. We conclude that *Geisleria sychnogonoides* was erroneously considered a pyrenocarpous taxon, because in dry conditions the apothecia are closed and not recognizable as such. The species usually grows on unstable soil and therefore often only develops young, more or less closed ascomata (yet with mature ascospores), and has also been confused with the superficially similar *Belonia incarnata*, in which the ascomata remain closed even when mature. *Geisleria sychnogonioides* has so far only been known as a rarely reported pioneer species from loamy soils in Europe and North America. Here it is reported to occur abundantly on lateritic soils in subtropical Brazil, suggesting that it is cosmopolitan and possibly common, but much overlooked.

Key words: crustose, lichen, perithecia, systematics, taxonomy, tropical

Accepted for publication 29 *August* 2013

Introduction

One of the most fundamental and phylogenetically constant characters in Ascomycota is the nature of the ascospore-producing fruiting bodies, which in most cases are either discocarpous (apothecioid) or pyrenocarpous (perithecioid). Apothecioid ascomata are typical of Pezizomycetes, Leotiomycetes, and subclass Lecanoromycetidae (Lecanoromycetes), whereas perithecioid ascomata are found throughout Sordariomycetes, Eurotiomycetes, and Dothideomycetes (Lumbsch & Huhndorf 2007; Schoch *et al.* 2009). Eurotiomycetes also includes lineages with completely closed ascomata (cleistothecia) (Geiser *et al.* 2006). For centuries, ascoma-types were the main division in the phylum Ascomycota, characterizing the Discomycetes (and Discolichenes) and the Pyrenomycetes (and Pyrenolichenes), respectively (Eschweiler 1824; Zahlbruckner 1907; see Lumbsch 2000), before it was replaced by a classification based on ascoma ontogeny and ascus type, distinguishing ascohymenial from ascolocular

A. Aptroot: ABL Herbarium, Gerrit van der Veenstraat 107, NL-3762 XK Soest, The Netherlands.
Email: andreaptroot@gmail.com
S. Parnmen, R. Lücking and H.T. Lumbsch: Department of Botany, The Field Museum, 1400 S. Lake Shore Drive, Chicago, Illinois 60605, USA.
E. Baloch: Department of Cryptogamic Botany, Swedish Museum of Natural History, P.O. Box 50007, SE-104 05 Stockholm, Sweden; and Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK.
P. Jungbluth: Departamento de Botânica, IBB, UNESP. Caixa Postal 510, CEP 18618-970, Distrito de Rubião Jr., Botucatu - SP, Brazil.
M. E. S. Cáceres: Departamento de Biociências, Universidade Federal de Sergipe, CEP: 49.500-000, Itabaiana, Sergipe, Brazil.

forms (Nannfeldt 1932; Luttrell 1951; Santesson 1952; Henssen & Jahns 1974). Yet the subgroups within this classification still relied heavily on whether the ascomata were apothecioid or perithecioid. The only group that was accepted rather early as having variable fruiting body morphology is the Arthoniomycetes (*Arthoniales*), ranging from typical apothecioid to lirelliform and stromatoid-perithecioid ascomata (Grube 1998; Ertz *et al.* 2009). This group was also considered intermediate between ascohymenial and ascolocular groups (Henssen & Thor 1994).

While perithecioid forms are occasionally nested within apothecioid lineages in other classes, such as Pezizomycetes (O'Donnell *et al.* 1997; Hansen & Pfister 2006; Lücking *et al.* 2009), phylogenetic studies have established Ostropomycetidae, and particularly *Ostropales*, as a prime example of repeated switches between apothecioid and perithecioid fruiting body types (Lumbsch *et al.* 2001, 2006, 2007; Kauff & Lutzoni 2002; Grube *et al.* 2004; Schmitt *et al.* 2005, 2006, 2009; Grube & Hawksworth 2007; Hawksworth & LaGreca 2007; Baloch *et al.* 2010; Rivas Plata *et al.* 2012, 2013). Currently, more than ten transitions from apothecioid to perithecioid forms are known in this subclass, including *Protothelenellaceae* and *Thelenellaceae*, several lineages within *Pertusariales* (*Coccotrema*, *Pertusaria* s. lat.), and a large number of lineages within *Ostropales*: *Ostropa* and *Robergea* in *Stictidaceae*, all taxa in *Porinaceae*, species of the former genus *Belonia* nested within *Gyalecta*, and in *Graphidaceae* species in the genera *Graphis* (*G. mexicana*), *Leucodecton* (*L. compunctum*, *L. compunctellum*), *Pseudoramonia*, *Ocellularia*, and *Thelotrema* (*T. porinoides*). In a number of cases, these species were originally described in, or temporarily referred to, typically perithecioid taxa, such as *Arthopyrenia*, *Porina*, and *Verrucaria*.

The genus *Geisleria* was described in the 19th century by Nitschke (Rabenhorst 1861) with a single species, *G. sychnogonioides* Nitschke, from northern Germany. It is usually characterized by immersed, seemingly perithecioid, non-carbonized ascomata, unitunicate asci, and hyaline 3-septate ascospores. The species was considered a typical pyrenocarpous fungus, lichenized with chlorococcoid algae, occurring on a rather unusual substratum for lichens, unstable loamy soils (Ernst 1993); it was once even recorded growing on a discarded army shirt covered with soil (Roux & Sérusiaux 2004). Such habitats are infrequently visited on lichen forays, and the species is relatively rarely reported. It is a pioneer species and often vanishes within a year or two from most of its known localities. It occurs in nature reserves, but more frequently along artificial ditches, in loam quarries or even in industrial areas. *Geisleria sychnogonioides* has so far been reported from Europe (France, Belgium, the Netherlands, Germany, Switzerland, Czech Republic) and North America (Roux & Sérusiaux 2004).

Four additional species have since been described in *Geisleria*, *viz.* *Geisleria alpina* Servít, which is a *Polyblastia* with a parasite (Swinscow 1967), *G. jamesii* Swinscow, which is now accepted as *Strigula jamesii* (Swinscow) R. C. Harris, and *G. sbarbaronis* Servít and *G. xylophila* Vězda, both known only from their types and not recently studied. *Geisleria sychnogonioides* was classified in the *Verrucariaceae* and even placed in *Verrucaria* (Stizenberger 1882), although it differs from the majority of the genera in this family by the persistent hamathecial filaments. More recently, the genus was synonymized with *Strigula* and the species recombined as *S. sychnogonioides* (Nitschke) R. C. Harris (Egan 1987), although the same author later stated that this generic placement is probably not correct (Harris 1995). Placement in *Strigula* was accepted by some authors (e.g. Roux & Sérusiaux 2004), but disputed by de Bruyn *et al.* (2008), who pointed out that the conidia lack the unique synapomorphy of *Strigulaceae*, the gelatinous appendices, a fact already noticed by Harris (1995). Thus, the systematic position of *Geisleria sychnogonioides* was considered uncertain, as it apparently showed no distinct synapomorphies with any known group of Ascomycota. Among pyrenocarpous lineages, lichenization with chlorococcoid green algae is only known from *Verrucariaceae*, *Protothelenellaceae* and *Thelenellaceae*.

Confusion is possible with the name *Thelenella sychnogonioides* (Zahlbr.) R. C. Harris, based on *Microglaena sychnogonioides* Zahlbr., which is a different pyrenocarpous lichen but also with chlorococcoid algae, in *Thelenellaceae*. The identical epithet in both taxa is independently based on a supposed similarity with the genus *Sychnogonia* Körb. [non *Sychnogonia* Trevis.], currently regarded as a synonym of *Muellerella* Hepp ex Müll. Arg. in the *Verrucariaceae*.

During recent years, the first author collected specimens of *Geisleria sychnogonioides* on various occasions, and attempts were made to sequence it, but without success. At the 6th EGBL (Encontro do Grupo Brasileiro de Liquenólogos) congress in Botucatu (São Paulo State, Brazil), the first author's attention was drawn to a degraded reddish laterite landscape covered by pale, roughly circular patches of several centimeters to decimeters in diameter. While it was unlikely that this unstable, urban substratum would support lichen growth, all patches examined contained pale, immersed perithecioid ascomata of minute size. Morphologically, these were indistinguishable from European material of *G. sychnogonioides*, and microscopical examination also failed to yield differentiating characters. We herewith report the species new to the Southern Hemisphere. Given its abundance at this locality, and the fact that this particular habitat is very common (and regrettably increasingly so) all over the tropics, we surmise that the species is thus cosmopolitan and common, but usually overlooked. DNA of the fresh material of the Brazilian specimen was successfully extracted and sequenced by the second author. After these results were obtained, the first author made collections of fresh material in the type region (northern Germany), and these were also successfully sequenced by the second author, confirming the identity of the Brazilian material and the phylogenetic position of the genus *Geisleria*.

Here we report on the phylogenetic placement of the genus *Geisleria* in the family *Stictidaceae* (*Ostropales*), a lineage to which it had never before been related and thus a highly unexpected result.

Materials and Methods

Material

The following material was used for the molecular phylogenetic study:

Geisleria sychnogonioides Nitschke: **Brazil:** *São Paulo*: Botucatu, near Pousada Mandala on SP-254, 850 m, 22°52′45″S, 48°29′16″W, on soil in cerrado area, 2012, *M. E. S. Cáceres & A. Aptroot* 13560 (ABL, F, SP): *Geisleria sychnogonoides3* in Table 1.—**Germany:** *Niedersachsen*: Moorbek N of Wildeshausen, W of Amelhausen, 52°94′73·35″N, 8°31′14·14″E, on soil of nature compensation area, 2013, *A. Aptroot* 70626: *Geisleria sychnogonoides1* in Table 1; Moorbek N of Wildeshausen, S of Glane, 52°91′62·88″N, 8°35′54·62″E, on sand in sand pit, *A. Aptroot* 70627: *Geisleria sychnogonoides2* in Table 1.

Taxa included in the analyses, along with GenBank accession numbers and collection information for newly sequenced samples, are listed in Table 1.

DNA extraction, amplification and sequencing

The Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, USA) was used to isolate DNA, following the manufacturer's instructions, except only 10–30 µl of extraction buffer and 10–30 µl dilution buffer were used; a 20 × DNA dilution was then used in subsequent PCR reactions. We assembled a three-locus data set consisting of mtSSU rDNA, nuLSU rDNA, and the protein-coding genes RPB1. Primers used to amplify fungal DNA are: 1) AL2R (Mangold *et al.* 2008) and LR6 (Vilgalys & Hester 1990); 2) mt SSU rDNA – mrSSU1, mrSSU3R (Zoller *et al.* 1999); 3) RPB1: gRPB1-A (Stiller & Hall 1997) and fRPB1-C rev (Matheny *et al.* 2002). PCR amplifications and cycle sequencing conditions were as described previously (Schmitt *et al.* 2010; Rivas Plata *et al.* 2013).

Phylogenetic analyses

Sequence alignments were carried out separately for each data set using Geneious Pro 5.4.3 (Drummond *et al.* 2011). The jModelTest Version 0.1.1 (Posada 2008) selected the following models as best fits for our data: GTR+G+I for nuLSU, RPB1, and GTR+G for mtSSU. The B/MCMC analysis was conducted on the concatenated data set using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). A run with 20 000 000 generations, starting with a random tree and employing 4 simultaneous chains, was executed. Every 100th tree was saved into a file. The first 500 000 generations (i.e. the first 5000 trees) were deleted as the 'burn in' of the chain. We used AWTY (Nylander *et al.* 2007) to compare splits frequencies in the different runs and to plot cumulative split frequencies to ensure that equilibrium was reached. Of the remaining 390 000 trees (195 000 from each of the parallel runs) a majority-rule consensus tree with average branch lengths was calculated using the sumt option of MrBayes. Posterior probabilities were obtained for each clade. Only clades that received bootstrap support equal or above 70% under ML and posterior probabilities ≥0·95 were considered as strongly

TABLE 1. *Specimens and GenBank accession numbers used in this study. Newly obtained sequences in bold.*

Species name	Family	Order	nuLSU	mtSSU	*RPB1*
Absconditella lignicola	*Stictidaceae*	*Ostropales*	FJ904669	FJ904691	KC191646
A. sphagnorum	*Stictidaceae*	*Ostropales*	AY300824	AY300872	KC191647
Acarosporina microspora	*Stictidaceae*	*Ostropales*	AY584643	AY584612	DQ782818
Agyrium rufum	*Agyriaceae*	*Agyriales*	EF581826	EF581823	EF581822
Ainoa mooreana	*Trapeliaceae*	Ostropomycetidae incertae sedis	AY212829	AY212851	DQ870928
Anisomeridium sp.	*Monoblastiaceae*	Dothideomycetes incertae sedis	JN887398	JN887410	–
Anzina carneonivea	*Trapeliaceae*	Ostropomycetidae incertae sedis	AY212829	AY212851	KC191648
Arctomia delicatula	*Arctomiaceae*	Ostropomycetidae incertae sedis	AY853355	AY853307	DQ870929
A. teretiuscula	*Arctomiaceae*	Ostropomycetidae incertae sedis	DQ007346	DQ007349	DQ870930
Arthopyrenia salicis	*Arthopyreniaceae*	Dothideomycetes	AY607730	AY607742	FJ941893
Aspicilia cf. cinerea	*Megasporaceae*	*Agyriales*	DQ780304	DQ780272	DQ870932
A. desertorum	*Megasporaceae*	*Agyriales*	KC020258	KC020252	KC020289
A. izcoana	*Megasporaceae*	*Agyriales*	AY853311	AY853359	DQ870934
Astrothelium cinnamomeum	*Trypetheliaceae*	Dothideomycetes incertae sedis	AY584652	AY584632	DQ782824
Baeomyces placophyllus	*Baeomycetaceae*	Ostropomycetidae incertae sedis	AF356658	AY300878	DQ870936
B. rufus	*Baeomycetaceae*	Ostropomycetidae incertae sedis	DQ871008	DQ871016	DQ870937
Carestiella socia	*Stictidaceae*	*Ostropales*	AY661687	AY661677	KC191649
Chapsa pulchra	*Graphidaceae*	*Ostropales*	KC020261	KC020255	KC020292
Chroodiscus parvisporus	*Graphidaceae*	*Ostropales*	KC020262	KC020256	KC020293
Circinaria caesiocinerea	*Megasporaceae*	*Agyriales*	DQ780303	DQ780271	DQ870931
C. contorta	*Megasporaceae*	*Agyriales*	DQ986782	DQ986876	DQ986852
C. fruticulosa	*Megasporaceae*	*Agyriales*	KC020259	KC020253	KC020290
C. hispida	*Megasporaceae*	*Agyriales*	DQ780305	DQ780273	DQ870933
Coccomycetella richardsonii	*Odontotremataceae*	*Agyriales*	HM244761	HM244737	KC191650
Coccotrema cucurbitula	*Coccotremataceae*	*Agyriales*	AF274092	AF329161	DQ870939
C. pocillarium	*Coccotremataceae*	*Agyriales*	AF274093	AF329166	DQ870940
Coenogonium leprieurii	*Coenogoniaceae*	*Ostropales*	AF465442	AY584698	–
C. luteum	*Coenogoniaceae*	*Ostropales*	AF279387	AY584699	–
C. pineti	*Coenogoniaceae*	*Ostropales*	AY300834	AY300884	–
Cryptodiscus gloeocapsa	*Stictidaceae*	*Ostropales*	AF465440	AY300880	KC191651
C. pini	*Stictidaceae*	*Ostropales*	HM244762	HM244738	KC191653
Dibaeis baeomyces	*Icmadophilaceae*	*Agyriales*	AF107555	AY300883	DQ842011
Diploschistes cinereocaesius	*Graphidaceae*	*Ostropales*	AY300835	AY300885	DQ870941
D. scruposus	*Graphidaceae*	*Ostropales*	AF279389	AY584692	DQ870943
Dyplolabia afzelii	*Graphidaceae*	*Ostropales*	HQ639628	HQ639594	KC020294
Geisleria sychnogonioides3			**KC689752**	**KC689751**	**KC689753**
G. sychnogonioides2			**KF220304**	**KF220306**	**KF220303**
G. sychnogonioides1			–	**KF220305**	**KF220302**
Glyphis cicatricosa	*Graphidaceae*	*Ostropales*	HQ639630	HQ639610	KC020296

Table 1. *Continued*

Species name	Family	Order	nuLSU	mtSSU	*RPB1*
Graphis scripta	*Graphidaceae*	*Ostropales*	DQ431937	DQ384870	DQ870947
Gregorella humida	*Arctomiaceae*	Ostropomycetidae incertae sedis	AY853378	AY853329	DQ870946
Gyalecta herculana	*Gyalectaceae*	*Ostropales*	FJ941886	–	FJ941896
G. russula	*Gyalectaceae*	*Ostropales*	HM244759	HM244735	FJ941897
G. flotowii	*Gyalectaceae*	*Ostropales*	HM244764	HM244740	KC191655
G. hypoleuca	*Gyalectaceae*	*Ostropales*	AF465453	HM244742	KC191656
G. truncigena	*Gyalectaceae*	*Ostropales*	HM244766	HM244743	KC191657
G. ulmi	*Gyalectaceae*	*Ostropales*	AF465463	AY300888	KC191658
Icmadophila ericetorum	*Icmadophilaceae*	*Agyriales*	DQ883694	DQ986897	DQ883723
Kirschsteiniothelia aethiops	*Kirschsteiniotheliaceae*	Dothideomycetes incertae sedis	AY016361	FJ190604	DQ471157
Laurera megasperma	*Trypetheliaceae*	Dothideomycetes incertae sedis	FJ267702	GU561847	–
Lobothallia radiosa	*Megasporaceae*	*Agyriales*	DQ780306	DQ780274	DQ870954
Myriotrema olivaceum	*Graphidaceae*	*Ostropales*	EU075627	EU075579	KC020298
Nadvornikia hawaiiensis	*Graphidaceae*	*Ostropales*	AY605080	EU075581	KC020300
Ocellularia chiriquiensis	*Graphidaceae*	*Ostropales*	EU075629	EU075582	KC020301
O. endoxantha	*Graphidaceae*	*Ostropales*	KC020263	KC020257	KC020302
Ochrolechia androgyna	*Ochrolechiaceae*	*Agyriales*	AY300846	AY300897	DQ870957
O. balcanica	*Ochrolechiaceae*	*Agyriales*	AF329171	AF329170	KC222183
O. frigida	*Ochrolechiaceae*	*Agyriales*	AY300847	AY300898	KC222184
O. oregonensis	*Ochrolechiaceae*	*Agyriales*	DQ780308	DQ780276	DQ870958
O. pallescens	*Ochrolechiaceae*	*Agyriales*	DQ780310	DQ780277	DQ870960
O. parella	*Ochrolechiaceae*	*Agyriales*	AF274097	AF329173	DQ870959
O. peruensis	*Ochrolechiaceae*	*Agyriales*	DQ780311	DQ780279	KC222185
O. sp.	*Ochrolechiaceae*	*Agyriales*	DQ986777	DQ986886	DQ986849
O. subpallescens	*Ochrolechiaceae*	*Agyriales*	GU980985	GU980978	GU981008
O. turneri	*Ochrolechiaceae*	*Agyriales*	AY568002	AY567982	DQ870961
O. upsaliensis	*Ochrolechiaceae*	*Agyriales*	GU980986	GU980979	GU981009
O. yasudae	*Ochrolechiaceae*	*Agyriales*	DQ986776	DQ986902	DQ986848
Odontotrema phacidiellum	*Odontotremataceae*	*Agyriales*	HM244769	HM244748	KC191661
O. phacidioides	*Odontotremataceae*	*Agyriales*	HM244770	HM244749	KC191662
Orceolina antarctica	*Trapeliaceae*	Ostropomycetidae incertae sedis	AF274115	AY212852	DQ870962
Orceolina kerguelensis	*Trapeliaceae*	Ostropomycetidae incertae sedis	AY212830	AF381561	DQ870963
Pertusaria albescens	*Ochrolechiaceae, Variolaria*	*Agyriales*	AF329176	AF329175	DQ870964
P. amara	*Ochrolechiaceae, Variolaria*	*Agyriales*	AF274101	AY300900	DQ973048
P. coccodes	*Pertusariaceae*	*Agyriales*	AF2792095	AY567984	DQ870966
P. corallina	*Ochrolechiaceae, Variolaria*	*Agyriales*	AY300850	AY300901	DQ870967
P. corallophora	*Ochrolechiaceae, Variolaria*	*Agyriales*	DQ780316	DQ780285	DQ870969
P. coronata	*Pertusariaceae*	*Agyriales*	AY300851	AY300902	DQ870968
P. gibberosa	*Pertusariaceae*	*Agyriales*	DQ780322	DQ780289	DQ870970

TABLE 1. *Continued*

Species name	Family	Order	nuLSU	mtSSU	*RPB1*
P. hermaka	*Pertusariaceae*	*Agyriales*	DQ780334	DQ780299	XXX NEW IS
P. lecanina	*Pertusariaceae*	*Agyriales*	AF279296	AY567991	DQ870972
P. leioplaca	*Pertusariaceae*	*Agyriales*	AY300852	AY300903	DQ870973
P. mammosa	*Ochrolechiaceae, Variolaria*	*Agyriales*	AY212831	AY212854	DQ870974
P. mesotropa	*Pertusariaceae*	*Agyriales*	DQ780325	DQ780292	DQ870975
P. ophthalmiza	*Ochrolechiaceae, Variolaria*	*Agyriales*	AY568006	AY567993	DQ870976
P. panyrga	*Ochrolechiaceae, Variolaria*	*Agyriales*	DQ780327	AY567994	DQ870977
P. paramerae	*Pertusariaceae*	*Agyriales*	DQ780326	DQ780293	GU981012
P. pertusa	*Pertusariaceae*	*Agyriales*	AF279300	AF381565	DQ870978
P. plittiana	*Pertusariaceae*	*Agyriales*	DQ780328	DQ780294	DQ870979
P. pustulata	*Pertusariaceae*	*Agyriales*	DQ780332	DQ780297	GU981013
P. scaberula	*Ochrolechiaceae, Variolaria*	*Agyriales*	AF274099	AF431959	DQ870980
P. subventosa	*Ochrolechiaceae, Variolaria*	*Agyriales*	AY300854	AY300905	DQ870981
Phlyctis agelaea	*Phlyctidaceae*	*Ostropales*	AY853381	AY853332	–
P. argena	*Phlyctidaceae*	*Ostropales*	DQ986771	DQ986880	KC191664
Phyllobathelium anomalum	*Strigulaceae*	Dothideomycetes incertae sedis	GU327722	GU327698	–
P. firmum	*Strigulaceae*	Dothideomycetes incertae sedis	GU327723	JN887413	–
Placopsis cribellans	*Trapeliaceae*	Ostropomycetidae incertae sedis	DQ871010	DQ871018	DQ870983
P. gelida	*Trapeliaceae*	Ostropomycetidae incertae sedis	AY212836	AY212859	DQ870984
P. santessonii	*Trapeliaceae*	Ostropomycetidae incertae sedis	AY212845	AY212867	DQ870986
Placynthiella icmalea	*Trapeliaceae*	Ostropomycetidae incertae sedis	AY212846	AY212870	DQ870985
P. uliginosa	*Trapeliaceae*	Ostropomycetidae incertae sedis	DQ986774	DQ986877	DQ986845
Porina aenea	*Porinaceae*	*Ostropales*	–	DQ168410	KC191665
P. byssophila	*Porinaceae*	*Ostropales*	–	HM244755	KC191666
Protothelenella sphinctrinoidella	*Protothelenellaceae*	Ostropomycetidae incertae sedis	AY607735	AY607747	DQ870989
P. corrosa	*Protothelenellaceae*	Ostropomycetidae incertae sedis	AY607734	AY607746	DQ870988
Pseudochapsa phlyctidioides	*Graphidaceae*	*Ostropales*	KC020260	KC020254	KC020291
Pycnotrema pycnoporellum	*Graphidaceae*	*Ostropales*	HQ639658	HQ639584	KC020299
Pyrenula aspistea	*Pyrenulaceae*	Chaetothyriomycetidae	JQ927470	JQ927462	–
P. nitida	*Pyrenulaceae*	Chaetothyriomycetidae	DQ329023	DQ328998	–
P. pseudobufonia	*Pyrenulaceae*	Chaetothyriomycetidae	–	AY584720	DQ840558
Racodium rupestre	?	Dothideomycetes	EU048582	EU048589	–
Rhabdodiscus subcavatus	*Graphidaceae*	*Ostropales*	EU075641	EU075595	KC020304
Rhexophiale rhexoblephara	*Gomphillaceae*	*Ostropales*	AY853391	AY853341	–
Rimularia insularis	*Trapeliaceae*	Ostropomycetidae incertae sedis	KC222205	KC222182	KC222188
R. psephota	*Trapeliaceae*	Ostropomycetidae incertae sedis	DQ871012	DQ871019	DQ870992
Schaereria corticola	*Agyriaceae*	Ostropomycetidae	AY300859	AY300909	DQ870993
Schizoxylon albescens	*Stictidaceae*	*Ostropales*	DQ401144	DQ401142	KC191667
Siphula ceratites	*Icmadophilaceae*	*Agyriales*	AY853394	AY853344	DQ986847

TABLE 1. *Continued*

Species name	Family	Order	nuLSU	mtSSU	*RPB1*
Stictis populorum	*Stictidaceae*	*Ostropales*	AY527327	AY300882	KC191668
S. radiata	*Stictidaceae*	*Ostropales*	AY300864	AY584727	–
Strigula jamesii	*Strigulaceae*	Dothideomycetes incertae sedis	JN887404	JN887416	–
S. nemathora	*Strigulaceae*	Dothideomycetes incertae sedis	JN887405	GU327701	–
Thamnolia vermicularis	*Icmadophilaceae*	*Agyriales*	AY961599	AY853345	DQ915599
Thecaria quassiicola	*Graphidaceae*	*Ostropales*	HQ639667	HQ639617	–
Thelotrema lepadinum	*Graphidaceae*	*Ostropales*	AY300866	AY300916	DQ973067
T. subtile	*Graphidaceae*	*Ostropales*	DQ871013	EU075607	DQ870998
T. suecicum	*Graphidaceae*	*Ostropales*	AY300867	AY300917	DQ870997
Topeliopsis decorticans	*Graphidaceae*	*Ostropales*	EU075654	EU075609	KC020288
Trapelia chiodectonoides	*Trapeliaceae*	Ostropomycetidae incertae sedis	AY212847	AY212873	DQ870999
T. placodioides	*Trapeliaceae*	Ostropomycetidae incertae sedis	AF274103	AF431962	DQ366259
Trapeliopsis flexuosa	*Trapeliaceae*	Ostropomycetidae incertae sedis	AF274118	AY212875	DQ871000
T. granulosa	*Trapeliaceae*	Ostropomycetidae incertae sedis	AF274119	AF381567	DQ871001
T. percrenata	*Trapeliaceae*	Ostropomycetidae incertae sedis	AF279302	AY212876	EF158853
Trypethelium nitidiusculum	*Trypetheliaceae*	Dothideomycetes	FJ267701	GU561848	–
Tubeufia cerea	*Tubeufiaceae*	Dothideomycetes incertae sedis	DQ470982	FJ190634	DQ471180
Varicellaria hemisphaerica	?	*Agyriales*	AF381556	DQ973000	DQ902341
V. lactea	?	*Agyriales*	AF381557	AF381564	DQ870971
V. velata	?	*Agyriales*	AY300855	AY300906	DQ870982
Verrucaria canella	*Verrucariaceae*	Chaetothyriomycetidae	EF643784	–	EF689787
V. funckii	*Verrucariaceae*	Chaetothyriomycetidae	EF105133	EF105155	–
Wawea fruticulosa	*Arctomiaceae*	Ostropomycetidae incertae sedis	DQ007347	DQ871023	DQ871005

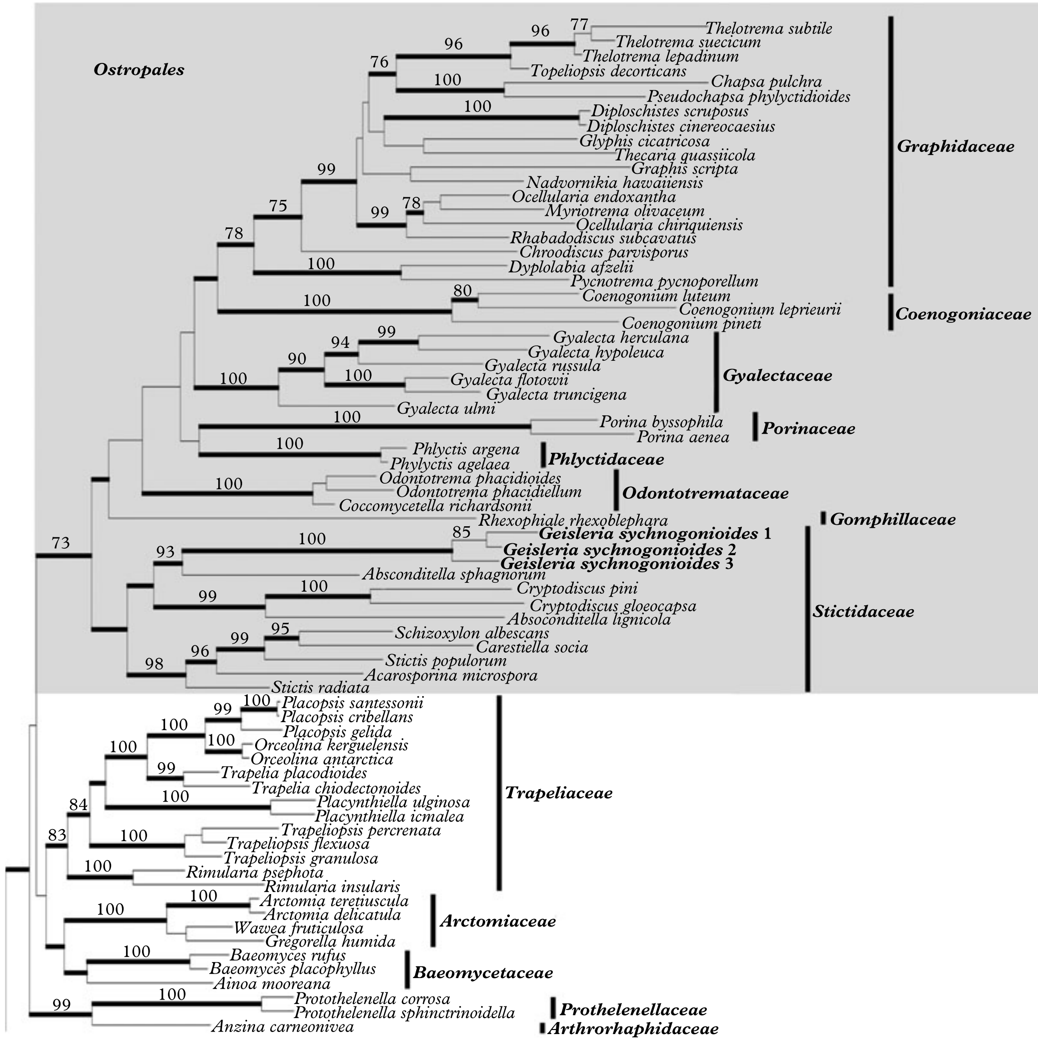

FIG. 1. Phylogenetic reconstruction of the placement of *Geisleria sychnogonioides* as sister to *Absconditella sphagnorum*. This is a maximum likelihood tree inferred from a concatenated alignment of mtSSU, nuLSU rDNA and *RPB1* sequences. Branches with bootstrap support ≥70% and posterior probabilities ≥0·95 are indicated in bold. ML-bootstrap values indicated at branches.

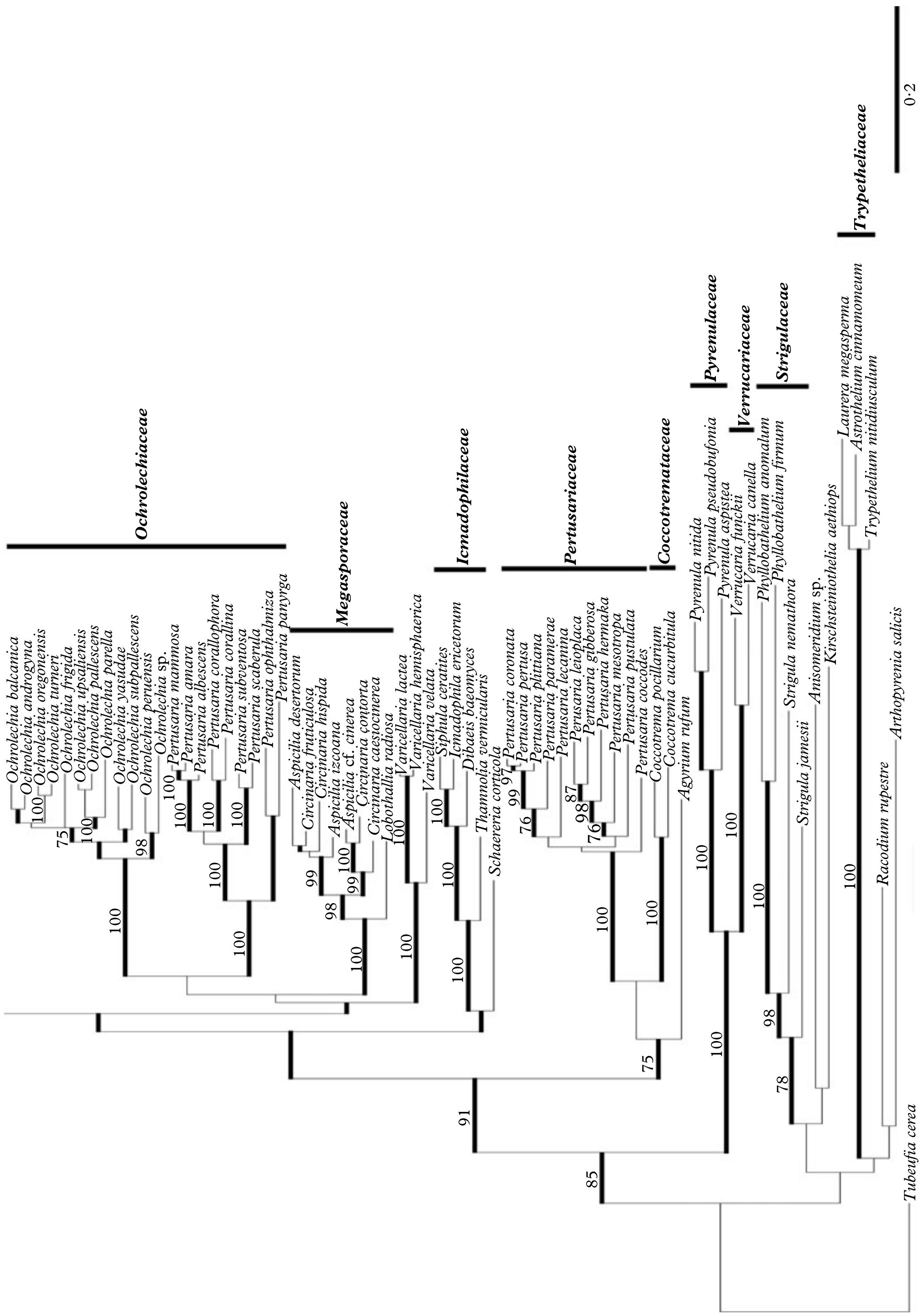

FIG. 1. *Continued*

supported. Phylogenetic trees were visualized using the program Treeview (Page 1996).

A maximum likelihood (ML) analysis was performed for each locus and the combined data set in RAxML 7.2.6 (Stamatakis 2006), using the GTRGAMMA model with 25 rate parameter categories. Support was then estimated by performing 1000 bootstrap pseudoreplicates (Felsenstein 1985). Only clades with bootstrap support equal or above 70% under ML and posterior probabilities ≥0·95 in the Bayesian analysis were considered as strongly supported.

Results and Discussion

Geisleria sychnogonioides clustered with high support within *Stictidaceae* in *Ostropales* (Fig. 1), close to *Cryptodiscus* Corda (Baloch *et al.* 2009) and *Absconditella* Vězda (Fig. 2A), with the type species of the latter sister to *Geisleria sychnogonioides*. Within *Stictidaceae*, this is the third apparently perithecioid lineage, besides *Ostropa* and *Robergea*, and the first outside the *Stictidaceae* core group (Lücking *et al.* 2011). This suggests yet another transition from apothecial to perithecioid ascomata within *Ostropales*. The relationship of *Geisleria* and *Absconditella* is comparable to what has been found for *Belonia* and *Gyalecta*, with the former producing perithecioid ascomata but being nested within the latter with apothecioid fruiting bodies. In the latter case, *Belonia* has been formally synonymized with *Gyalecta* (Baloch *et al.* 2013). Here we do not propose that *Absconditella* be synonymized with *Geisleria*, nor do we combine the two genera and propose to conserve the name *Absconditella*. The reasoning behind this is that the two (out of 11) species of *Absconditella* sequenced so far do not cluster together, rendering the genus paraphyletic, and that a larger sampling seems advisable (just as discussed by Baloch *et al.* 2009) before a decision can be made about the monophyletic groups in the clade now consisting of the genera *Absconditella*, *Cryptodiscus* (including *Bryophagus*) and *Geisleria*. Figure 1 also shows the phylogenetic position of the families in which *Geisleria* was previously classified, *viz.* *Verrucariaceae* (Eurotiomycetes) and *Strigulaceae* (Dothideomycetes), as well as representative groups from the Ostropomycetidae and some other groups with a phylogenetic position in between, in order to show how distant *Geisleria* is after all from the groups in which it was previously classified.

The phylogenetic position of *Geisleria* at first is entirely unexpected, given its previous classification as a pyrenocarpous lichenized fungus. However, anatomical study of the sequenced material revealed that its ascus type is ostropalean, with a ring-shaped structure projecting from the tholus down into the lumen (Fig. 2B). The same applies to the hamathecium and ascospores. In addition, the photobiont is morphologically similar to the one found in *Absconditella*. A subsequent study of the isotypes of *Geisleria sychnogonioides* in W, and additional material of the species collected in various parts of Europe, revealed that the species actually produces typical gyalectoid apothecia, identical to those of *Absconditella* (Fig. 3A & C), and its anatomical features support placement within that lineage. In the original exsiccate (Rabenhorst, *Lichenes Europaei* 574; Fig. 3: upper left), Nitschke described the ascomata as subglobose apothecia and noted that fully grown apothecia only appear after a while, according to his interpretation after fertilization ("*nach der Befruchtung*"). There are potentially three reasons why subsequent authors classified this taxon as having perithecia: 1) in dry conditions, the apothecia shrink and resemble young, more or less closed apothecia with narrow pores (Fig. 3B); 2) even young, closed apothecia (Fig. 3D) produce mature ascospores, a fact we interpret as an adaptation of the species growing on unstable soil, ensuring successful reproduction and propagation in early developmental stages; 3) several specimens in material identified as *Geisleria sychnogonioides* actually belong to *Belonia incarnata* Th. Fr. & Graewe ex Th. Fr. (Fig. 2C & D), a perithecioid species nested within *Gyalecta* (Baloch *et al.* 2010, 2013). It appears that these two species have been confused repeatedly. However, they can be readily separated by their substratum (debris instead of soil in the latter), thallus morphology (cartilaginous in the latter), and ascoma ontogeny (remaining closed in the latter), as well as other features.

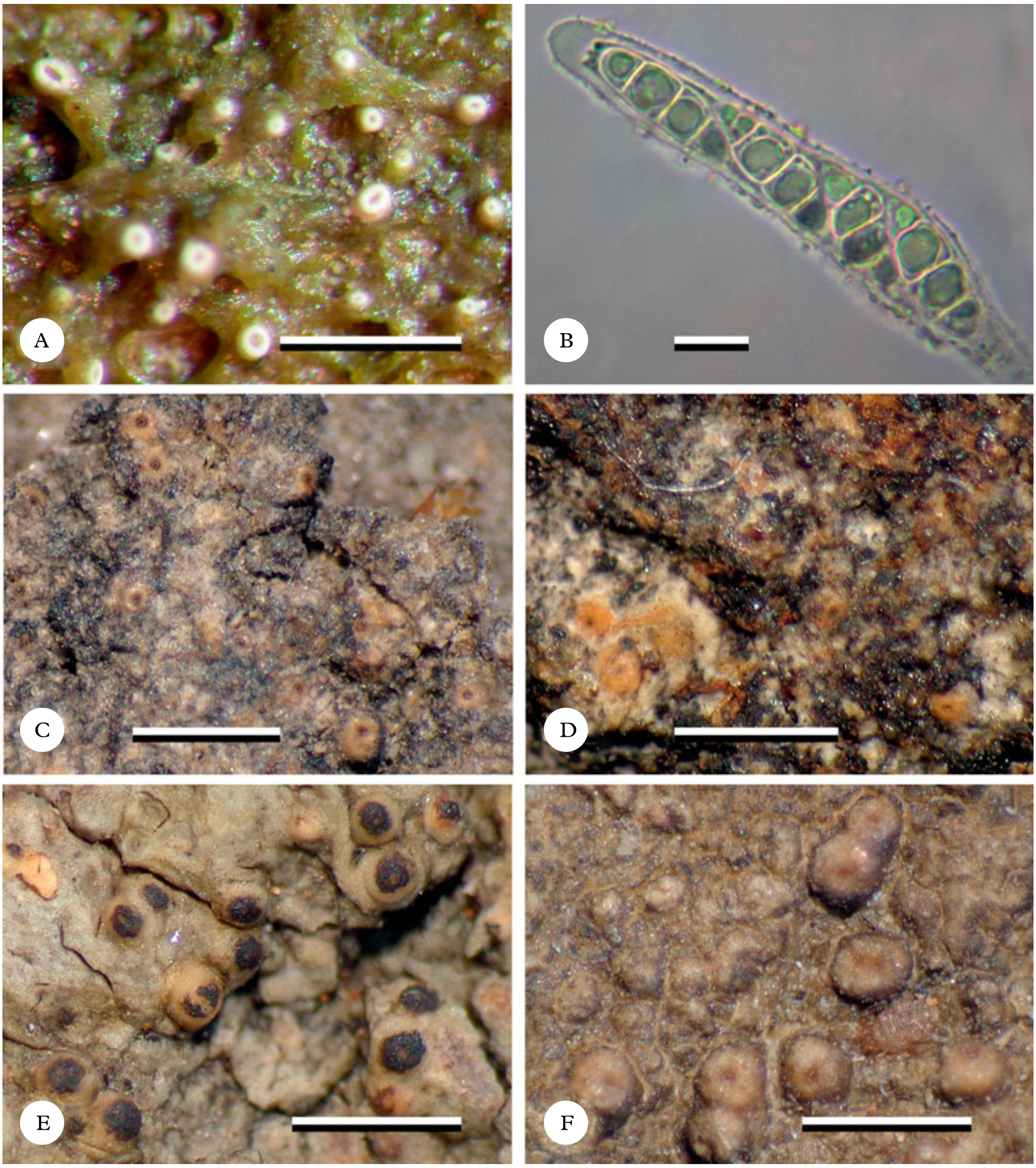

FIG. 2. A, *Absconditella delutula*, thallus and apothecia (Netherlands, *Aptroot* 70517, ABL); B, *Geisleria sychnogonioides*, ascus (Brazil, *Cáceres & A. Aptroot* 13560, F); C & D, *Belonia incarnata*, thallus and (closed) apothecia (D a specimen identified as *G. sychnogonioides* in W); E, *Belonia herculana* (W), thallus and perithecioid ascomata; F, *B. russula* (W), thallus and perithecioid ascomata. Scales: A, C–F = 1 mm; B = 10 µm. In colour online.

Geisleria sychnogonioides could thus be interpreted as *Absconditella*, with its fruiting bodies already producing mature ascospores in young, still closed stages, whereas open apothecia, which the species is capable of developing, are only seen in specimens of a certain age, including the rather well-developed type material. This situation is thus slightly

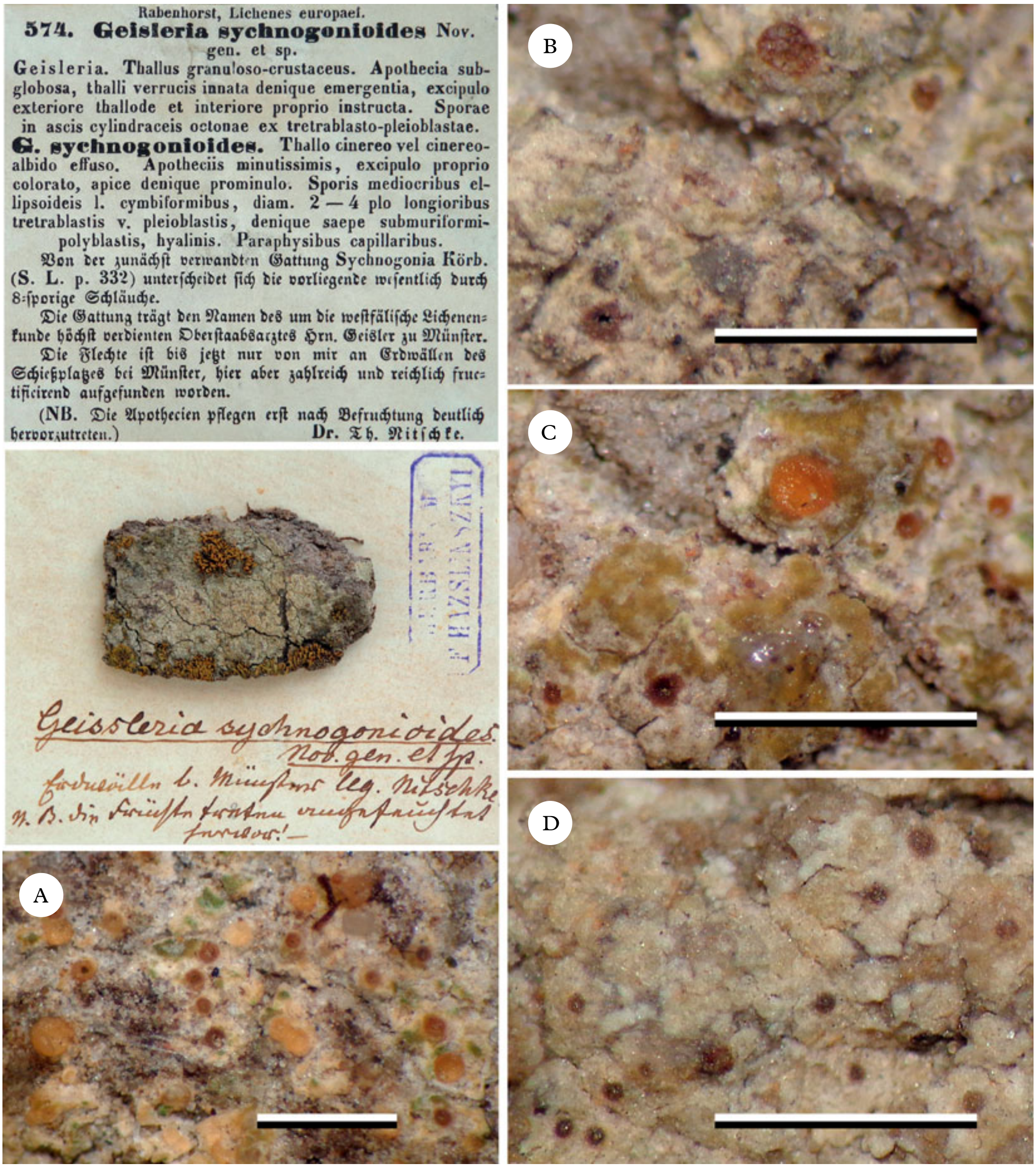

FIG. 3. *Geisleria sychnogonioides* (isotypes in W). Upper left, original label of Rabenhorst's exsiccata no. 574. Middle left, isotype specimen distributed separately from exsiccata. A, non-exsiccate isotype, thallus and closed and fully open apothecia (hydrated); B & C, exsiccate isotype, in dried and hydrated condition showing difference in size and appearance of mature apothecia; D, non-type specimen with young, closed apothecia only (producing mature ascospores). Scales: A–D = 1 mm. In colour online.

different from the distantly related, perithecioid forms of *Gyalecta* previously classified in *Belonia* (Baloch *et al.* 2010, 2013), in which even mature ascomata remain closed (Fig. 2E & F), and it could be interpreted as an intermediate evolutionary step between hemiangiocarpy (apothecia closed when young and mature ascospores only seen in open apothecia) and angiocarpy (apothecia remaining closed throughout their development).

We are grateful to CAPES for providing funding to PJ to enable AA, RL and MESC to attend the EGBL6 meeting in Botucatu where the material was collected. Funding provided by the National Science Foundation (NSF), DEB-1025861 to The Field Museum (PI HTL, CoPI RL, "ATM – Assembling a taxonomic monograph: the lichen family *Graphidaceae*") covered the laboratory costs. The sequences of *Geisleria* were generated in the Pritzker Laboratory for Molecular Systematics at The Field Museum (Chicago); the authors thank I. Schmitt for allowing them to use these unpublished sequences. EB thanks the Swedish Research Council (grants VR 621-2006-3760 and VR 621-2009-537, PI: Mats Wedin). AA thanks the Stichting Hugo de Vries-Fonds for a travel fund.

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