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Molecular phylogeny of *Heteroplacidium*, *Placidium*, and related catapyrenioid genera (Verrucariaceae, lichen-forming Ascomycota)[1]

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- *Premise of the study:* Verrucariaceae is a fascinating lineage of lichenized fungi for which generic and species delimitation is problematic due to the scarcity of discriminating morphological characters. Members of this family inhabit rocks, but they further colonize soils, barks, mosses, and other lichens. Our aim is to contribute to the DNA-based inference of the Verrucariaceae tree of life and to investigate characters that could be useful for proposing a more natural classification. We focused on catapyrenioid genera, which are often part of biological soil crusts, a cryptogam-dominated ecosystem contributing to soil formation and stabilization in arid environments. Understanding their evolution and taxonomy is essential to assess their roles in these fragile and important ecosystems.
- *Methods:* A multigene phylogeny of Verrucariaceae including catapyrenioid genera is presented. We further examined the phylogenetic relationships among members of *Heteroplacidium* and *Placidium*. The evolution of selected characters was inferred using the latter phylogeny.
- *Key results: Anthracocarpon* and *Involucropyrenium* were closely related to *Endocarpon*. *Placidium* comprised two monophyletic clades sister to *Heteroplacidium*. Inferred ancestral states of diagnostic characters revealed that the type of medulla and the pycnidia location were homoplasious within the *Placidium* clade. In contrast, the presence of rhizines was a synapomorphy for *Clavascidium*.
- *Conclusions:* Our results provide new information on the usefulness of characters for delineating groups in Verrucariaceae. Taxonomic changes are proposed to reflect more natural groupings: *Heteroplacidium podolepis* is transferred to *Placidium*, and *Clavascidium* is recognized as a different genus. Eight new combinations are proposed for *Clavascidium*.

Key words: *Anthracocarpon*; *Catapyrenium* s.l.; character evolution; *Clavascidium*; *Involucropyrenium*; medulla; new combinations; pycnidia position; rhizines.

Verrucariaceae is a cosmopolitan family of mostly lichenized species including ca. 55 genera and ca. 930 species (Kirk et al., 2008; Lumbsch and Huhndorf, 2010). Members of this family inhabit an unusually broad range of nutrient-poor habitats such as rock surfaces, but some also colonize soils, trees (bark or leaves), and other lichens or bryophytes (Breuss, 1994, 1996; Döbbeler, 1997; Navarro-Rosinés et al., 2007). Many species are adapted to dry environments and are components of biological soil crusts in semiarid regions (Belnap and Lange, 2001). They play an important role in ecosystem functioning by affecting soil nutrient cycling, stability, and infiltration; by influencing the establishment of vascular plants; and by serving as habitats for a large number of arthropods and microorganisms (Hale, 1983; Belnap and Lange, 2001; Bowker et al., 2011). Therefore, establishing a knowledge base for identifying and classifying these lichens and for elucidating their evolution is of great importance for further ecological studies. However, because of their reduced morphology (e.g., endolithic, epilithic crusts, or parasitic lifestyles), Verrucariaceae are extremely deficient in readily observable, taxonomically useful characters for delimiting genera. Additionally, many of the morphological traits are symplesiomorphic or homoplastic, but clear synapomorphies are rare in the family (Gueidan et al., 2007, 2009; Savić et al., 2008).

Catapyrenioid genera are characterized by a squamulose thallus (i.e., composed of scales, Fig. 1) and have been historically classified within *Catapyrenium* s.l. and *Endocarpon*. However, thallus growth habits in Verrucariaceae have evolved multiple times (Gueidan et al., 2007). The genus *Placidium* was resurrected in an attempt to reflect the variability among the squamulose taxa included in *Catapyrenium* s.l., a group that

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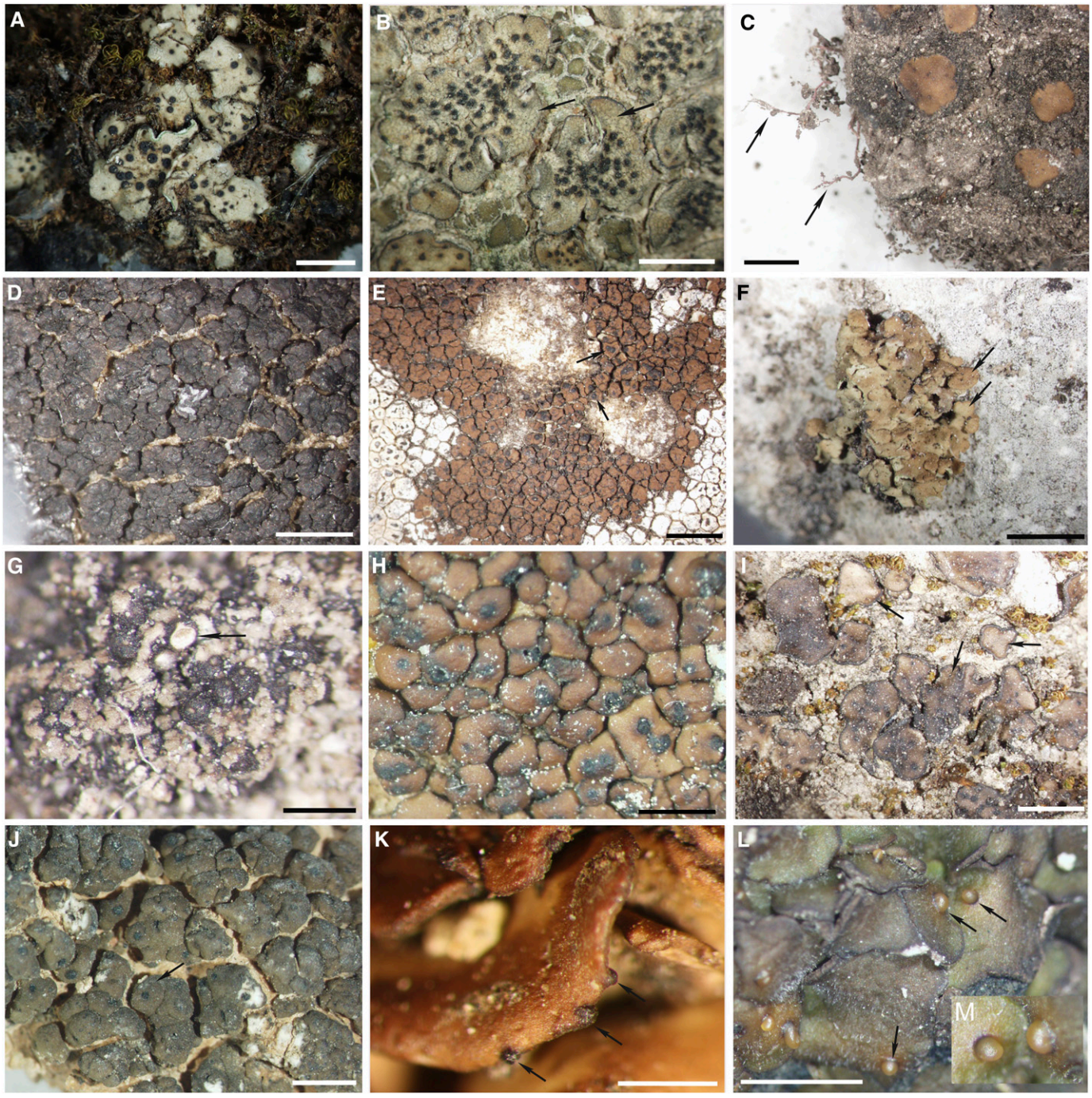

Fig. 1. Members of *Catapyrenium* s.l. are often components of biological soil crusts, and they exert a great influence on ecosystem functioning. Photographs show phenotypic variation in thallus morphology. (A) *Anthracocarpom virescens*. (B) *Clavascidium lacinulatum* var. *atrans* (arrows point to some squamules). (C) *Clavascidium lacinulatum* var. *erythrostratum* with dispersed squamules and rhizines (arrows). (D) *Heteroplacidium congestum*. (E) *Heteroplacidium fusculum*, areolate thallus with perithecia (arrows). (F) *Heteroplacidium imbricatum* with imbricate squamules (arrows) forming cushion-like thallus. (G) *Involucropyrenium pusillum*, cross section of a perithecium (arrow) covered by involucrellum. (H) *Placidium acarosporoides*. (I) *Placidium michelii* with dispersed squamules (arrows). (J) *Placidium podolepis* with small squamules (arrow). (K) *Placidium rufescens*, marginal pycnidia (arrows). (L) *Placidium squamulosum*, laminal pycnidia (arrows). (M) Detail of pycnidia from L. Scale bars A–F, H, I, K = 2 mm; G, J = 1 mm; L = 3 mm. Black dots over the squamules could be either perithecia or pycnidia, but usually they are externally indistinguishable.

Breuss (1996) split into eight genera: *Anthracocarpon* Breuss, *Catapyrenium* Flot. (*Catapyrenium* s.str.), *Clavascidium* Breuss, *Heteroplacidium* Breuss, *Involucropyrenium* Breuss, *Neocatapyrenium* H. Harada, *Placidium* A. Massal., and *Scleropyrenium* H. Harada (Fig. 1). This classification was mainly based on combinations of characters such as pycnidium type (i.e., asexual fruiting bodies; Figs. 1K, 1L, 2D, 2E), ascus shape, ascospore arrangement, thallus upper cortex structure (Fig. 2A–C)

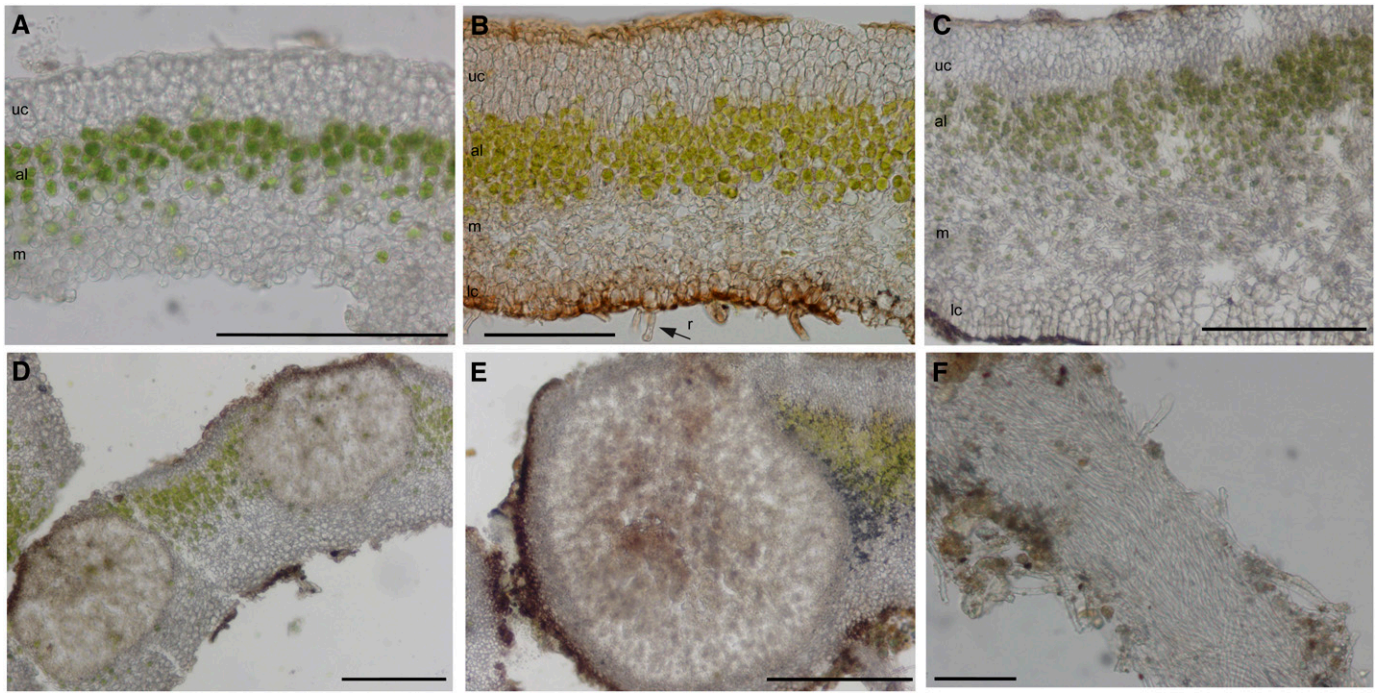

Fig. 2. The phylogenetic distribution of characters traditionally used for delimiting genera and species in *Catapyrenium* s.l. had not, until now, been analyzed among these species. Light micrographs show anatomy of catapyrenioid Verrucariaceae, focusing on the studied characters. Different types of medulla. (A) Paraplechtenchymatous medulla in *Heteroplacidium divisum* (bar = 130 µm). (B). Mixed type medulla in *Placidium squamulosum* (bar = 200 µm). (C) Prosoplechtenchymatous medulla *in Placidium velebiticum* (bar = 200 µm). Pycnidia position. (D) Laminal pycnidia in *Heteroplacidium imbricatum* (bar = 150 µm). (E) Marginal pycnidia in *Placidium semaforonense* (bar = 200 µm). (F) Detail of a rhizine in *Placidium semaforonense* (bar = 150 µm). al = algal layer, lc = lower cortex, m = medulla, r = rhizohyphae, uc = upper cortex.

and anchoring organs (e.g., rhizines or rhizohyphae, Figs. 1C, 2B, 2F), as well as presence/absence of an involucrellum (i.e., tissue surrounding the perithecium wall, Fig. 1G) (Harada, 1993; Breuss, 1996). As for other members of the Verrucariaceae, species of *Catapyrenium* s.l. have perithecia (i.e., subglobose or flask-like fruiting bodies opening by an ostiole) with short pseudoparaphyses and periphyses (i.e., sterile hyphae attached to the inner perithecium wall), bitunicate asci (i.e., with two functional wall layers) and lack interascal filaments (Janex-Favre, 1971). Taxa were included in *Catapyrenium* s.l. based on the following combination of characters: squamulose thallus, simple ascospores, and the absence of algae inside the perithecia (Hawksworth et al., 1980; Wirth, 1980; Clauzade and Roux, 1985; Breuss and Hansen, 1988; Breuss, 1990; Smith et al., 2009). However, these features were shown (Gueidan et al., 2007) to be symplesiomorphic and retained in distantly related lineages within the Verrucariaceae (simple ascospores and the absence of algae inside the perithecia), or to be homoplasious (squamulose thallus).

Recent molecular studies have shown that members of *Catapyrenium* s.l. are distributed in different lineages across the family Verrucariaceae (Gueidan et al., 2007, 2009; Savić et al., 2008; Prieto et al., 2010b). Lineage 4 sensu Gueidan et al. (2007) includes members of *Catapyrenium* s.s. (Prieto et al., 2010b), whereas *Heteroplacidium* (Fig. 1D–F), *Placidium* (including *Clavascidium umbrinum*, Fig. 1B, C, H–L) and one member of *Neocatapyrenium* belong to Lineage 3, together with other members of the Verrucariaceae (Gueidan et al., 2007). The only genus not supported by these studies was *Clavascidium,* which was synonymized with *Placidium* (Gueidan et al., 2009). Under this revised, phylogenetically based classification *Placidium* and *Heteroplacidium* are sister groups.

Placidium and *Heteroplacidium* differ mainly in their thallus anatomy: the thallus of *Heteroplacidium* is almost completely paraplechtenchymatous (Fig. 2A), whereas thallus differentiation is more heterogeneous in *Placidium* (Fig. 2B, C). Moreover, the thallus is crustose-areolate to squamulose for *Heteroplacidium* (Fig. 1D–F), with small squamules or areoles (1–3 mm in diameter), whereas it is strictly squamulose in *Placidium* (Fig. 1H–L), with squamules of (1) 2–8 (15) mm in width. Species delimitation in *Heteroplacidium* and *Placidium* is based on combinations of some of the same characters used at the generic level, such as pycnidia position, medulla type, and thallus-anchoring structure (e.g., rhizines or rhizohyphae, Figs. 1C, 2B, 2F) and, in addition, on differences in thallus thickness, and ascospore size (e.g., Breuss, 2001a, b, 2002; Prieto et al., 2010a).

All these characters can occasionally overlap or be ambiguous at both taxonomic ranks. However, three of these traits (pycnidia position, medulla type, and rhizines presence/absence) are more clearly defined and particularly useful in delimiting species (Figs. 1, 2); therefore, they were one of the focal points of this study. Most genera within *Catapyrenium* s.l. have laminal pycnidia. Only *Placidium* comprises species with marginal or laminal pycnidia (Figs. 1K, 1L, 2E), a very useful character to delimit species in this genus (e.g., Breuss, 2002, 2010; Prieto et al., 2010a). To our knowledge, the position of pycnidia has not previously been used to delimit species in a lichen genus. Although the type of medulla is occasionally difficult to distinguish, it is a very good diagnostic character to delimit genera or

species in *Heteroplacidium* and *Placidium*. Three types of medulla are present among members of *Catapyrenium* s.l. (including *Heteroplacidium* and *Placidium*). The prosoplechtenchymatous medulla is characterized by loosely interlaced hyphae with elongated cells and without or with only isolated globular cells, which then are not noticeable in a microscopic preparation (Fig. 2C). According to Hannemann (1973), other plechtenchymas are derived from this type. The mixed-type medulla ("Mischtyp" sensu Breuss, 1990) refers to a medulla in which globular cells are so numerous that they can be clearly seen in section (Fig. 2B). The paraplechtenchymatous medulla has flattened, very tightly arranged, globular cells with small interhyphal spaces (Fig. 2A). Finally, presence/absence of rhizines (i.e., root-like structures composed of conglutinated hyphae, Figs. 1C, 2F), constitutes a good character to delimit *Placidium* species (e.g., Breuss, 2002, 2010; Prieto et al., 2010a). The distribution of these characters among the taxa within the *Placidium* group sensu Gueidan et al. (2007), i.e., a clade comprising the two genera *Heteroplacidium* and *Placidium*, and their usefulness in delimiting natural groups has not been studied.

The current study has two purposes. The first is to investigate the phylogenetic history of the genus *Placidium* and its relatives, including analysis of the placement of members of *Catapyrenium* s.l. that had not previously been sampled (i.e., *Anthracocarpon* and *Involucropyrenium*). The second is to evaluate the usefulness of the three morphological characters in circumscribing species within *Heteroplacidium* and *Placidium*.

MATERIALS AND METHODS

***Taxon and gene sampling*—**Our first set of analyses was based on the nuLSU rDNA and the RNA polymerase II largest subunit (*RPB*1, regions A–D and D–G), hereafter called the Verrucariaceae Data Set (VDS). For this set of analyses, we included members of the family Verrucariaceae belonging to Lineage 3 sensu Gueidan et al. (2007), and we added new sequences of *Anthracocarpon*, *Heteroplacidium*, *Involucropyrenium* and *Placidium*. In total, the VDS included 83 specimens of Verrucariaceae; 25 of which were sequenced for the first time as part of this study (Appendix 1). Sequences from the remaining 58 specimens were downloaded from GenBank. A second set of analyses was performed using the nuITS, nuLSU, and *RPB*1 (region A–D) sequences from 62 specimens belonging to *Heteroplacidium* and *Placidium* (hereafter, the *Placidium* group data set [PGDS]) (Appendix 1). A total of 128 sequences were generated for this study.

***Morphological study*—**Cross sections (14–16 μm thick) of thalli and perithecia were cut with a freezing microtome or by hand. Sections were observed and measured in water or occasionally were mounted in lactophenol cotton blue. Species identifications were based on general keys (Nimis and Martellos, 2004; Smith et al., 2009), monographs of *Catapyrenium* s.l. (Breuss, 1990, 1993, 1996, 2001b; Prieto et al., 2010a) and publications of individual species descriptions (e.g., Breuss, 1988, 1992, 1998).

***DNA isolation and sequencing*—**DNA isolation, PCR amplification, and PCR product purification and sequencing were performed following the methodology of Prieto et al. (2010b), except for the *RPB1* region A–D. This region was amplified using primers *RPB*1-Af (Stiller and Hall, 1997) and *RPB*1-6-R1asc (Hofstetter et al., 2007). Amplifications were performed in 25-μL volumes containing one microliter of a 1/10 or 1/100 dilution of genomic DNA and the following reaction mixture: 2.50 μL PCR buffer (with 15 μmol/L $MgCl_2$, Abgene, Rochester, New York, USA), 2.50 μL BSA (10 mg/mL), 2.50 μL dNTP (2 mmol/L), 2.00 μL primers (10 mmol/L), 0.15 μL *Taq* polymerase (5U/μl, Denville, NJ) and water to a total volume of 25.00 μL.

Amplifications were carried out in a PTC-200 Peltier thermal cycler (MJ Research, Waltham, Massachusetts, USA) and performed using the following program: initial denaturation of 3 min at 95°C, followed by 35 cycles of the following steps: 95°C for 45 s, 52°C for 90 s, 72°C for 90 s, followed by a final extension at 72°C for 10 min.

***Sequence alignment*—**Each sequence fragment was subjected to BLAST searches for a first verification of their identities. Subsequently, they were assembled and edited using the programs SeqNavigator 1.0.1 (Applied Biosystems) and Sequencher version 4.1 (Genes Codes Corp., Ann Arbor, Michigan, USA). Sequences were aligned manually with the program MacClade 4.01 (Maddison and Maddison, 2001), with the help of amino acid sequences for protein coding loci and, for ribosomal loci, with the help of the secondary structure of the nuLSU from *Saccharomyces cerevisiae* (Cannone et al., 2002) following the method described in Kjer (1995). Ambiguous regions (sensu Lutzoni et al., 2000) and introns were delimited manually and excluded from the phylogenetic analyses.

***Phylogenetic analyses*—**The combinability of the data sets was assessed by comparing well-supported clades among single-gene trees (Mason-Gamer and Kellogg, 1996). Each locus was subjected to a bootstrap maximum likelihood (ML) analysis involving 1000 pseudoreplicates using the program GARLI v. 0.951 (Zwickl, 2006) and a GTRMIX model. Because no conflict was detected using a 75% bootstrap value threshold, where a monophyletic group would be supported with bootstrap values ≥75% with one locus and the same group of taxa would be supported (≥75%) as nonmonophyletic with another locus, it was assumed that loci were congruent and could be concatenated. The concatenated data set was subjected to the same ML analysis as described, except that a search was done on the original concatenated data set and bootstrap values were reported on the most likely tree derived from the analysis of the original concatenated data set.

The evolutionary models for Bayesian analyses were selected using the Akaike information criterion (AIC) as implemented in the program MrModeltest 2.2 (Nylander, 2004). The GTR+I+G model was used for all partitions (nuLSU, nuITS, *RPB1* first, second and third codon positions). Data sets were analyzed using the program MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). Two analyses of four chains were run for 2 million generations starting from an initial random tree, and trees were sampled every 100 generations. To determine whether the chains had converged, verify that mixing was appropriate, and choose a suitable burn-in, we plotted the log-likelihood values against the generation time. We assumed stationarity of the chains when log-likelihood values reached the same stable equilibrium value for each independent run (Huelsenbeck and Ronquist, 2001). We also tested convergence with the AWTY option (Nylander et al., 2008). A burn-in sample of 750 and 500 trees for VDS and PGDS, respectively, were discarded for each run. The remaining trees (pooled from both independent runs) were used to estimate branch lengths with the SUMT command of MrBayes, and posterior probabilities (PPs) were calculated with the majority rule consensus tree command in the program PAUP* v. 4.0b10 (Swofford, 2002).

***Character evolution*—**We inferred ancestral states and traced the evolution of medulla type, position of pycnidia, and presence of rhizines for the *Heteroplacidium-Placidium* group (PGDS) using two methodologies and the last 8000 trees that resulted from the first run from the Bayesian analysis of the concatenated data set. Bayesian reconstruction was performed using the program SIMMAP v.1 Beta 2.3 (Bollback, 2006). We used the option MULTIPLE MAPPING with the number of repetitions set to 100 over three different morphological priors to test the influence on the results. Maximum likelihood ancestral state reconstruction was performed with the program Mesquite 2.74 (Maddison and Maddison, 2010) with the ML model Mk1. An asymmetry likelihood ratio test conducted in Mesquite indicated that the fit of the asymmetrical model was not significantly better than the single rate (Mk1) model, so the latter was applied.

RESULTS

***Verrucariaceae data set (VDS)*—**The combined data matrix included 4245 characters (1276 for nuLSU and 2969 for *RPB1*) after exclusion of ambiguous regions and introns. We included both regions of *RPB1* (A–D and D–G) although our newly obtained sequences were incomplete and only comprised the region A–D. Missing nucleotides represented ca. 30% of the data set. The best tree resulting from the ML search is presented in Fig. 3.

Our results confirm those previously obtained by Gueidan et al. (2007) and Savić et al. (2008). The monophyly of the

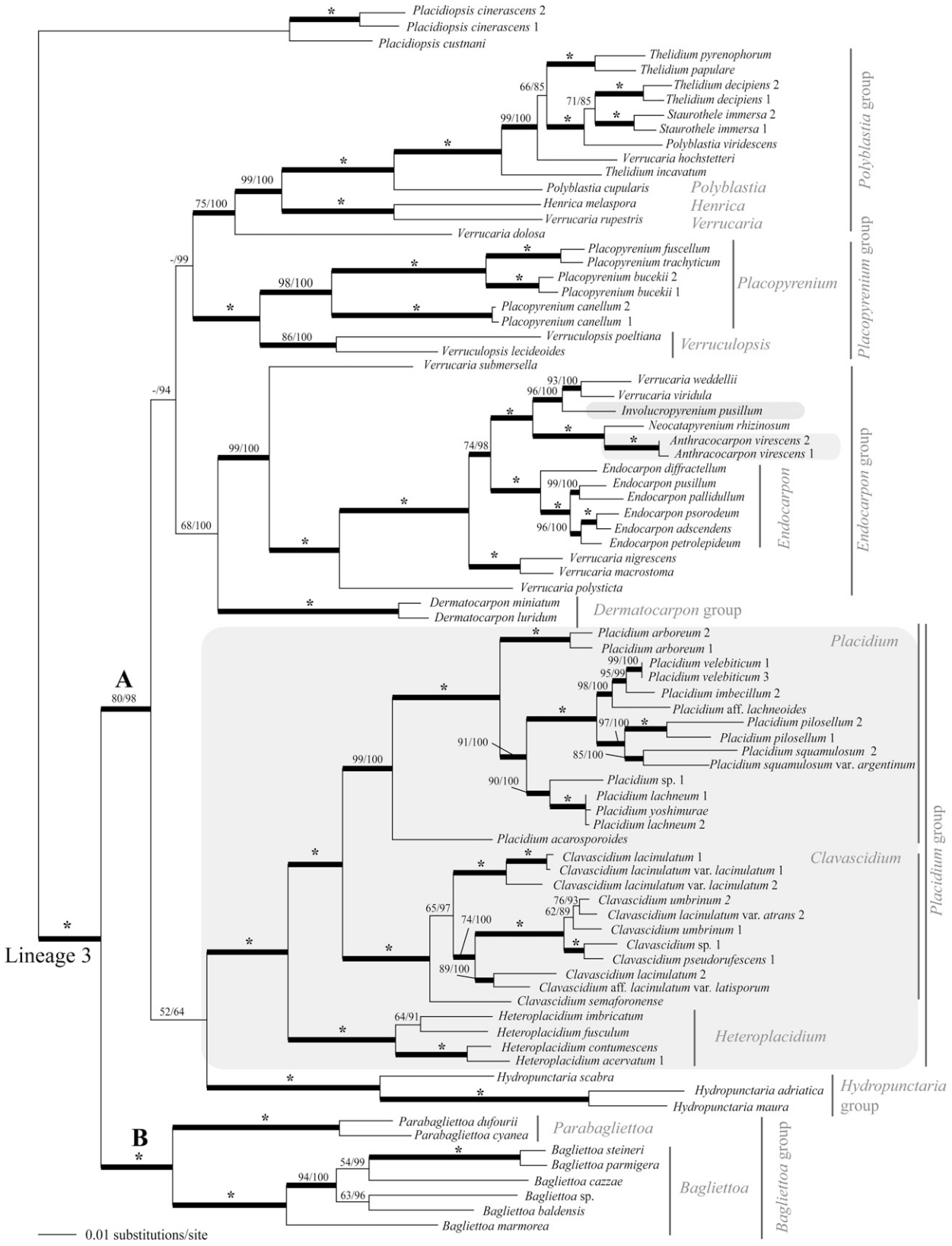

Fig. 3. Phylogenetic relationships of catapyrenioid species within the *Verrucariaceae* based on maximum likelihood analysis of nuLSU and *RPB1* from 83 representative specimens (VDS). Support values are ordered as ML BS/PP. Internodes with ML BS ≥70% and PP ≥95% are highlighted with thicker lines. An asterisk over a bold branch indicates that this internode has a BS value and PP of 100%. Members of *Catapyrenium* s.l. included in this study are highlighted with gray boxes. The suprageneric group system mainly follows Gueidan et al. (2007).

Dermatocarpon, *Endocarpon*, *Placidium*, *Placopyrenium*, *Polyblastia*, and *Hydropunctaria* groups together is for the first time well supported (80% ML BS and 98% PP, clade A in Fig. 3). The sister relationship of this clade with the *Bagliettoa* group confirms the results of Gueidan et al. (2007) (100% both ML BS and PP, clade B, Fig. 3). Groups within clade A are highly supported in our analyses, but relationships among them are not (Fig. 3); i.e., the sister relationship of the *Polyblastia* and *Placopyrenium* groups was only supported by Bayesian analysis, and the monophyly of the *Endocarpon* and *Dermatocarpon* groups was supported in Bayesian analysis but received 68% support in likelihood analyses. Relationships within the *Polyblastia* group do not contradict results from Savić et al. (2008).

The members of *Anthracocarpon* and *Involucropyrenium,* included here in a phylogenetic study for the first time, were nested in the *Endocarpon* group, with high support values (100% both ML BS and PP). Both genera belong to a clade that contains *Neocatapyrenium rhizinosum* and two *Verrucaria* species (*V. viridula* and *V. weddellii*). Although only one member of *Involucropyrenium* was included in this study, due to the lack of *RPB1* sequences for the combined matrix, a preliminary analysis based on nuLSU sequences showed that the genus is nested polyphyletically within the *Endocarpon* group (M. Prieto et al., unpublished data).

The sister relationship of *Heteroplacidium* and *Placidium* sensu Gueidan et al. (2009) is highly supported (100% both ML BS and PP). Moreover, the first split within the latter, corresponding to nonrhizinate and rhizinate *Placidium* species, is highly supported (Figs. 3, 4). Because both clades are monophyletic and can be easily differentiated by the presence of rhizines, we propose that *Clavascidium* (synonymized as *Placidium* by Gueidan et al., 2009) be adopted as a valid generic name for species of *Placidium* with rhizines (see Taxonomic section).

Placidium group data set (PGDS)— The combined data matrix included 317 nuITS, 1118 nuLSU and 1115 *RPB1* region A–D characters after exclusion of ambiguous regions and introns. In total, 2550 unambiguously aligned sites were included for the ML and Bayesian analyses. The majority rule consensus tree of 39000 sampled trees with the Bayesian analysis was similar to the ML topology, and no conflict was detected. The best tree from the ML analysis is presented in Fig. 4 with branch lengths and support values.

Clade B is strongly supported by both analyses (Fig. 4) and contains members of *Heteroplacidium* s.s. All relationships within this group are well resolved and supported. Within the *Placidium* clade sensu Gueidan et al. (2009) (clade A), two highly supported clades correspond to species with and without rhizines (clades D and C, respectively). The nonrhizinate clade contains *P. michelii* (type of *Placidium*) and a basal grade, which includes *Placidium acarosporoides* and *Heteroplacidium podolepis*. Clade D contains members of *Placidium* with rhizines, which are combined here as members of *Clavascidium*. The occurrence of selected anatomical and morphological characters widely used to distinguish species of *Clavascidium*, *Heteroplacidium*, and *Placidium* are shown in Fig. 4.

Character evolution— Ancestral character states were inferred for 10 well-supported nodes within the *Heteroplacidium-Clavascidium-Placidium* clade (Table 1, Fig. 4). The results from the two methodologies support a single origin of rhizines in the ancestral lineage prior to the diversification of extant *Clavascidium* species forming clade D. The type of medulla could not be reconstructed unequivocally for the ancestor of the *Heteroplacidium-Clavascidium-Placidium* group or for the ancestor of *Placidium* and *Clavascidium*. The *Heteroplacidium* ancestor most likely had a paraplechtenchymatous medulla. The first unequivocal origin of a prosoplechtenchymatous medulla seems to have occurred during the early evolution of lineage E. The ancestor of clade K was reconstructed as having a mixed medulla. Pycnidia position was reconstructed as laminal for the common ancestor of the three genera with a switch from laminal to marginal pycnidia during the early evolution of lineage E (Table 1, Fig. 4). The ancestor of *Clavascidium* (clade D) is also reconstructed as having laminal pycnidia, and only one switch from laminal to marginal pycnidia is observed in this clade (Fig. 4).

Taxonomy—*Clavascidium lacinulatum* (Ach.) M. Prieto *comb. nov.* Basionym: *Endocarpon hepaticum* var. *lacinulatum* Ach., Lich. Univ.: 299 (1810)
MycoBank no.: MB 563527

Clavascidium lacinulatum var. *atrans* (Breuss) M. Prieto *comb. nov.* Basionym: *Placidium lacinulatum* var. *atrans* Breuss, in Lendemer, Opusc. Philolichenum 1: 54 (2004)
MycoBank no.: MB 563528

Clavascidium lacinulatum var. *erythrostratum* (Breuss) M. Prieto *comb. nov.* Basionym: *Placidium lacinulatum* var. *erythrostratum* Breuss, Bryologist 103(4): 705 (2000)
MycoBank no.: MB 563529

Clavascidium lacinulatum var. *latisporum* (Breuss) M. Prieto *comb. nov.* Basionym: *Catapyrenium lacinulatum* var. *latisporum* Breuss, Stapfia 23: 94 (1990)
MycoBank no.: MB 563530

Clavascidium semaforonense (Breuss) M. Prieto *comb. nov.* Basionym: *Catapyrenium semaforonense* Breuss, Stapfia 23: 112 (1990)
MycoBank no.: MB 563531

Clavascidium pseudorufescens (Breuss) M. Prieto *comb. nov.* Basionym: *Placidium pseudorufescens* Breuss, in Nash et al., Lich. Fl. Gr. Sonoran Desert Region 1: 391 (2002)
MycoBank no.: MB 563532

Clavascidium imitans (Breuss) M. Prieto *comb. nov.* Basionym: *Catapyrenium imitans* Breuss, Linzer Biol. Beitr. 24(2): 813 (1992)
MycoBank no.: MB 563533

Clavascidium krylovianum (Tomin) M. Prieto *comb. nov.* Basionym: *Dermatocarpon krylovianum* Tomin, *Trudy Tomsk. Gos. Univ.* 116: 149 (1951)
MycoBank no. MB 563534

Placidium podolepis (Breuss) M. Prieto *comb. nov.* Basionym: *Catapyrenium podolepis* Breuss, Pl. Syst. Evol. 185: 29 (1993)
Synonym: *Heteroplacidium podolepis* (Breuss) Breuss, Ann. Naturhist. Mus. Wien, Ser. B, Bot. Zool. 98 (Suppl.): 40 (1996)

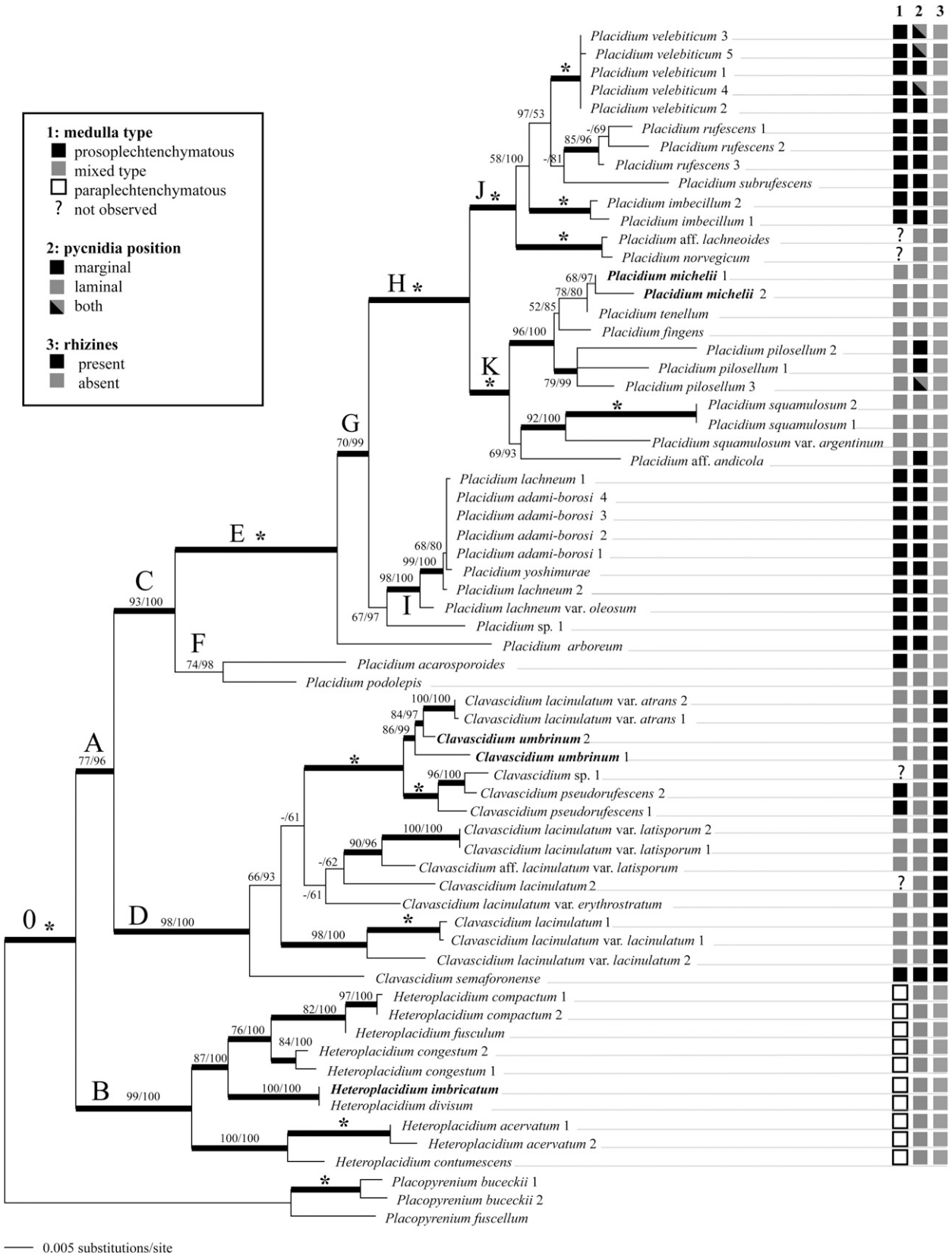

Fig. 4. Phylogenetic relationships among members of the *Placidium* group, based on maximum likelihood analysis of concatenated nuITS, nuLSU, and *RPB1* data sets (PGDS). Support values are ordered as ML BS/PP. Internodes with ML BS ≥70% and PP ≥95% are highlighted with thicker lines. An asterisk over a bold branch indicates that this internode has a BS value and PP of 100%. Type species of a genus are in bold.

TABLE 1. Ancestral state probabilities for three diagnostic characters: (A) medulla type, (B) pycnidia position, and (C) presence/absence of rhizines at 10 nodes shown in Fig. 4. Results are presented for the two methods used: Bayesian method (analyzed with SIMMAP) and maximum likelihood (analyzed with Mesquite). When no unequivocal states were reconstructed a dash (—) is reported.

A) Medulla type

	Bayesian (SIMMAP)			ML (Mesquite)		
Node	Para	Proso	Mixed type	Para	Proso	Mixed type
0	0.2360	0.5370	0.2271	—	—	—
A	0.0024	0.7921	0.2055	0.1487	0.4669	0.3843
B	**0.9991**	0.0006	0.0003	**1.0000**	0.0000	0.0000
C	0.0002	0.9386	0.0611	0.0520	0.5949	0.3530
D	0.0012	0.4022	0.5966	0.0186	0.2744	0.7068
E	0.0000	**0.9998**	0.0001	0.0000	**1.0000**	0.0000
G	0.0000	**1.0000**	0.0000	0.0002	**0.9980**	0.0016
H	0.0005	0.9463	0.0532	0.0018	**0.9505**	0.0476
J	0.0002	**0.9929**	0.0069	0.0001	**0.9985**	0.0013
K	0.0000	0.0001	**0.9999**	0.0003	0.0080	**0.9916**

B) Pycnidia position

	Bayesian (SIMMAP)			ML (Mesquite)		
Node	Laminal	Marginal	Both	Laminal	Marginal	Both
0	**0.9937**	0.0050	0.0008	**0.9844**	0.0000	0.0000
A	**0.9981**	0.0018	0.0001	**0.9800**	0.0000	0.0000
B	**0.9999**	0.0000	0.0000	**1.0000**	0.0000	0.0000
C	**0.9642**	0.0352	0.0006	0.9116	0.0000	0.0000
D	**0.9540**	0.0452	0.0008	0.9200	0.0649	0.0150
E	0.0042	**0.9955**	0.0003	0.0000	**0.9813**	0.0000
G	0.0001	**0.9999**	0.0000	0.0000	**0.9986**	0.0000
H	0.1386	0.8611	0.0003	0.2278	0.7572	0.0086
J	0.2879	0.7115	0.0060	0.2001	0.7797	0.0200
K	0.8444	0.1555	0.0000	0.5154	0.4759	0.0086

(C) Rhizines

	Bayesian (SIMMAP)		ML (Mesquite)	
Node	Absent	Present	Absent	Present
0	**0.9798**	0.0202	**0.9936**	0.0000
A	**0.9675**	0.0325	**1.0000**	0.0000
B	**0.9999**	0.0001	**1.0000**	0.0000
C	**0.9989**	0.0011	**1.0000**	0.0000
D	0.0010	**0.9990**	0.0000	**1.0000**
E	**0.9999**	0.0001	**1.0000**	0.0000
G	**1.0000**	0.0000	**1.0000**	0.0000
H	**1.0000**	0.0000	**1.0000**	0.0000
J	**1.0000**	0.0000	**1.0000**	0.0000
K	**1.0000**	0.0000	**1.0000**	0.0000

Notes: Para: paraplechtenchymatous, Proso: prosoplechtenchymatous

MycoBank no.: MB 563535

Type: Argentina, prov. La Rioja, Westfuss der Sierra de Famatina, SW-Fuss des cerro Cachiyuyo, saisontrockenes Flusstal 9 km N Villa Castellí, 1500 m, 09/02/1990, W. & S. Till, LI! 297365.

Remarks: This species (Fig. 1J), combined into *Heteroplacidium* by Breuss in 1996, is here transferred to the genus *Placidium* based on molecular and morphological data. Although it is similar to members of *Heteroplacidium*, its medulla is not fully paraplechtenchymatous, but instead corresponds to a mixed-type medulla. All studied specimens of *P. podolepis* had clavate asci.

Specimens examined: Yemen, Socotra, Prov. Aden. Wadi Ayeb Valleyinland, SW of Hadibu and S of Mt. Asher, on rocks, 80 m, B. Mies & C. Printzen, 15/01/1994, LI 12927. Yemen, Djebel Urays, middle Wadi Asurai, c. 1 km north of the camp, close to step, western wall, on small 45°-inclined boulder near the ground, rel. shaded, c. 250 m, lava, M. Schultz, 17/03/2002, LI 512045. Kuwait, Khiran, G. Brown, 13/02/1997.

DISCUSSION

Phylogenetic position of catapyrenioid genera within Verrucariaceae—Members of the *Endocarpon* group (Fig. 3), including *Anthracocarpon*, *Endocarpon*, *Involucropyrenium*, *Neocatapyrenium*, and a subset of *Verrucaria* species, are characterized by the presence of *Endocarpon*-type pycnidia (Gueidan et al., 2007 and present study). The monophyletic group composed of *Involucropyrenium pusillum*, *Verrucaria*

viridula, and *V. weddellii* (Fig. 3) shares the presence of an involucrellum. However, this feature is not unique to this clade. In other lineages of the *Endocarpon* group, some species have an involucrellum (e.g., *Verrucaria submersella*, *Verrucaria macrostoma*, and *Verrucaria nigrescens*). Preliminary analyses based on nuLSU (including more species from this genus) showed *Involucropyrenium* as polyphyletic, but we prefer to wait for more data before proposing new combinations for *V. weddellii* and *V. viridula*.

Neocatapyrenium rhizinosum and *Anthracocarpon virescens* (type species of *Anthracocarpon*) are similar species, both having carbonaceous rhizines and carbonaceous or black perithecium walls. A broader phylogenetic study that incorporates more members of *Neocatapyrenium* [including the type *N. cladonioideum* (Vain.) Harada] needs to be undertaken due to the high variability observed within the genus (Breuss, 2005; Prieto et al., 2010a).

***Phylogeny and classification of the* Placidium *group*—**The close relationship of *Heteroplacidium* and *Placidium* is nearly in accordance with the traditional (morphologically and anatomically based) classification of Breuss (1996). Although both genera are very closely related, molecular data supports the distinction of both genera as monophyletic entities, and so far, only a few minor taxonomic adjustments are required, including the transfer of *Heteroplacidium podolepis* to the genus *Placidium*.

Clade B contains only members of *Heteroplacidium* (Fig. 4). The recent combination of *H. compactum* (Roux, 2008), a facultative parasite, is supported here, and the species is sister to *H. fusculum* (another parasitic species). Although their recognition as two distinct species has been controversial (see Breuss [1994] and Prieto et al. [2010a] for further details), our results show genetic distances similar to, or larger than, other closely related and well-established species within the *Placidium* group. Both species are closely related to (the saxicolous) *H. congestum*, in a clade together with the type species of this genus (*H. imbricatum*) and its close relative *H. divisum*. The close relationship between these two genera that was suggested by previously observed morphological similarities between *H. acervatum* and *H. contumescens* (Prieto et al., 2010a) is confirmed by the present study.

Two distinct sister groups can be distinguished within the *Placidium* clade sensu Gueidan et al. (2009). Clade D is composed of members of *Clavascidium* characterized by the presence of rhizines and includes species with clavate to (sub) cylindrical asci (Fig. 4). The latter character was traditionally used for separating *Placidium* from the rest of genera included in *Catapyrenium* s.l., especially to distinguish *Clavascidium* from *Placidium* (Breuss, 1996). However, both ascus types are present in the current delimitation of *Placidium*, although species with clavate asci are mainly found within *Clavascidium*. This group includes the type species of *Clavascidium* (*C. umbrinum*) and members of the four varieties described for *Placidium lacinulatum* (Breuss, 2002; Lendemer, 2004), here transferred to *Clavascidium*. Although the classification at the species level does not agree fully with the resulting monophyletic groups, our results support a clade including specimens with black rhizines and dark exciples (*C. lacinulatum* var. *atrans*, *C. umbrinum, C. pseudorufescens*, and *Clavascidium* sp.). The variability of *Clavascidium lacinulatum* and the varieties included in the mentioned species has been previously discussed (Breuss, 2002) in relation with the existence of intermediate forms of the different varieties. Although this clade is not fully resolved, the recognition of varieties of *C. lacinulatum* as distinct species could be one step toward reconciling the taxonomy of this group with its phylogenetic history. *Clavascidium semaforonense*, the only species of the genus with marginal pycnidia is distantly related to the rest of species with laminal pycnidia.

Clade C (Fig. 4) is composed of species lacking rhizines, including the type species of *Placidium* (*P. michelii*). In a first revision by Breuss (1996), *Placidium acarosporoides* was transferred to *Heteroplacidium* but transferred back to *Placidium* (Breuss, 2002) because of its complex thallus anatomy and cylindrical asci in early stages of its development. Its sister species, *Heteroplacidium podolepis* is here transferred to the genus *Placidium,* although asci in this species are clavate. Therefore, both types of asci are found in this lineage that evolved from the first diversification within *Placidium* (clade C). Both species have relatively small squamules, with an areolate appearance of the thallus (Fig. 1H, J) and rhizohyphae confined to central parts of the squamules, giving an appearance of central peduncles. These characteristics appear to be intermediate phenotypes between *Heteroplacidium* and *Placidium*, explaining the difficulty of assigning these two species to one of these two genera. However, having a completely paraplechtenchymatous thallus is diagnostic for *Heteroplacidium*.

Clade E includes species with cylindrical asci, at least at an early developmental stage. *Placidium arboreum*, a corticolous species, is sister to the remaining members of clade E. Clade I is characterized by the presence of a prosoplechtenchymatous medulla and a lower cortex composed of anticlinally arranged cells and includes *Placidium adami-borosi* and the closely related *P. lachneum* and *P. yoshimurae*. Differences between *P. adami-borosi* and *P. lachneum* are scarce and based mainly on the size of the squamules and the ecology (Breuss, 1990). Moreover, *P. lachneum* var. *oleosum* seems to be part of a genetically distinct lineage from the rest of individuals included in clade I, which comprises the type variety of *P. lachneum* and *P. adami-borosi*. The lack of specimens representing these varieties prevented us from revising the taxonomy within this clade, which will be the subject of a forthcoming study. *Placidium* sp. 1, a species similar to *P. lachneum* but with broader ascospores and thinner rhizohyphae, is sister to clade I, although this relationship is not fully supported.

The first divergence within group H encompasses group J, with all members having a prosoplechtenchymatous medulla, and group K (which includes the type species of *Placidium, P. michelii*), with species sharing a mixed-type medulla. The position of pycnidia on the thallus is not as well segregated within group H, and some species are polymorphic. Our results support the recognition of *P. pyrenaicum* as *P. velebiticum* (Fig. 4) as proposed by Prieto et al. (2010a). No clear anatomical differences could be found between these two species, except for the position of their pycnidia. However, this trait was sometimes found to be polymorphic within a single individual and even on the same squamule.

Ancestral state reconstruction of anatomical characters—*Medulla type*—This character is particularly important for species delimitation within the genera *Clavascidium* and *Placidium* (e.g., Breuss, 1990, 1996, 2002), as well as to distinguish these two genera from *Heteroplacidium* (Breuss, 1996). However, we cannot infer which type of medulla was present in the common ancestor of *Heteroplacidium, Clavascidium*, and *Placidium*. The ancestor of *Heteroplacidium* is reconstructed as having a paraplechtenchymatous medulla, thus confirming the

taxonomical value of this type of medulla. The ancestor of *Clavascidium* and *Placidium* was not reconstructed unequivocally for this character (Table 1). However, both analyses support an early origin of the prosoplechtenchymatous medulla within the genus *Placidium* (clade E). In clade H, the two main types of medulla seem to be fully segregated, with a prosoplechtenchymatous medulla for clade J, and a mixed type medulla for clade K derived from a common ancestor with the prosophechtenchymatous type. Although the type of medulla is here shown to be quite labile within *Clavascidium* and *Placidium*, the paraplechtenchymatous medulla seems fixed within *Heteroplacidium.* The prosoplechtenchymatous medulla was suggested by Hannemann (1973) as being the most ancestral from which the rest of plechtenchymas evolved. Although we cannot support or reject this hypothesis, our results showed a trend for ancestors with prosoplechtymatous medulla in the *Placidium* clade.

The importance of thallus morphology for water relations was documented by various authors (Larson, 1981; Rundel, 1982). Specifically, Sancho and Kappen (1989) demonstrated that the structure of the medulla was a key factor in water saturation and loss rate. However, the type of medulla correlates with other anatomical characteristics. How combinations of these characters might be adaptively advantageous needs to be studied.

Pycnidia position—Several interesting observations can be made with regard to pycnidia position within the *Placidium* clade: *Clavascidium* (clade D) only contains one taxon with marginal pycnidia, so that marginal pycnidia are rare in this group. Specimens with marginal pycnidia are common in the *Placidium* clade (C). In general, species with a prosoplechtenchymatous medulla are mostly characterized by marginal pycnidia. Likewise, species with mixed medulla have mostly laminal pycnidia. Therefore, it is possible that the evolution of these two traits is correlated.

Pycnidia position has not been evaluated from an evolutionary point of view before this study. Differences in their position on thalli have only been observed in *Clavascidium* and *Placidium*, and they are always laminal in the rest of members of Verrucariaceae. This character is helpful to delimit species within both genera, although it is homoplasious.

Rhizines presence/absence—The presence of rhizines clearly constitutes a synapomorphy for *Clavascidium* (clade D) supporting its acceptance as a separate genus from *Placidium*. In Verrucariaceae, a great variability in attachment organs are present, e.g., stipe-like holdfasts, rhizohyphal wefts or umbilicus, among others. These character states seem to be fixed in some genera within Verrucariaceae and can be used as taxonomical characters to define some groups (e.g., umbilicus in *Dermatocarpon* or constricted bases, stipes or rhizine-like structures in *Placopyrenium*) but can be variable within other groups (e.g., *Endocarpon* or *Verrucaria*). According to this, the evolutionary value of this trait should be analyzed within each group individually.

Recent molecular studies have shown that many conventionally used morphological characters were unreliable for defining natural groupings in Verrucariaceae (Gueidan et al., 2007, 2009; Savić et al., 2008; Muggia et al., 2010; Prieto et al., 2010b). Additional characters are now needed to refine and clarify, when possible, the generic delimitation within Verrucariaceae. Our present study contributes to this endeavor, bringing a new insight on the taxonomy of the squamulose taxa and the evolution of morphological characters used to circumscribe them. However, additional investigations need to be undertaken to propose a more appropriate generic classification in Verrucariaceae, e.g., by studying the *Endocarpon* or the *Staurothele* groups sensu Gueidan et al. (2009), which still deserve a thorough molecular phylogenetic revision, by widening the taxon sampling or by incorporating more members of the polyphyletic *Verrucaria* in the phylogenies.

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APPENDIX 1. Specimens used for this study with GenBank accession numbers. Entries for newly obtained sequences are in boldface. Specimen data are given for newly produced sequences with country of origin, collection number and location of voucher. Names followed by an asterisk are the result of recent taxonomic changes (Prieto et al. 2010a and this study). Data set 1 corresponds to the Verrucariaceae data set, and 2 corresponds to the *Placidium* group data set. Herbaria acronyms refer to University of Arizona, USA (ARIZ); University of Colorado Museum, USA (COLO); Duke University, USA (DUKE); Oberösterreichischen Landesmuseums Linz, Austria (LI) and Real Jardín Botánico, Spain (MA).

Species	Collection number	GenBank Accession number			Data set	Origin
		nuITS	nuLSU	*RPB1*		
***Anthracocarpon virescens* (Zahlbr.) Breuss 1**	**M. Prieto 530 (MA)**	**—**	**GU228948**	**GU229015**	**1**	**Spain**
***A. virescens* (Zahlbr.) Breuss 2**	**M. Prieto 531 (MA)**	**—**	**GU228946**	**GU229010**	**1**	**Spain**
Bagliettoa baldensis (A. Massal.) Vězda	—	—	EF643786	EF689744	1	—
B. cazzae (Zahlbr.) Vězda & Poelt	—	—	EF643789	EF689745	1	—
B. marmorea (Scop.) Gueidan & Cl. Roux	—	—	EF643800	EF689802	1	—
B. parmigera (Steiner) Vězda & Poelt	—	—	EF643805	EF689746	1	—
B. sp. [*Verrucaria calciseda* auct. pp]	—	—	EF643788	EF689790	1	—
B. steineri (Kusan) Vězda	—	—	EF643809	EF689809	1	—
***Clavascidium* aff. *lacinulatum* var. *latisporum** (Breuss) M. Prieto**	**M. Prieto 1704 (MA)**	**GU228959**	**GU228911**	**GU229020**	**1, 2**	**Mexico**
*C. lacinulatum** (Ach.) M. Prieto 1	—	EF469155	EF469158	EF689765	1, 2	—
*C. lacinulatum** (Ach.) M. Prieto 2	—	—	EF643762	EF689766	1, 2	—
***C. lacinulatum* var. *atrans** (Breuss) M. Prieto 1**	**M. Prieto 1702 (MA)**	**GU228958**	**GU228912**	**—**	**2**	**Mexico**
***C. lacinulatum* var. *atrans** (Breuss) M. Prieto 2**	**M. Prieto 1703 (MA)**	**GU228957**	**GU228910**	**GU229019**	**1, 2**	**Mexico**
***C. lacinulatum* var. *erythrostratum** (Breuss) M. Prieto**	**ARIZ 551162**	**GU228965**	**—**	**—**	**2**	**USA**
***C. lacinulatum* var. *lacinulatum** (Ach.) M. Prieto 1**	**M. Prieto 552 (MA)**	**GU228960**	**GU228913**	**GU229003**	**1, 2**	**Spain**
***C. lacinulatum* var. *lacinulatum** (Ach.) M. Prieto 2**	**ARIZ 551097**	**GU228964**	**GU228923**	**GU229011**	**1, 2**	**USA**
***C. lacinulatum* var. *latisporum** (Breuss) M. Prieto 1**	**ARIZ 551314**	**—**	**GU228934**	**—**	**2**	**Mexico**
***C. lacinulatum* var. *latisporum** ((Breuss) M. Prieto 2**	**ARIZ 551304**	**—**	**GU228933**	**—**	**2**	**Mexico**
***C. semaforonense** (Breuss) M. Prieto**	**M. Prieto 63 (MA)**	**GU228961**	**GU228930**	**GU229002**	**1, 2**	**Spain**
C. sp. 1	**—**	EF469156	EF469159	EF689750	1, 2	—
***C. pseudorufescens** (Breuss) M. Prieto 1**	**LI 566404 (MA)**	**GU228966**	**GQ344563**	**GU229001**	**1, 2**	**USA**
***C. pseudorufescens** (Breuss) M. Prieto 2**	**LI 127885 (MA)**	**GU228963**	**GU228945**	**—**	**2**	**France**
C. umbrinum (Breuss) M. Prieto 1	**LI 350628**	**GU228962**	**GU228924**	**GU229021**	**1, 2**	**Mexico**
C. umbrinum (Breuss) M. Prieto 2	**—**	—	EF643749	EF689749	1,2	—
Dermatocarpon luridum (With.) J. R. Laundon	—	—	EF643750	EF689751	1	—
D. miniatum (L.) Mann	—	—	EF469160	EF689752	1	—
Endocarpon adscendens (Anzi) Müll. Arg.	—	—	EF643751	EF689753	1	—
E. diffractellum (Nyl.) Gueidan & Roux	—	—	EF643773	EF689776	1	—
E. pallidulum (Nyl.) Nyl.	—	—	DQ823097	DQ840552	1	—
E. petrolepideum (Nyl.) Nyl.	—	—	EF643752	EF689754	1	—
E. psorodeum (Nyl.) Th. Fr.	—	—	EF643753	EF689755	1	—
E. pusillum Hedw.	—	—	EF643754	EF689756	1	—
Henrica melaspora (Taylor) Savić & Tibell	—	—	148628047	149794932	1	—
***Heteroplacidium acervatum* (Breuss) Breuss 1**	**LI 271015**	**GU228954**	**GQ344564**	**GU229000**	**1, 2**	**Spain**
***H. acervatum* (Breuss) Breuss 2**	**M. Prieto 399 (MA)**	**GU228955**	**GU228932**	**—**	**2**	**Spain**
H. compactum **(A. Massal.) Gueidan & Cl. Roux 1**	**M. Prieto 1607 (MA)**	**GU228949**	**GU228916**	—	**2**	**Spain**
H. compactum **(A. Massal.) Gueidan & Cl. Roux 2**	**M. Prieto 1701 (MA)**	**GU228952**	**GU228918**	—	**2**	**Spain**
H. congestum **(Breuss & McCune) Breuss 1**	**LI 552268**	**GU228950**	**GU228920**	—	**2**	**USA**
H. congestum **(Breuss & McCune) Breuss 2**	**LI 297536**	**GU228951**	**GU228919**	—	**2**	**USA**
H. contumescens (Nyl.) Breuss	—	—	EF643755	EF689757	1, 2	—
H. divisum **(Zahlbr.) Breuss**	**LI 218428**	**GU228953**	**GU228915**	—	**2**	**Italy**
H. fusculum (Nyl.) Gueidan & Cl. Roux	—	—	EF643793	EF689794	1, 2	—
H. imbricatum (Nyl.) Breuss	—	—	EF643756	EF689758	1, 2	—
Hydropunctaria adriatica (Zahlbr.) C. Keller & Gueidan	—	—	EF643783	EF689786	1	—
H. maura (Wahlenb.) C. Keller	—	—	EF643801	EF689803	1	—
H. scabra (Vězda) C. Keller	—	—	EF643808	EF689808	1	—
Involucropyrenium pusillum **Breuss & Türk**	**M. Prieto 1269 (MA)**	—	**GU228947**	**GU229016**	**1**	**Portugal**
Neocatapyrenium rhizinosum (Müll. Arg.) Breuss	—	—	EF643757	EF689759	1	—
Parabagliettoa cyanea (A. Massal.) Gueidan & Cl. Roux	—	—	EF643790	EF689791	1	—
P. dufourii (DC.) Gueidan & Cl. Roux	—	—	EF643792	EF689793	1	—
Placidiopsis cinerascens **(Nyl.) Breuss 1**	**MA 16308**	—	**GQ344570**	**GU228999**	**1**	**Spain**
P. cinerascens **(Nyl.) Breuss 2**	**MA 16315**	—	**GQ344572**	**GU228998**	**1**	**Spain**
P. custnani (A. Massal.) Körb.	—	—	EF643758	EF689760	1	—
Placidium acarosporoides (Zahlbr.) Breuss	—	—	EF643760	EF689762	1, 2	—
P. adami-borosi **Szatala 1**	**LI 297343**	**GU228988**	**GU228944**	—	**2**	**France**
P. adami-borosi **Szatala 2**	**LI 285596**	**GU228987**	**GU228943**	—	**2**	**Italy**
P. adami-borosi **Szatala 3**	**LI 285591**	**GU228986**	**GU228942**	—	**2**	**Italy**
P. adami-borosi **Szatala 4**	**Aptroot S/N**	**GU228985**	**GU228936**	—	**2**	**Italy**
P. **aff.** *andicola* **(Breuss) Breuss**	**LI 512226**	**GU228983**	**GU228941**	**-**	**2**	**Chile**
P. **aff.** *lachneoides* **(Breuss) Breuss**	**M. Prieto 564 (MA)**	**GU228972**	**GU228906**	**GU229005**	**1, 2**	**Spain**
P. arboreum (Schwein. ex E. Michener) Lendemer 1	—	—	EF643765	EF689767	1	—
P. arboreum **(Schwein. ex E. Michener) Lendemer 2**	**COLO 479327**	**GU228995**	—	**GU229006**	**1, 2**	**USA**
P. fingens **(Breuss) Breuss**	**LI 271006**	**GU228989**	**GU228935**	—	**2**	**Spain**

APPENDIX 1. Continued.

Species	Collection number	GenBank Accession number			Data set	Origin
		nuITS	nuLSU	*RPB1*		
P. imbecillum **(Breuss) Breuss 1**	**M. Prieto 664 (MA)**	**GU228980**	—	—	**2**	**Spain**
P. imbecillum **(Breuss) Breuss 2**	**LI 364261**	**GU228979**	**GU228940**	**GU229012**	**1, 2**	**Austria**
P. lachneum **(Ach.) B. de Lesd. 1**	**M. Prieto 322 (MA)**	**GU228982**	**GU228926**	**GU229018**	**1, 2**	**Spain**
P. lachneum (Ach.) B. de Lesd. 2	—	—	EF643761	EF689764	1, 2	—
P. lachneum **var.** *oleosum* **(Breuss) Breuss**	**M. Prieto 105 (MA)**	**GU228981**	**GU228929**	—	**2**	**Spain**
P. michelii **A. Massal. 1**	**M. Prieto 702 (MA)**	**GU228992**	**GU228908**	—	**2**	**Spain**
P. michelii **A. Massal. 2**	**M. Prieto 1356 (MA)**	—	**GU228909**	—	**2**	**Spain**
P. norvegicum **(Breuss) Breuss**	**M. Prieto 330 (MA)**	**GU228973**	**GU228904**	—	**2**	**Spain**
P. pilosellum **(Breuss) Breuss 1**	**M. Prieto 3 (MA)**	**GU228993**	**GU228925**	**GU229013**	**1, 2**	**Spain**
P. pilosellum **(Breuss) Breuss 2**	**M. Prieto 439 (MA)**	**GU228968**	**GU228907**	**GU229008**	**1, 2**	**Spain**
P. pilosellum **(Breuss) Breuss 3**	**M. Prieto 128 (MA)**	**GU228967**	—	—	**2**	**Spain**
*P. podolepis** **(Breuss) M. Prieto**	**LI 297365**	**GU228956**	**GU228917**	—	**2**	**Argentina**
P. rufescens **(Ach.) A. Massal. 1**	**M. Prieto 10 (MA)**	**GU228970**	**GU228931**	—	**2**	**Spain**
P. rufescens **(Ach.) A. Massal. 2**	**M. Prieto 522 (MA)**	—	**GU228927**	—	**2**	**Spain**
P. rufescens **(Ach.) A. Massal. 3**	**M. Prieto 164 (MA)**	**GU228971**	**GU228914**	—	**2**	**Spain**
P. **sp. 1**	**Aptroot 56961**	**GU228997**	**GU228901**	EF689769	1, 2	**China**
P. squamulosum **(Ach.) Breuss 1**	**M. Prieto 336 (MA)**	**GU228994**	—	—	**2**	**Spain**
P. squamulosum **(Ach.) Breuss 2**	**M. Prieto 339 (MA)**	**GU228969**	**GU228928**	**GU229009**	**1, 2**	**Spain**
P. squamulosum **var.** *argentinum* **(Räsänen) Breuss**	**M. Prieto 1705 (MA)**	**GU228991**	**GU228922**	**GU229014**	**1, 2**	**Argentina**
P. subrufescens **(Breuss) Breuss**	**Aragón 33/04 (MA)**	**GU228974**	**GU228903**	—	**2**	**Spain**
P. tenellum **(Breuss) Breuss**	**MA 16300**	**GU228990**	**GQ344562**	—	**2**	**Spain**
P. velebiticum **(Zahlbr. ex Zschacke) Breuss 1**	**M. Prieto 607 (MA)**	**GU228996**	**GU228902**	**GU229022**	**1, 2**	**Spain**
P. velebiticum **(Zahlbr. ex Zschacke) Breuss 2**	**LI 551990**	**GU228978**	**GU228939**	—	**2**	**Slovenia**
*P. velebiticum** (*pyrenaicum*) **3**	**LI 297363**	**GU228975**	**GU228921**	**GU229023**	**1, 2**	**Spain**
*P. velebiticum** (*pyrenaicum*) **4**	**LI 285722**	**GU228977**	**GU228938**	—	**2**	**Spain**
*P. velebiticum** (*pyrenaicum*) **5**	**LI 285716**	**GU228976**	**GU228937**	—	**2**	**Spain**
P. yoshimurae **(H. Harada) Breuss**	**LI 316277**	**GU228984**	**GU228905**	**GU229007**	**1, 2**	**Japan**
Placopyrenium bucekii (Nádv. & Servít) Breuss 1	—	EU010245	EF643767	EF689771	2	—
P. bucekii (Nádv. & Servít) Breuss 2	—	EU010246	EF643768	EF689772	2	—
P. canellum (Nyl.) Gueidan & Cl. Roux 1	—	—	EF643784	EF689787	1	—
P. canellum (Nyl.) Gueidan & Cl. Roux 2	—	—	EF643785	EF689788	1	—
P. fuscellum (Turner) Gueidan & Cl. Roux	—	EU010255	EF643794	EF689795	1, 2	—
P. trachyticum **(Hazsl.) Breuss**	**DUKE 139586**	—	**GQ344561**	**GU229017**	**1**	**Belgium**
Polyblastia cupularis A. Massal.	—	—	EF643769	EF689773	1	—
P. viridescens Zschacke	—	—	EF643771	EF689774	1	—
Staurothele immersa (A. Massal.) Dalla Torre & Sarnth. 1	—	—	EF643776	EF689779	1	—
S. immersa (A. Massal.) Dalla Torre & Sarnth. 2	—	—	EF643777	EF689780	1	—
Thelidium decipiens (Hepp) Kremp. 1	—	—	EF689782	EF689859	1	—
T. decipiens (Hepp) Kremp. 2	—	—	EF643778	EF689781	1	—
T. incavatum Nyl. ex Mudd	—	—	EF643780	EF689783	1	—
T. papulare (Fr.) Arnold	—	—	EF643781	EF689784	1	—
T. pyrenophorum (Ach.) Körb.	—	—	EF643782	EF689785	1	—
Verrucaria dolosa Hepp	—	—	EF643791	EF689792	1	—
V. hochstetteri Fr.	—	—	EF643795	EF689796	1	—
V. macrostoma Dufour ex DC.	—	—	EF643799	EF689801	1	—
V. nigrescens Pers.	—	—	EF643804	EF689806	1	—
V. polysticta Borrer	—	—	EF643807	EF689807	1	—
V. rupestris Schrad.	—	—	EU598724	EU723786	1	—
V. submersella Servít	—	—	EF643797	EF689799	1	—
V. viridula (Schrad.) Ach.	—	—	EF643814	EF689814	1	—
V. weddellii Servít	—	—	EF643812	EF689812	1	—
Verruculopsis lecideoides (A. Massal.) Gueidan & Cl. Roux	—	—	EF643798	EF689800	1	—
V. poeltiana (Clauzade & Cl. Roux) Gueidan, Nav.-Ros. & Cl. Roux	—	—	EF643822	EF689822	1	—