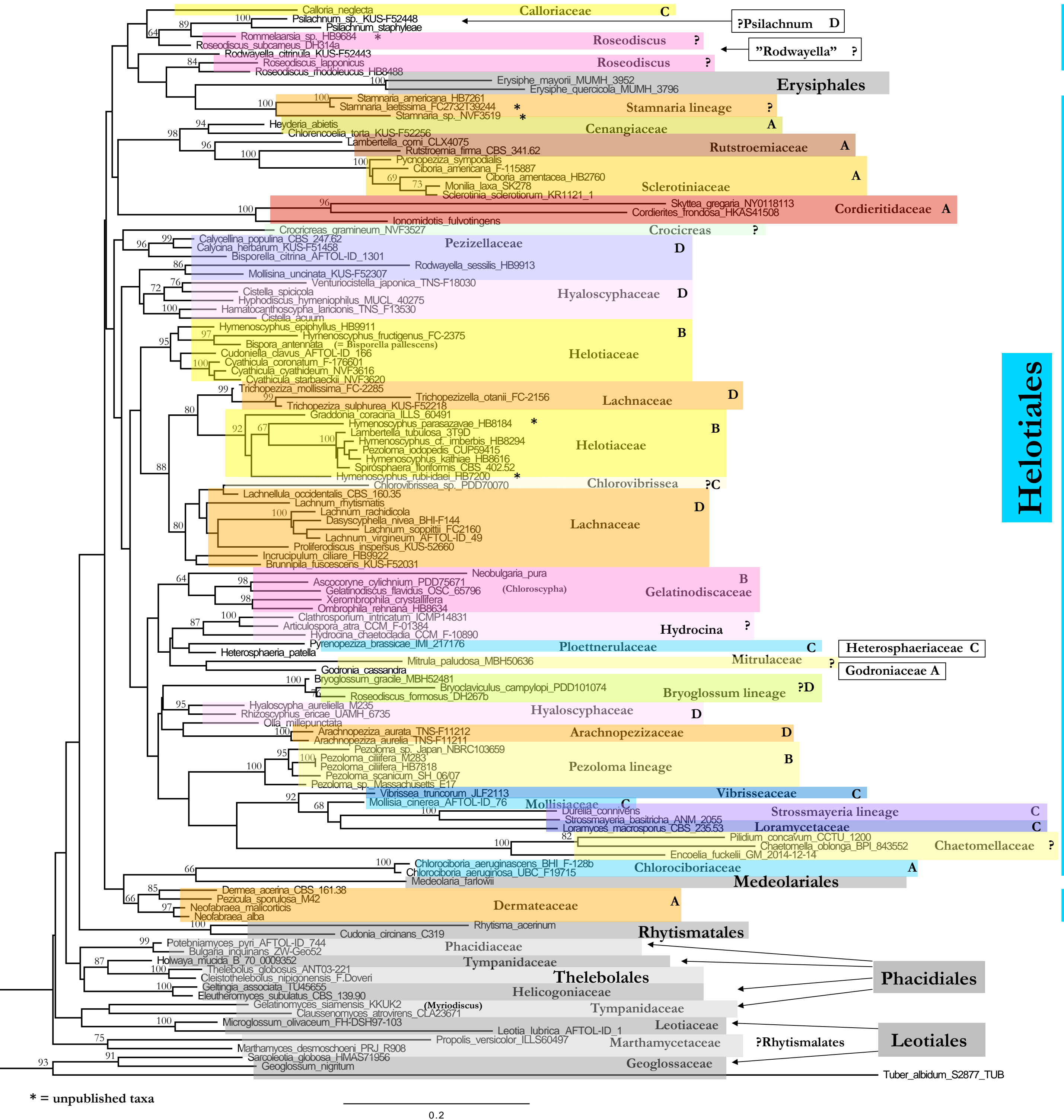


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INTRODUCTION - This study arose from a compilation of the non-lichenized discomycetes for the 13th edition of 'A. Engler's Syllabus of Plant Families', in which some new families were established and forgotten ones resurrected. It includes recent, partly unpublished results and tries to combine morphological and molecular data. 24 families and some undescribed lineages are here proposed in the Helotiales sensu stricto (excluding Leotiales, Phacidiales, and some other orders). Paraphyletic groups are accepted whenever obvious morphological support is noted. A pragmatic approach based mainly on morphology tries to arrange these 24 families in 4 main groups (A-D). Our phylogenetic analysis hardly represents these groups, although it shows high bootstrap support for most of the proposed families. The lack of support at the basal nodes of the tree indicates limitations of our data, which might be

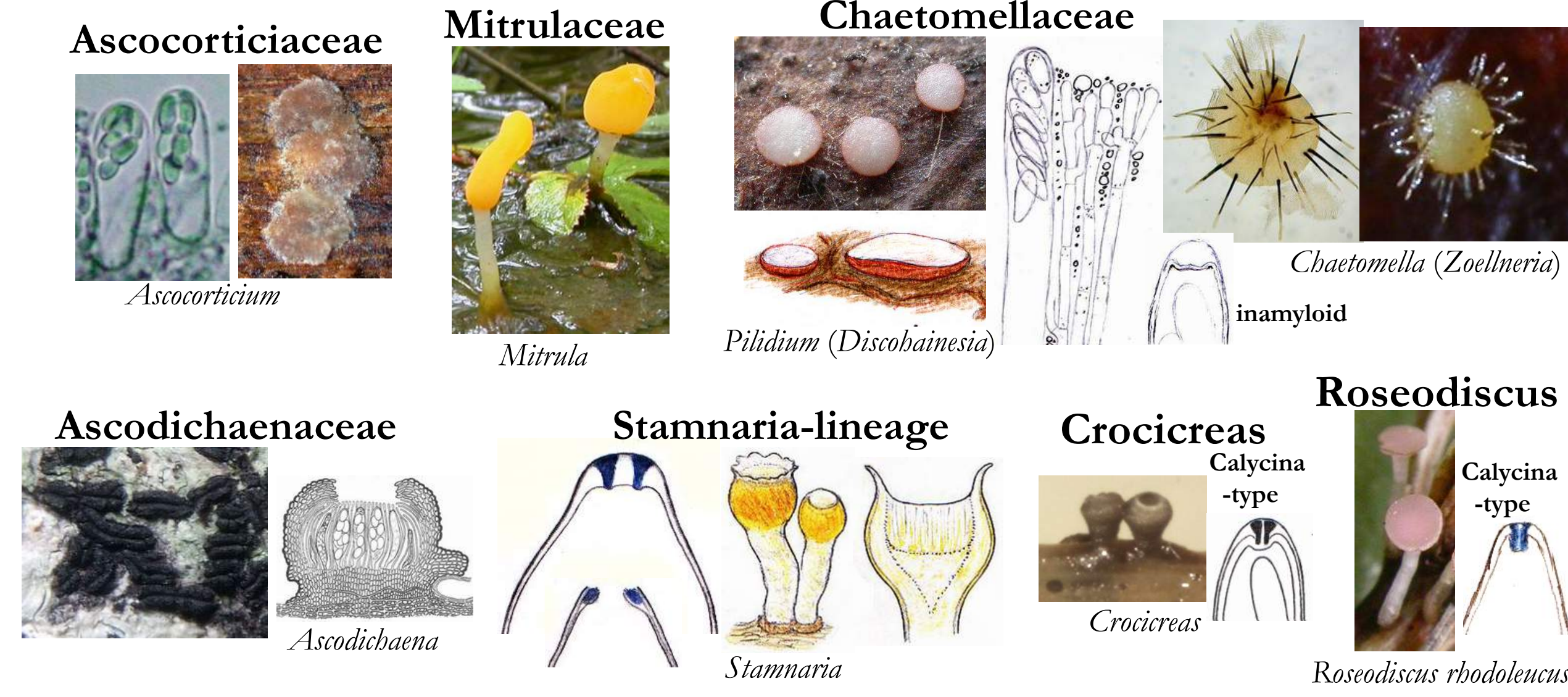
due to taxon sampling errors and/or lack of more gene regions. We consider this presentation as a stimulus for further work, which should include more, also protein-coding, gene regions. The families are mostly well represented with high bootstrap support, and also some family associations show high support. Some unexpected results are worth of mention: (1) Rosodiscus requires restriction to the type C. gramineum, while the large genus Cyathia is unrelated to them; (2) encoelioid taxa turned out to belong in various families, E. fucicili in Chaetomellaceae, E. fascicularis in Sclerotiniaceae, E. tilacea in Rutstroemiaceae, E. heteromera and E. fimbriata in Cordieritidaceae, and the type E. fufuracea in Cenangiaceae. A large number of helotiacean genera are still unsequenced, and even some sequenced ones cannot presently be placed in any of the presently recognized families.

ML phylogenetic analysis based on ITS+LSU rDNA



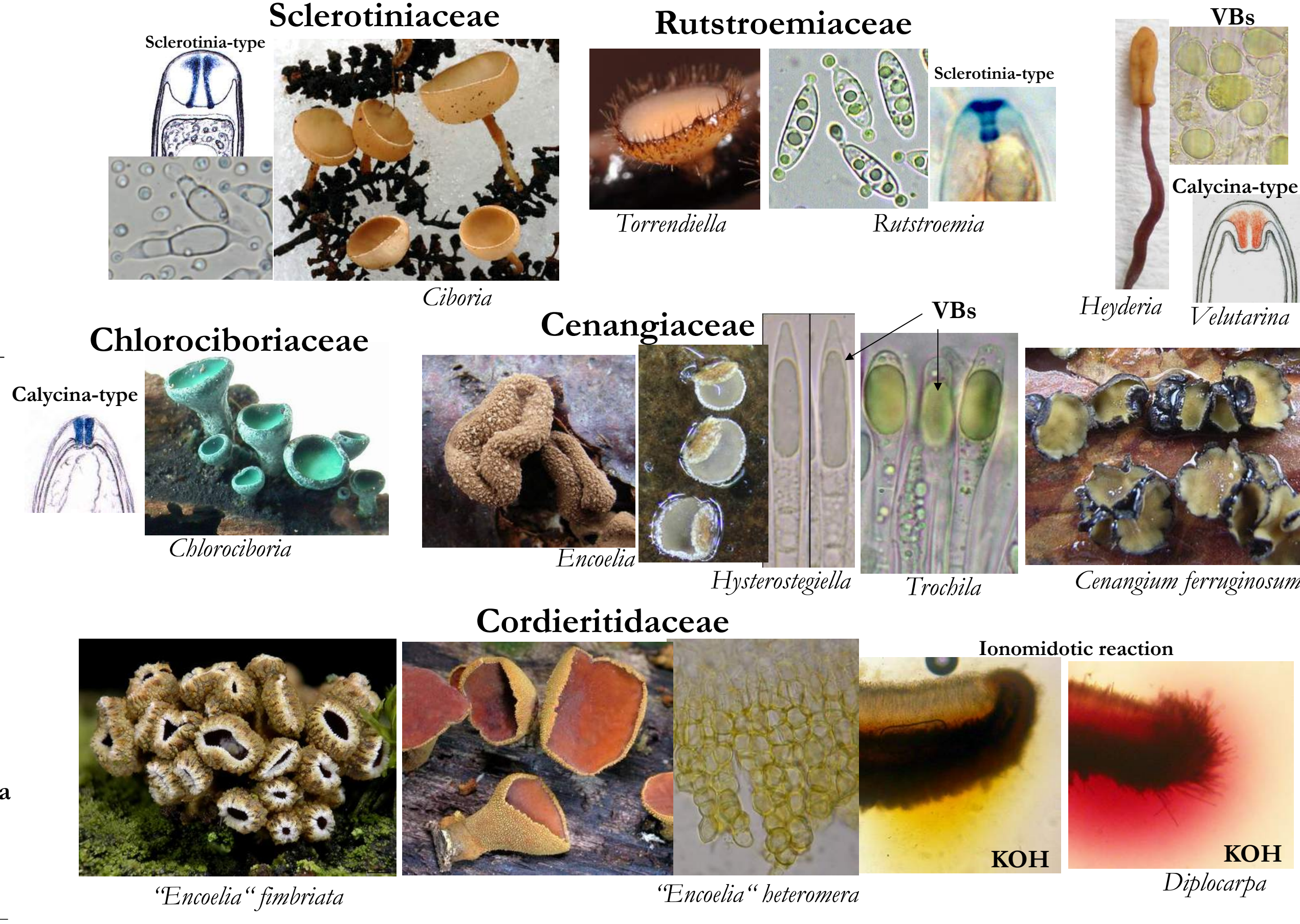
INCERTAE SEDIS

Fam. Ascocorticiaceae J.Schröt. - 3 genera (3): Ascocortium (= Ascodontium, Laticaria), Ascocortidium, Ascovorus. Fam. Ascodichaenaceae D. Hawksw. & Sherwood - 2 genera (4): Ascodichaena, Delphinia. Fam. Chaetomellaceae Baral, P.R. Johnst. & Rossmann - 4 genera (c. 80): Chaetomella (= Zoellneria), Ptilidium (= Discobainesia), Sphaerographium, Synchaetomella. Fam. Mitrulaceae Rchb. - 1 genus (5): Mitrula. Stammaria lineage - 1 genus (c. 7): Stammaria (= Titaeospora).



GROUP A - Sclerotiniaceae and encoelioid taxa

Subgroup I - Ionomidiotic reaction always absent, VBs partly present. Fam. Rutstroemiaceae Holst-Jensen, L.M. Kohn & T. Schumacher - 6 genera (c. 55): e.g. Lambertella, Lanzzia, Rutstroemia (= Psidium), Torrendiella. Fam. Sclerotiniaceae Whetzel - 28 genera (c. 150): e.g. Botrytis (= Botrytinia), Ciberinia, Moellerodiscus, Montinia, Myopappus (= Redheadii), Myriostroma, Piccomphala, Sclerotinia, Sclerotinia, Sclerotinia. Fam. Cenangiaceae Rehm (= Hemiphaciaceae Korf) - 15 genera (c. 70): e.g. Cenangium, Cenangium, Cenangium, Crumenolopsis, Encoelia, Heyderia, Hysterostegella, Rhododactylus, Sarcothelia, Trochilia, Velutaria.



Subgroup II - polyphyletic group comprising ionomidiotic taxa without VBs. Fam. Dermateaceae Fr. - 3 genera (c. 70): Derma, Neobulbina, Peziza. Fam. Cordieritidaceae (Sacc.) Sacc. - 14 genera (c. 115): e.g. Ameghinella, Coniherites, Diploarpha (= Ionomidiotic), Limoniella, Rhombocarpus, Skytta, Unguiculariopsis. Fam. Godroniaceae Baral - 4 genera (c. 45): Ascocarp (= Botrydium), Atropellis, Godronia (= Fucella), Gremmeniella (= Brunboschia). ? Fam. Chlorociboriaceae Baral & P.R. Johnst. - 1 genus (c. 22): Chlorociboria (= Dactyloina).

GROUP B - Helotiaceae taxa

Fam. Gelatinodiscaceae S.E. Carp. - 8 genera (c. 55): e.g. Ascovoryne (= Caryne), Ascotremella, Chlorocypha (= Gelatinodiscus), Neobulbina, Omphophila, Xeromorphophila. Fam. Helotiaceae Rehm - c. 18 genera (c. 180): e.g. Bispora (= Bispora), Bryoscyphus, Cudoniella, Cyathicilia (= Glarea), Diciphalospora, Gleetonia (= Endocoditium), Graddonella, Hymenoscyphus, Hymenotremella, Phaeobotryella, Symphyosira (= Symphyosira). Fam. Roesleriaceae Y.J. Yao & Spooner - 2 genera (c. 7): Roesleria, Roeslerina. ? Discinella-Pezoloma lineage - 2-3 gen. (c. 15): Discinella (= Geosoryne), Pezoloma (= Articulospora).

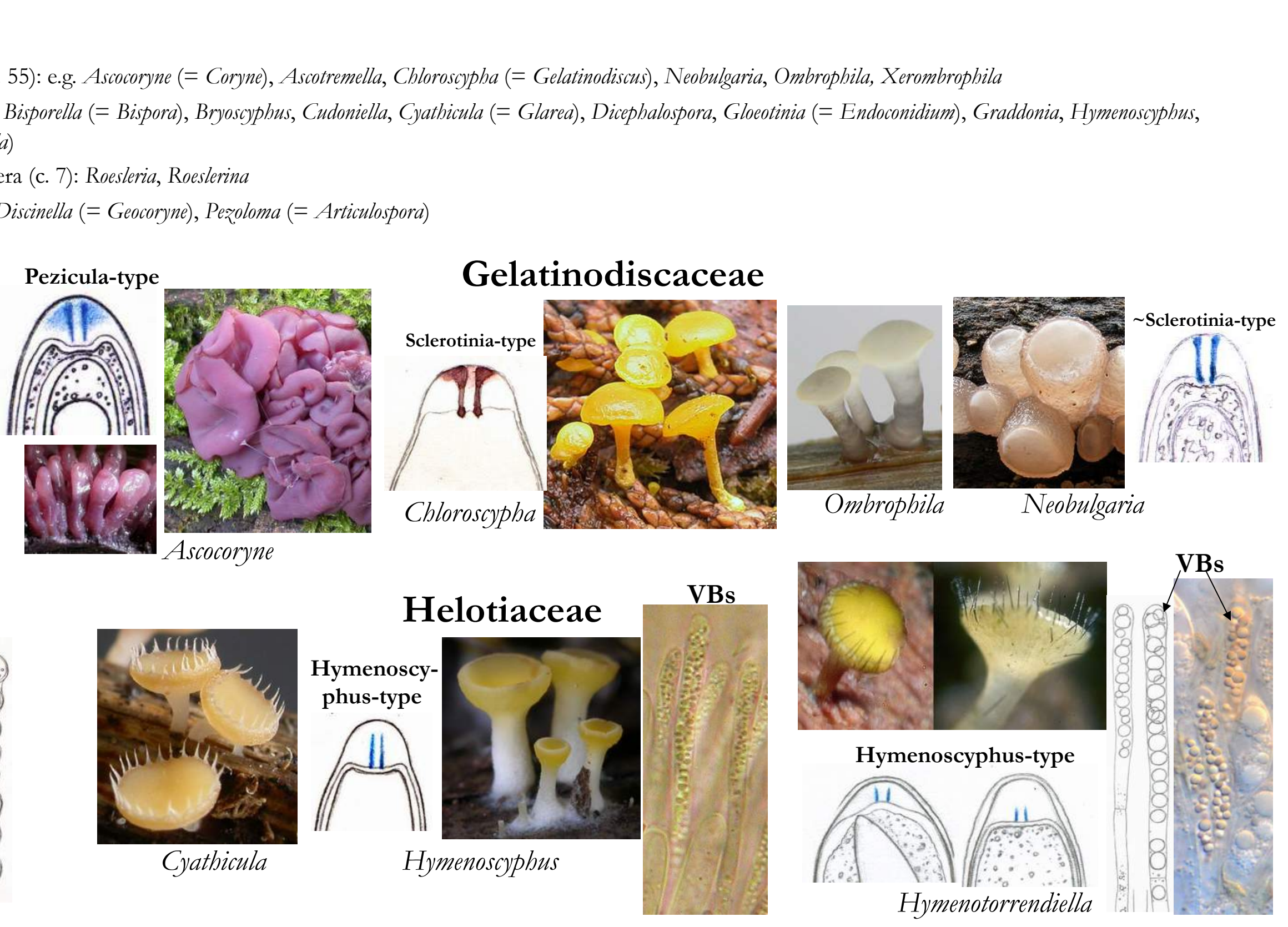


Table of selected characters of families and lineages here recognized within the Helotiales.

Table with columns for Lineage, inc.s., devel., stipe, erump., stroma, exc., hairs, cryst., ionom., par., VBs, amyl., ring, croz., sheath, lipid, asex., codg., lifest., desicc. and rows for various fungal families and lineages.

Development: Cor = corticoid; Cs = clavate-stipitate; Pr/Me = apothecoid; opening in the pro- (Pr) or mesothelium (Me) phase; Cl = cleistothecial; Maz = mazacium. Stipe: s = sessile; st = stipitate. Erumpent: er = erumpent; su = superficial. Pr/Me: dark stratified host tissue present (+) or absent (-). Excipulum: g = globose, p = prismatic, i = intricate. Hairs: h = hairs, i = teeth. Crystals: octahedral crystals present (+) or absent (-). Ionomidiotic: KOH releasing pigment into medium (ionomidiotic reaction), present (+) or absent (-). Paraphysis shape, c = cylindrical to clavate; l = lanceolate. VBs: refractive vacuolar bodies in paraphyses/excipulum cells present (+) or absent (-). Anolyiditic: iodine reaction of apical ring (Apar), B = hemianthoid (red, types RB/RR), - = inamyloid. Ring: apical ring type, S = Sclerotinia-type, C = Calycina-type, Pe = Pezizella-type, Pl = Plectonera-type, H = Hymenoscyphus-type, V = Vibrissae-type. Crozieria at the ascus base present (+) or absent (-). Sheath around ascospores, either gelatinous or separating after discharge (ap) = terminal gelatinous appendages, amy = amyloid sheath. Lipid: relative lipid content of ascospores, 1 = low, 2 = medium, 3 = high. Asexual state: h = hymenocarpous, pe = penicillate, s = synnematous/prothothal, h = acervular, p = precondial. Conidiogenesis: h = holoblastic, p = phallic, a = arthric. Lifestyle: s = saprobic, p = parasitic (or mycorrhizal). Desiccation tolerance: ser = desiccation-tolerant (seric substrate); hyg = desiccation-sensitive (hygic substrate).

COMMENTS ON SOME CHARACTERS

Ascus structure: Striking correlations between apical ring types and molecular data disclosed various misplaced taxa. E.g., species previously placed in Torrendiella (Rutstroemiaceae, Sclerotinia-type) had to be transferred to the unrelated new genus Hymenotremella (Helotiaceae, Hymenoscyphus-type). Or Bispora citrina and allies (Calycina-type) were transferred to Calycina (Pezizellaceae), whilst the type B. pallidissima (telomorph of Bispora antennata, Hymenoscyphus-type) clustered in Helotiaceae. Vacuoles: Presence vs. absence of refractive vacuoles (= VBs, living state!) correlates with phylogenetic lineages, permitting separation at the family level: VBs present: Mollisiaceae, Pezizellaceae, Cyathicilia (Helotiaceae), Cenangiaceae (major part). VBs absent: Plectonera-type, Hyaloscyphaceae, Cordieritidaceae.

Ionomidiotic reaction (IR): The extrusion of KOH-soluble yellow to red-brown pigment shows rather high correlation with phylogenetic groups. It occurs mainly in Cordieritidaceae, Dermateaceae, and Godroniaceae. IR was so far never observed in association with VBs (note that the yellow extrusion in Mollisia is a colour reaction of VBs, no IR).

PHYLOGENETIC ANALYSIS

ITS and LSU rDNA sequences were generated or downloaded from GenBank. Sequences were aligned using MUSCLE 3.7 and further hand-aligned in Se-AL v2.0a1. Ambiguously aligned regions were removed from the dataset. Phylogenetic inferences were estimated under maximum likelihood (ML) using RAxML 7.2.8 with a GTR+G model of nucleotide substitution. Rapid bootstrapping was implemented with 1000 replicates. The best ML tree (-ln = 32060.593754) and bootstrap values >= 60% are shown. Our ITS+LSU dataset includes 122 taxa; 50 sequences (ITS, LSU) were newly generated.

FUTURE DIRECTIONS

Sample more taxa and include more loci in the tree (SSU rDNA, RPB2).

ACKNOWLEDGEMENTS

Sarah Verhaeghen is thanked for technical assistance, and Guy Marson for supplying unpublished sequences.

GROUP C - Mollisiaceae taxa

subgroup I - VBs usually absent. Fam. Heterosphaeriaceae Rehm - 1 genus (7): Heterosphaeria (= Heterosphaera). Hysteropezizella lineage - 5-7 genera (42): e.g. Calyia, Coronellaria, Hysterospora, Hysteropezizella. Fam. Calloriaceae Marchand - c. 15 genera (c. 110): e.g. Calloria (= Cylindrocolla), Diplonaria, Dubevina, Eisporella, Laetina, Navelia, Naviopsis, Plectonera. Fam. Drepanopezizaceae Bat. & H. Maia - c. 10 genera (c. 52): e.g. Blumeriella (= Phloeosporidia), Diploarpha (= Marsominia), Drepanopeziza (= Gleetosporidiella), Lepitrotachia (= Sporoneva), Pseudopeziza, Spilopodia (= Melanidiscus). Fam. Plectonera-type Kirschst. (= Pyrenopezizaceae Ruhland ex Velen.) - c. 7 genera (2125): e.g. Dennisodiscus, Lasionomollisia, Oculimaula (= Helgardia), Pirottaea (= Plectonera), Pyrenopeziza (= Cadophora).

subgroup II - VBs present

Fam. Mollisiaceae Rehm - c. 11 genera (2190): e.g. Mollisia (= Belonopsis, Cystodendron, Huglandia, Phaeomollisia, Phialocephala, Tapesia), Niptera (= Nimbomollisia), Sautomollisia. Fam. Loramycetaceae Dennis ex Digby & Goos - 2 genera (4): Loramycetes, Obiectodiscus. Fam. Vibrissaceae Locq. ex Korf - 3-4 genera (c. 35): Leucovibrissa, Pocillum, Vibrissa (= Anariza). Strossmayeria lineage - 3 genera (235): Durella, Gorgoniceps, Strossmayeria (= Pseudospinopa).

GROUP D - Hyaloscyphaceae taxa

Fam. Arachnopezizaceae Hosoya, J.G. Han & Baral - 5 genera (c. 30): Arachnopeziza, Arachnospiza, Austropeziza, Eriopeziza, Parachnopeziza. Fam. Lachnaceae Raitv. - 16 gen. (c. 250): Albotricha, Belonidium, Brunnipila, Capnitricha, Dasycephala, Eriosephella, Incrucipulum, Lachnellula, Lachnum, Lasiolebotium, Neodasycephala, Perrotia, Proliferodiscus, Salenopeziza, Trichopeziza, Trichopezizella. Fam. Hyaloscyphaceae Nannf. - 26 genera (c. 220): e.g. Amnidisca, Cistella, Dematiocypha (= Haplographium), Echinella, Fuscolachnum, Graddonella, Hyalopeziza, Hyaloscypha (= Cheimorhynchella, Fuscoscypha, Parorbilopsis, Rhizoclyphus), Hylodiscus (= Catemphera), Inaripila, Olla, Protomyxaria, Unguicularia, Urvellia, Urvellia, Urvellia. Fam. Pezizellaceae Velen. - 18 genera (c. 170): e.g. Allophylaria, Antinna, Calycellina (= Chaetochalara, Phialina), Calycina (= Chalora, Gemmina), Cylindaria, Hamatocanthoscypha, Micropeziza (= Calloriella), Mollisia, Phaeoscypha, Psilachnum, Rodwayella (= Kermelia), Velutaria (= Tapesia). ? Bryoglossum lineage - 2 genera (3): Bryoglossum, Bryoglossum.

