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Application of ultrastructural and molecular data in the taxonomy of helotialean fungi
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Dissertation was accepted for the commencement of the degree of Doctor philosophiae in botany and mycology at the University of Tartu on August 25, 2016 by the Scientific Council of the Institute of Ecology and Earth Sciences, University of Tartu.

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Commencement: Room 218, 40 Lai Street, Tartu, on 11 November 2016 at 10.15 a.m.

Publication of this thesis is granted by the Institute of Ecology and Earth Sciences, University of Tartu

ISSN 1024-6479

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University of Tartu Press
www.tyk.ee
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The author’s contribution to the publications
Kadri Pärtel was responsible for developing the research ideas and writing all the manuscripts; where transmission electron microscopy (TEM) was used (II–VI), K. Pärtel conducted all laboratory work and analyses. A. Raitviir, K. Põldmaa and H.-O. Baral contributed to the conceptual aspects of the research questions and writing. K. Pärtel and H.-O. Baral contributed to the morphological studies in paper I. A. Raitviir participated in the identification of species, which ultrastructure was observed. H. Tamm contributed to the molecular laboratory work and phylogenetic analyses.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSE</td>
<td>dark septate endophyte</td>
</tr>
<tr>
<td>EcM</td>
<td>ectomycorrhiza</td>
</tr>
<tr>
<td>ErM</td>
<td>ericoid mycorrhiza</td>
</tr>
<tr>
<td>INSD</td>
<td>international nucleotide sequence databases</td>
</tr>
<tr>
<td>I+</td>
<td>ascus apex amyloid (blue/red in iodine solution)</td>
</tr>
<tr>
<td>I–</td>
<td>ascus apex inamyloid (not stained in iodine solution)</td>
</tr>
<tr>
<td>IR</td>
<td>ionomidotonic reaction: fungal tissue extracting pigments in aqueous potassium hydroxide solution (KOH)</td>
</tr>
<tr>
<td>LM</td>
<td>light microscopy</td>
</tr>
<tr>
<td>LUG</td>
<td>Lugol’s solution, aqueous solution of iodine and potassium iodide</td>
</tr>
<tr>
<td>MLZ</td>
<td>Melzer’s solution, aqueous solution of chloral hydrate, potassium iodide, and iodine</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>rDNA</td>
<td>regions coding for ribosomal RNA (ribonucleic acid):</td>
</tr>
<tr>
<td>ITS</td>
<td>internal transcribed spacer: ITS1, ITS2 flanking 5.8 subunit in rDNA</td>
</tr>
<tr>
<td>18S</td>
<td>small subunit (SSU)</td>
</tr>
<tr>
<td>28S</td>
<td>large subunit (LSU)</td>
</tr>
<tr>
<td>rpb</td>
<td>RNA polymerase II subunit</td>
</tr>
<tr>
<td>tef</td>
<td>translation elongation factor</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>VB</td>
<td>vacuolar body</td>
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1. INTRODUCTION

Fungi represent a diverse group of eucaryotic organisms that were classified as a separate kingdom fifty plus years ago (Whittaker 1969). Mycology, initially a descriptive subdiscipline of botany, has increasingly been developed in terms of experimental and molecular science. Advances in methodology have allowed researchers to more precisely characterize and catalogue fungi, which remains an essential task of mycologists. The general principle of fungal systematics is based on the common origin of taxa, the hypotheses of evolutionary history, which are modeled using genetic data. The implementation of DNA-based methods of phylogenic classification has been especially important regarding ascomycetes (=phylum Ascomycota), the most species rich group of fungi.

Molecular studies are accumulating new evidence on the phylogenetic relationships of previously poorly explored lineages. This development is based not only on visible fruitbodies, but on data from analyses of their habitat, such as soil, water, and host plants. Although much regarding the life-histories of fungi remains unknown, DNA analysis has been confirmed as an important tool for providing essential information about the life of fungi, in addition to the traditional knowledge based on fruitbodies (Stajich 2015). However, many branches are missing or unnamed in the current Fungal Tree of Life (e.g. Fig. 4 in LoBuglio & Pfister 2010). The aim of this study was to investigate one of the “weak branches” of the Fungal Tree of Life, the helotialean fungi, which are mostly cupulate ascomycetes, that are as common as mushrooms. However, owing to their scattered growth and small size (often less than 2mm in fruitbody diameter), recording them in nature is difficult, despite the fact that some are brightly coloured.

The helotialean fungi belong to the class Leotiomycetes, where limitation of genera and higher taxa are not well agreed. Owing to the different interpretation of characteristics of these fungi, the morphological description of individual species often differ drastically, although some standard practise for describing species has been suggested (e.g. Huhtinen 1994). The search for information about species description often ends up in returning ambiguous information, especially where descriptions lack detailed illustrations and one must imagine the appearance of the fungus. Due to the scarcity of taxonomic keys, the process of species identification is often time-consuming and unproductive.

The molecular identification of helotialean fungi is critical, because the key users of taxonomic information (e.g. ecologists, evolutionary biologists, and plant pathologists) need DNA barcode markers for species identification. DNA-based methods will also help to characterise the biodiversity of helotialean fungi, especially their symbiotic relationships with plants. Current state, when several morphological criteria that once defined a taxon have been deposed, indicates the need for building a new classification based upon phylogenetic data. To ascertain new diagnostic characters, a critical revision of morphological characters using complemented technical possibilities (e.g. electron
micscopy) is essential. The main research questions concern the delimitation of families and genera considering their phylogeny. In the following section, the selected groups of helotiallean fungi with their main characters, are introduced. The original multigene phylogeny and electron microscopy results are then presented and discussed.

The current confusion regarding the order level delimitation of the studied fungi, necessitated using the informal name “helotiallean fungi”. This indicates that at present, Helotiales represents a polyphyletic group, largely circumscribed using characters that have evolved as a result of convergent evolution (e.g. Wang 2006a). The family names used in the original papers III–VI do not correspond to those in this thesis, because the classification of the studied genera was changed based upon the subsequently published data of other researchers and one’s own results.

1.1. Overview of study groups and taxonomic problems

1.1.1. Class Leotiomycetes O.E. Erikss. & Winka 1997

The class Leotiomycetes includes over 1000 genera of inoperculate ascomycetes (Johnston et al. 2015). Their ascus apex lacks a lid or operculum, and they ejaculate spores via an alternative mechanism, which is mainly annular in structure. The sister class of the Leotiomycetes is the perithecial Sordariomycetes (Spatafora et al. 2006, Schoch et al. 2009a). Although these classes largely deviate morphologically, it has been presumed that they evolved from a common ancestor: a nonlichenized saprotroph with unitunicate inoperculate asci (Zhang & Wang 2015). The estimated time of diversification is about 300 million years ago, during the Permian-Carboniferous geological period, as there are no known fossil records of Leotiomycetes (Beimforde et al. 2014).

The class Leotiomycetes was described by Eriksson and Winka (1997), and includes the orders Helotiales, Rhytismatales, Erysiphales, Thelebolales, Leotiales, Phacidiales, Cyttariales, and Medeolariales. The last two orders are monotypic, whereas several others contain numerous members (Kirk et al. 2008). The current classification of the Leotiomycetes (Lumbsch & Huhndorf 2010, public databases Mycobank and Index Fungorum) and its accepted orders, are based mainly on the traditional morphological characters of the teleomorph. Often these characters have not confirmed phylogenetically informative and the present Leotiomycetes classification is not congruent with recently presented phylogenies (Wang et al. 2006a, b; Schoch et al. 2009a; Hustad & Miller 2011; Lantz et al. 2011; Han et al. 2014; Crous et al. 2014). These phylogenies show both polyphyly or paraphyly among many of Helotiales taxa, intermixed between other orders, where monophyly is well supported for Cyttariales, Erysiphales, Rhytismatales, Phacidiales.
The proposed classifications are sometimes controversial and often very
difficult to interpret when one compares morphology and phylogeny. For
example, the Erysiphales (powdery mildews), which are chasmothecial epiphytic
leaf parasites (Webster & Weber 2007), have very different morphology com-
pared to apothecial Helotiales, but phylogenies (Wang et al. 2006a, b) have
supported its placement among these. In contrast, members of Geoglossaceae,
morphologically similar to Helotiales, have been excluded from this order, with
a new class Geoglossomycetes created based on molecular phylogenies (Schoch
et al. 2009b).

The biogeography of Leotiomycetes is also important to understand their
diversity (Zhang & Wang 2015). In the Erysiphales, different lineages are
geographically isolated, with two basal lineages diverged to South American
and eastern Asia (Takamatsu 2004). The Cyttariales is restricted to the southern
hemisphere, where Cyttaria spp. have coevolved with its host Nothofagus
(Peterson et al. 2010).

1.1.2. Order Helotiales Nannf. 1932

Based on a macroscopical study of fungal fruitbodies, A. J. Retzius (1769)
introduced the genus Lachnum; subsequently E. Fries (1822) established among
many others, the genus names Mollisia and Encoelia. Based on micromorpho-
logy, during the second half of the 19th century the Friesian genera were divided
into numerous smaller ones by Fuckel (1869), Karsten (1871), and Boudier
(1885). At the beginning of the 20th century, J. A. Nannfeldt (1932) established
the basis of generic level taxonomy in the order Helotiales.

Helotiales is an order with worldwide distribution, including approximately
300 genera and 3000 species (Baral 2016). The order is diverse both ecologi-
cally and regarding the “general habitus” of fruitbodies. The most common
fruitbodies are non-stromatic sessile or stipitate cupulate-discoid apothecia, but
other types include semi-immersed, turbinate, funnel-shaped, clavate (Baral
2016, Spooner 1987), or exceptionally cleistothecial (e.g. Bicornispora, see
Galán et al. 2015). Stipes, if present, are mostly central and cylindrical. The
apothecia may be scattered singly or variously aggregated, and be soft or
leathery tough. Some examples of the different lineages studied for this thesis
are illustrated in Fig. 1. The asci develop in the hymenium amongst the longi-
tudinal sterile paraphyses, and the receptacle tissues are usually well developed.

Nannfeldt (1932) distinguished six families in the order Helotiales, of
which the Geoglossaceae, Orbiliaceae and Phacidiaceae have been updated to a
higher taxonomical rank today (Eriksson et al. 2003, Schoch et al. 2009b, Crous
et al. 2014). The other three (Helotiaceae, Hyaloscyphaceae, and Dermateaceae)
remain families within the Helotiales. However, the original concept of
classifying these families based on their excipular structure, hairs, and ascus and
paraphyses features (like introduced in Cannon & Kirk 2007), has been
discredited by molecular phylogeny (Wang 2006a, Han et al. 2014, Crous et al.
2014), because members of these families have been divided into many separate lineages. Baral (2016) recently differentiated a total of 25 Helotiales families, many of which were resurrected from historical families. In the Helotiales, the classification of approximately 90 genera are incertae sedis (Lumbsch & Huhndorf 2010; Baral 2016).

a) Family Dermateaceae Fr. 1849

Members of the Dermateaceae (sensu Nannfeldt 1932) were defined by the parenchymatous cells of the outer excipulum and the sessile apothecia. Based on phylogenies globose excipular cells was considered to be homoplasious character. Wang (2006a, b) revealed two distinct lineages which form the Dermateaceae s. str and Mollisia complex. The first lineage includes the plant endophyte-parasites Dermea Fr., Pezicula Tul. & C. Tul., and Neofabraea H.S. Jacks., and the family name Dermateaceae should be restricted to those genera according to Verkley (1999) and Abeln (2000). This family is a quite well studied monophyletic group (Verkley 1999; Abeln et al. 2000; Jong et al. 2001; Chen et al. 2015).

The second of Wang’s lineages (2006a) contained Mollisia in the larger clade Loramyces-Mollisia-Vibrisea. Mollisioids are soft (as indicated by their name), with mostly sessile discoid apothecium (Fig. 1e) and rounded or rectangular brown-walled excipular cells (Nauta 2010). The mollisioid fungi lack any modern revision based on morphology and there is also a shortage of molecular studies. Mollisia, with >120 species (Kirk et al. 2008), is “notorious” in terms of species misidentifications. Morphologically similar genera include the Tapesia (Pers.) Fuckel with the subiculum under the apothecia, and the septate-spored Niptera Fr. and Belonopsis (Sacc.) Rehm (Nannfeldt 1985; Nauta & Spooner 1999). Pyrenopeziza Fuckel is another species-rich genus, but with more erumpent apothecia than Mollisia (Greenleaf & Korf 1980, Gremmen 1958, Hüttner 1958, Gminder 1996). Despite Pyrenopeziza and Mollisia being extremely similar macromorphologically, they are not genetically closely related. Anamorph features correspond to separate clades of the teleomorph of these fungi. Cadophora-like producing solitary phialids are related to Mollisia dextrinospora Korf (note: the morphology of this species is similar to Pyrenopeziza), and Phialocephala-like producing complex heads of multiple phialids are related to Mollisia spp. (Day et al. 2012). Baral (2016) resurrected the family Ploettnerulaceae Kirschst. which includes Pyrenopeziza and several other lineages traditionally connected with the Dermateaceae.

The Mollisiaceae comprises genera with high ecological plasticity, such as the Phialocephala, which includes species that are either frequent root endophytes, leaf endophytes, saprobes, or parasites (e.g. on grasses) (Zaffarano et al. 2010; Queloz et al. 2011, Wong et al. 2015, Tanney et al. 2016). Often the morphology is highly reduced and the lifecycle lacks the sexual state, such with in the Phialocephala fortinii complex as characterized by melanized septate hyphae (Grüning et al. 2008).
Fig. 1 Apothecia of helotialean fungi.  

a Ciboria batschii with sclerotia, TU104222.  
b Rutstroemia firma, TU104493.  
c Chlorencoelia versiformis, TU107606.  
d Encoelia furfuracea, TU104599.  
e Mollisia lividofusca, TU104358.  
f Calycina citrina, TU109158.  
g Lachnum brevipilum, TU109185.  
h Capitotricha bicolor, TU104600.  
i Trichopeziza mollissima, TU104372.  
j Hymenoscyphus fraxineus, TU104160.  
k Ionomidotis irregularis, TAAM198450.  
l Chlorociboria aeruginascens.  

Scale bar: a–d = 5mm, e–j = 1mm, k = 1cm. Authors of images V. Liiv: a, c, i, j, l; K. Põldmaa: d; H. Tamm: e, f, I. Zettur: k.
b) Family Helotiaceae Rehm 1892
Currently the Helotiaceae is the most heterogeneous family of the Helotiales in terms of morphology and ecology, and comprises 117 genera and 826 species (Kirk et. al. 2008). Previously, Nannfeldt (1932), Dennis (1978), and Korf (1973) distinguished up to 10 intrafamiliar subdivisions, but none have been systematically evaluated using molecular phylogenetics. Several lineages (Ascocoryne-Neobulgaria, Calycina, Chlorociboria, Cordierites, Hymenoscyphus-Cudoniella, Stamnaria, Strossmayeria, and Mitrula) have been recognized using phylogenies (Hustad & Miller 2011, Baral et al. 2013, Crous et al. 2014, Baral et al. 2015b). Chlorociboriateae Baral & P.R. Johnst. has recently been described for the Chlorociboria lineage (Baral 2015a) and Pezizellaceae Velen. emended for Calycina-Calycellina-Mollisina lineage (Baral 2016). Some species-rich genera of the Helotiaceae, such as Crocicreas Fr. (Carpenter 1981) and Hymenoscyphus Gray (Lizoñ & Kučera 2014), have yet to be critically revised.

Morphological features of the family Helotiaceae, according to the original description (Rehm 1892) are sessile or stipitate, fleshy or cartilagineus apothecia, with most having ecal excipulum of the textura oblita. Korf (1973) emphasized long-celled excipulum, rarely of t. prismatica or angularis, gelatinized apothecia, and medullary excipulum of the t. intricata for the Helotiaceae. The subfamily Encoelioideae Nannf. was distinguished from the Ciborioideae by the former’s longevity and the leathery consistency of their apothecia. The outside of the Encoelioideae apothecium seems mealy (Fig. 1d), because the outermost cells of the ecal excipulum are loosely aggregated. Several of these genera had previously been assigned to the family Cenangiaceae Rehm. Encoelioideae (sensu Korf 1973) was distinguished from other members of the Helotiaceae mainly by the characters of the excipulum.

Encoelia (Fr.) P. Karst. is a large heterogeneous genus with members that have tough apothecia with a coarse outside, and usually erumpent from bark (Korf 1973). Peterson and Pfister (2010) revealed that the genus Encoelia is polyphyletic, with three species included in their four gene phylogenetic analysis falling into two distinct groups: E. heteromera (Mont.) Nannf. with E. helvola (Jungh.) Overeem near the Cordierites Mont. (Helotiaceae); and E. fascicularis (Alb. & Schwein.) P. Karst. in the Sclerotiniaceae. However, Encoelia furfuracea (Roth) P. Karst., a type species of the genus, has been neglected in recent phylogenetic and morphological studies, despite being commonly found in Europe and North America.

c) Families Hyaloscyphaceae Nannf. 1932 and Lachnaceae (Nannf.) Raithv. 2004
The family Hyaloscyphaceae was established by Nannfeldt (1932) for taxa with hairy apothecia and was originally divided into tribes: 1) Arachnopezizeae: apothecia arising from the subiculum; 2) Hyaloscyphae: small-sized apothecia, mainly cylindrical paraphyses, hairs of various shape; and 3) Lachnaceae: relatively large apothecia, hairs multisepate and granulated (Fig. 1g–h), lanceolate paraphyses. The first multigene phylogenetical work of hyaloscyphoid fungi–
based on Asian data—suggested this family is polyphyletic (Han et al. 2014). The Hyaloscyphaceae was emended by Raitviir (2004), who excluded genera of the Lachneae as a distinct family of its own (Lachnaceae), which was later supported by the multigene phylogenetic study of Hosoya et al. (2010).

*Lachnum* Retz. is a world-wide genus with approximately 250 species (Kirk et al. 2008). These have cupulate +/- stipitate apothecia with hyaline or pigmented hairs often bearing crystals in apices, and lanceolate paraphyses. Raitviir’s (1970) idea that species with totally or partially smooth-walled hairs belonging to separate genera (*Albotricha* Raitv., *Belonidium* Mont. & Durieu, *Dasyscyphella* Tranzschel and *Trichopezizella* Dennis ex Raitv.), was confirmed by the exclusion of further taxa from *Lachnum*, based on morphological characters. New genera have been proposed for fungi with thick-walled hairs, e.g. *Capitotricha*, *Brunnipila*, and *Incrucipulum* by Baral (1985), and for those with thin-walled melanin-containing hairs (*Fuscolachnum* J.H. Haines, Haines 1989). *Albotricha* have hairs that bear amorphous reactive resinous matter that does not dissolve in Melzer reactive (MLZ) (Raitviir 1970). *Lachnellula* P.Karst. contains 40 species (Kirk et al. 2008) and is macromorphologically very similar to *Lachnum* segregates, in contrast to latter, *Lachnellula* asci arise from unique open croziers, the stipe is short, and the paraphyses cylindrical. Baral (2000) emphasized the desiccation-tolerance of the apothecia in *Lachnellula* spp.

*Trichopeziza* (Fig. 1i) and *Trichopezizella* were assigned by Raitviir (1987) into a different subfamily (Trichopezizelloideae), which were also recently excluded from the emended Lachnaceae (Hosoya et al. 2010). In contrast to the Lachnaceae, the *Trichopeziza* spp. have long, smooth, densely septate, and relatively thick (up to 2µm) hairs of a yellow–reddish–brownish pigment.

### 1.2. Characters in systematics of Helotiales

#### 1.2.1. Characters of apothecium

Traditional morphological characters used to identify the Helotiales are the shape and measurements of the hymenium components of apothecia: asci, ascospores, and paraphyses (Nannfeldt 1932, Korf 1973, Spooner 1987, Pfister & Kimbrough 2001). The structure that supports hymenial part of the apothecium is called the excipulum with layers of different hyphal types distinguished (*textura* type), and the presence of exudate or/and gel noted. The characters of hairs covering the external part of the apothecium of numerous helotialesan taxa have been of important diagnostic value (hairs’ density, length, shape, septation, presence of crystals or other external substances, and refractivity). The ornamented hairs, which seem punctate or granulate in LM, and the crystals in the central part of the hair apex have been observed under scanning electron microscopy (Hein 1980, Horner et al. 1983).
Baral (1992) has drawn the attention to the cell components, such as the amount of lipid bodies in ascospores, or vacuolar bodies in vegetative cells. The special components of vacuoles, the refractive vacuolar bodies, occurring on external parts of apothecia, contain a colloidal substance, which reflects the light during microscopical observations (Baral 1992).

Isolation of helotialean fungi into pure culture is not routinely applied and many of them do not produce conidiomata or any other asexual structures in culture, and/or the ascospores do not germinate on standard culture media.

1.2.2. Ascus and its apical apparatus

The ascus is the largest distinct cell in the fruitbodies of ascomycetes, resembles a fluid-filled sac, and its growth is determinate in contrast to unspecialized vegetative hyphae (Read & Beckett 1996). Asci develop from ascogenous hyphae growing out of an ascogonium (Wilson 1952). The essential biological processes karyogamy, meiosis, and mitosis, are conducted in the asci (Bellemère 1994). In Helotiales, the result of these processes, the ascospores, are forcibly ejected and carried via air to new substrata or habitats. For example, *Sclerotinia sclerotiorum* eject thousands of ascospores synchronistically, when a blast of air is created that carries the spores away (Roper et al. 2010).

At the top of the ascus is a trigger (a ring-shaped structure) and the structure of the ascus apex has been shown to be quite complex under TEM, and is called the ascus apical apparatus (Verkley 1992). This apparatus is responsible for the ejaculation of ascospores. This is a fast process, because it is important to cross the stagnant layer of air surrounding the fruitbodies, however the pressure is controlled to avoid rupture of the ascus (Fritz et al. 2013, Trail & Seminara 2014). It is assumed that glucose or glycerol provides the osmotically active solute responsible for the increase in turgor pressure just before discharge (Read & Beckett 1996). In helotialean fungi, the opening is in most cases via eversion of an apical ring (annulus) (Verkley 1995b).

Ascus characteristics have long been used in ascomycete systematics, first the shape and size, then the number of ascospores (mostly 8) per ascus, and the arrangement of spores (uniseriate, biseriate, or overlapping) (Bellemère 1994). The presence of croziers at the ascus base, next to the ascogenous hyphae, is constant for a taxon (Huhtinen (1990). Boudier (1879) argued for use of iodine solution for studying the ascus apex in detail, as he found this method useful in classifying apothecial ascomycetes (Discomycetes). The iodine reaction, however, has not become a standard component in all descriptions of taxa in the helotialean fungi.

The helotialean fungi differ in their ascus apices, with an apical ring not always present. When a ring is absent, the apical wall may be thickened (reviewed in Verkley 1995b). The annulus (the apical ring), usually reacts to iodine, and according to Baral (1987a) this species-specific reaction is either: 1) negative in iodine reagents = inamyloid, I–; 2) stains blue in Melzer (MLZ) and
Lugol (LUG) solution = euamyloid, I+ bb; 3) stains reddish in LUG = hemiamyloid, I+ rb (red at high, blue at low concentrations) or I+ rr (red); in addition, hemiamyloid apex stains blue in MLZ after KOH pretreatment. Ascus shapes change before the liberation of spores (Bellemère 1994). Mature asci are usually apically blunt, while the lateral wall becomes thinner and apical ring height decreases compared to juvenile asci. In some species, e.g. *Lachnellula occidentalis*, inamyloid and amyloid asci are intermixed in the hymenium of one apothecium (Baral & Matheis 2000). However, amyloidity type is mostly constant in a species, and the annulus shape and (approximate) type can be described using LM. Based on observations of different ascal apices, some taxa have been critically studied and taxonomic recombinations proposed (Triebel & Baral 1996, Johnston et al. 2014, Sandoval-Leiva 2014).

The pioneer of comparative TEM studies of the ascus appical apparatus in helotialean fungi was Bellemère (1977). Before him only single species of Sclerotiniaceae (*Ciboria acerina* by Corlett & Elliott 1974 and *Dumontinia tuberosa* by Schoknecht 1975) and Bulgariaceae (Bellemère 1969) had been studied. Later studies by G. J. M. Verkley revealed specific ascus apical apparatus in several families of Helotiales (Verkley 1992; 1993a, b; 1994; 1995a, b; 1996 and 2003). Verkley’s comparative treatment of 26 genera (resulting in 14 different ascus types) in his doctoral thesis (1995b) suggested that ultrastructural studies offer a promising approach in refining the taxonomy of the Helotiaceae, Geoglossaceae and Sclerotiniaceae, via observing methodically each developmental stage (juvenile, immature, and mature) of the ascus lateral wall structure and mode of dehiscence.

### 1.3. Ecology of Leotiomycetes

Leotiomycetes inhabit very different ecological niches, from marine to terrestrial, and from soil to the crowns of trees. A reference-based overview of substrata/hosts and putative lifestyles of this fungal class is presented in Table 1. Ecology has been neglected by most earlier taxonomists, and fruitbody specimens in fungal collections had often been detached from the substrate, and or the plant species upon which they were growing, was not identified. Teleomorphs and anamorphs of helotialean fungi are most frequently observed on different parts of plants, and the formers’ lifestyle is either saprobic, parasitic, or symbiotic. In the case of foliicolous fungi, it has been shown with molecular and cultivating methods, that still attached senescent living leaves are hosts for endophytic Leotiomycetes, and that the same species later act as initial decomposers (Koukol & Baldrian 2012, Voříšková & Baldrian 2013) by producing extracellular enzymes (Korkama-Rajala et al. 2008, Žifčáková et al. 2011).
Table 1. Distribution of putative lifestyles and substrates among the lineages of the Leotiomycetes. Taxa are assigned into families according to the results of the phylogenetic analysis (I) and a recent classification by Baral (2016) in which old family names have been resurrected.

<table>
<thead>
<tr>
<th>Lifestyle</th>
<th>Substrate/host</th>
<th>Examples</th>
<th>References</th>
<th>(Putative) lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saprobes</td>
<td>dead leaves</td>
<td><em>Heyderia</em>, Rutstroemiaceae, Lachnaceae, Pezizellaceae, Helotiaceae and many others</td>
<td>Hansen &amp; Knudsen 2000, Dennis 1978</td>
<td>Various, see examples</td>
</tr>
<tr>
<td></td>
<td>dead stems of herbaceous plants</td>
<td><em>Crocicreas</em>, Lachnaceae, Mollisiaceae, Helotiaceae s.l., Hyaloscyphaceae and many others</td>
<td>Hansen &amp; Knudsen 2000, Dennis 1978</td>
<td>Various, see examples</td>
</tr>
<tr>
<td></td>
<td>decaying wood</td>
<td>Most families include lignonicolous members</td>
<td>Hansen &amp; Knudsen 2000, Dennis 1978</td>
<td>many</td>
</tr>
<tr>
<td></td>
<td>algae, <em>Phaeofucaceae</em></td>
<td><em>Calycina maritima</em></td>
<td>Baral &amp; Rämä 2015</td>
<td>Pezizellaceae</td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td><em>Hyphodiscus spp.</em></td>
<td>Han et al. 2014</td>
<td><em>Hyphodiscus</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Moserella</em></td>
<td>Pöder &amp; Scheuer 1994</td>
<td>unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Ionomidotis pro parte</em></td>
<td>Zhuang 1988 a</td>
<td>Cordieritiaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Unguiculariopsis, Skyttea</em></td>
<td>Suja et al. 2015</td>
<td>Cordieritiaceae</td>
</tr>
<tr>
<td>Dung</td>
<td></td>
<td><em>Coprotnia</em></td>
<td>Dumont 1975</td>
<td>Sclerotiniaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Thelebolales</em></td>
<td>Landvik et al. 1998</td>
<td>Thelebolales</td>
</tr>
<tr>
<td>Soil</td>
<td></td>
<td><em>Phaeohelotium geogenum</em>, <em>Discinella Podophacidium xanthomelum</em></td>
<td>Hansen &amp; Knudsen 2000</td>
<td>Helotiaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>unknown</td>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>Mosses</td>
<td></td>
<td><em>Bryoscyphus</em>, <em>Hymenoscyphus, Mniaeia</em></td>
<td>Stenroos et al. 2010</td>
<td>Pezizellaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Helotiaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Mniaeia</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hyaloscypha hepatica</em></td>
<td>Baral et al. 2009</td>
<td>Hyaloscyphaceae</td>
</tr>
<tr>
<td></td>
<td>submerged wood</td>
<td><em>Vibrissea</em></td>
<td>Hustad &amp; Miller 2011</td>
<td>Vibrisseaceae</td>
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<tr>
<td></td>
<td>leaves</td>
<td><em>Hymenoscyphus fraxineus</em></td>
<td>Baral &amp; Benmann 2014</td>
<td>Helotiaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Kohninia linnaeicola</em></td>
<td>Holst-Jensen et al. 2004</td>
<td>Sclerotiniaceae</td>
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<tr>
<td></td>
<td></td>
<td><em>Pyrenopeziza brassicae</em></td>
<td>Li et al. 2003</td>
<td>Ploettnerialceae</td>
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<td></td>
<td><em>Rhytismatales</em></td>
<td>Lantz et al. 2011</td>
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<td></td>
<td></td>
<td><em>Erysiphales</em></td>
<td>Braun &amp; Cook 2012</td>
<td>Erysiphales</td>
</tr>
<tr>
<td>Parasites</td>
<td>branches of trees</td>
<td><em>Neofabraea, Pezicula</em></td>
<td>Abeln et al. 2000</td>
<td>Dermateaceae s.s.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Cyttaria</em></td>
<td>Peterson &amp; Pfister 2010</td>
<td>Cyttariaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Gremmeniella</em></td>
<td>Baral 2015a</td>
<td>Godroniaceae</td>
</tr>
<tr>
<td></td>
<td>mosses</td>
<td><em>Discinella, Pezoloma</em></td>
<td>Kowal et al. 2015</td>
<td>Pezoloma</td>
</tr>
<tr>
<td></td>
<td>plant roots</td>
<td><em>Roesleria</em></td>
<td>Kirchmair et al. 2008</td>
<td>Roesleria</td>
</tr>
<tr>
<td></td>
<td>fruits, <em>Rosaceae</em> and others</td>
<td><em>Monilinia</em></td>
<td>Holst-Jensen et al. 1997</td>
<td>Sclerotiniaceae</td>
</tr>
<tr>
<td>Lifestyle</td>
<td>Substrate/host</td>
<td>Examples</td>
<td>References</td>
<td>(Putative) lineage</td>
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<td>-----------</td>
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<tr>
<td>Parasites</td>
<td>herbs, Liliaceae</td>
<td>Medeolaria</td>
<td>LoBuglio &amp; Pfister 2010</td>
<td>Medeolariales</td>
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<td></td>
<td><em>Equisetum</em></td>
<td>Roseodiscus, Stamnaria</td>
<td>Baral &amp; Krieglsteiner 2006</td>
<td>Roseodiscus, Stamnaria</td>
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<tr>
<td></td>
<td><em>Oidiodendron, Amorphotheca, Myxotrichium</em></td>
<td>Wang 2006a</td>
<td>Myxotrichaceae</td>
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<td>Symbionts, endophytes</td>
<td><em>Meloniomyces variabilis, Pezoloma ericae</em></td>
<td>Hambleton &amp; Sigler 2005, Hambleton et al. 1999</td>
<td>Pezoloma</td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td><em>Sarcotrochila, Cenangium, Rhabdocline</em></td>
<td>Grünig et al. 2009</td>
<td>Cenangiaceae</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Phaeomollisia Phialocephala</em></td>
<td>Tanney et al. 2016</td>
<td>Mollisiaceae</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rhytismataceae, Cryptomycetaceae</em></td>
<td>Lantz et al. 2011</td>
<td>Rhytismales</td>
<td></td>
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<tr>
<td>Symbionts, mycorrhizal</td>
<td><em>arbutoid ErM subtype</em></td>
<td>Leotia cf. lubrica</td>
<td>Kühndorf et al. 2015</td>
<td>Leotiaceae</td>
</tr>
<tr>
<td></td>
<td><em>ErM+DSE</em></td>
<td>Acephala applanata</td>
<td>Lukešová et al. 2015, Hambleton &amp; Sigler 2005, Walker et al. 2011</td>
<td>Mollisiaceae, Pezoloma</td>
</tr>
<tr>
<td>aeroaquatic</td>
<td><em>Gyoeffyella, Tricladium, Ypsilina, Filosporella, Lemonierra Rhynchosporium Tetractadium Flagelliospora</em></td>
<td>All Baschien et al. 2013</td>
<td>Pezoloma, ploeotneruloid, Tetractadium, Phacidiales</td>
<td></td>
</tr>
</tbody>
</table>
Nowadays, fungal ecology is usually based on genetic data. Mycorrhizal or endophytic lifestyle, existence in soil or in aquatic environments, and either as mycelium or single conidia, are easily detected using modern DNA sequencing techniques, and such kinds of studies are increasing in number. DNA from the fruitbodies of numerous taxa are sequenced, though a large part of described taxa still lack molecular data. Therefore, it is difficult to refresh taxonomical information regarding the source organism of unidentified strains, resulting in them being named as “Leotiomycetes/Helotiales sp.” or “uncultured ascomycetes” in international nucleotide sequence databases (INSD).

Based on Tedersoo et al. (2014) the phylogenetic diversity of Leotiomycetes in soil is high. According to their analyses of global soil samples, Leotiomycetes represent 7.1% of all fungal groups detected in this environment, and are relatively more diverse in arctic tundra (approximately 25% from total sequences of that biome). In addition, proportions of Leotiomycetes in boreal and southern temperate forests, and grassland–shrublands, exceed the global average proportion, and are least abundant in tropical savannas (Tedersoo et al. 2014). Leotiomycetes can survive in extreme habitats, such as the acidotolerant anamorphic fungus *Soosiella minima* Hujslová & M. Kolářík recently discovered in acidic soil with a pH of <3 (Hujslová et al. 2014). The ectomycorrhizal helotialean fungi often lack latin names and known teleomorphs, and most probably have evolved independently in different geographical regions (Tedersoo 2010). In mycorrhizal symbiosis members of Leotiomycetes do not form the typical Hartig net, with the one exeption being *Leotia lubrica* that forms arbutoid mycorrhiza (Kühendorf et al. 2015).

Globally distributed root endophytes associated with ectomycorrhizae dominate among the soil-inhabiting Leotiomycetes. These are referred to as root associated fungi or dark septate endophytes (Queloz et al. 2011). Quite common among these are the *Phialocephala-Acephala* and *Rhizoscyphus-Melinomyces* complexes, which are host generalists (Vrålstad et al. 2002, Tedersoo et al. 2009). Their effects upon the plant partner range from neutral to negative, and are strain-dependent (Tellenbach et al. 2011, Reininger et al. 2012). It has been shown that the fungi forming ericoid or orchidoid mycorrhiza largely overlap (Bergero 2000, Chambers et al. 2008, Kohout et al. 2012).

One important role of Leotiomycetes in plant communities is the decomposition of plant matter. This process involves many fungi, with early decomposers (in woody substrates often opportunistic basidiomycetes) are gradually replaced by ascomycetes. For instance, *Chlorociboria aeruginascens* causes soft rot while colonizing fallen trunks that have previously been degraded by white rot fungi (Richter & Glaeser 2015). The enzymatic activities of Leotiomycetes as wood-decomposers are not well known. *Calycina citrina* (Hedw.) Gray (Fig. 1f) and *Bulgaria inquinans*, have been associated with low levels of soft rot decay (Worrall et al. 1997), as has *Phialocephala dimorphosphora* (Held 2013).

More than 100 Leotiomycetes species live in aquatic habitats. Many of these that live in freshwater also inhabit terrestrial habitats, such as waterlogged
wood (Dennis 1978, Pfister & Kimbrough 2001). Many species in the Loramyctaceae-Vibrissaceae-Mollisaceae clade, especially in an asexual state, are found on submerged wood or other substrate in aquatic environments (Baschien et al. 2013, Sandoval-Leiva et al. 2014).

Apothecia of encoelioid species mostly form on the bark or wood of various tree species (I Table S3) and saprotrophic or sometimes parasitic lifestyles have been suggested for these fungi (Torkelsen & Eckblad 1977). Encoelia furfuracea grows on recently died standing branches of Corylus.

1.4. Aims

The general aim of the present thesis was to contribute to establishing a phylogeny-based classification of the Leotiomycetes, especially of helotialean fungi.

In particular, the aims were following:
1) to evaluate the applicability of morphological and ultrastructural characters in the systematics of helotialean fungi (species, genus, family level) focusing on genera of the family Lachnaceae, as well as of mollisioid and encoelioid fungi;
2) to reveal the phylogenetic affinities of species included in the subfamily Encoelioideae, and particularly in the genus Encoelia, with a special focus on its type species, E. furfuracea;
3) to establish a phylogeny-based classification of the taxa previously incorporated to Encoelioideae;
4) to synthesize the information on the ecology of members of different helotialean lineages by analyzing together ITS rDNA sequences originating from fruitbodies, culture isolates and complex biological samples using sequences obtained in this study as well as those available in INSD.
2. MATERIALS AND METHODS

2.1. Materials

The studied fungal specimens for encoelioid phylogeny (I) were obtained from the fungaria (acronyms according to Thiers, 2015): BPI, C, CUP, DAOM, FH, K, LD, M, NY, O, OULU, QCNE, S, TAAM, TNS, and TU, and from the private collections of H.-O. Baral, G. Marson, J. H. Petersen, I. Wagner, and E. Rubio Domínguez. For the TEM research (II–VI), living apothecia were collected from nature and kept alive in plastic boxes until fixation. During winter, prefrozen substrates, such as dead Rubus idaeus canes and stems of Filipendula ulmaria, were incubated in vegetation chambers under artificial light and humid conditions (on wetted filter paper) at 20 °C, in order to obtain living apothecia for fixation. For original voucher specimens preserved in TAAM and TU collections, the exhaustive data were entered into the PlutoF platform (https://plutof.ut.ee/ Abarenkov et al. 2010) and data are partly accessible via the public website (https://natarc.ut.ee/en/seenekogud.php).

2.2. Methods

For identification purposes morphological characters were recorded in all studied specimens using LM. After the reconstruction of the multigene phylogeny (I), the features distinguishing monophyletic clades, were outlined for each taxon. The ascus apical apparatus and apothecial hair wall ultrastructure were selected for TEM observations because the resolution of LM was insufficient for characterising these and additional details were expected to be found in their ultrastructure.

2.2.1. Light microscopy

The morphology of the living apothecia and anamorphs was mostly studied with the specimens mounted in tap water. Dry specimens were rehydrated and mounted in a 3% aqueous potassium hydroxide solution (KOH). For staining specific structures, cotton blue (CB, in lactic acid), cresyl blue (CRB, in water), Melzer’s regent (MLZ) and Lugol’s solution (IKI) were used. Ionomidotic reaction (IR) was tested in encoelioid specimens (I) by applying a 3–10% aqueous KOH solution to a water mount of apothecial fragments. Microphotographs and measurements of structural elements were taken from freehand sections or squash mounts using a Nikon 80i microscope.
2.2.2. Transmission electron microscopy

For TEM analysis (II–VI), taxa were sampled from the Lachnaceae and Mollisiaceae, and *Encoelia furfuracea*, whose ultrastructure had not previously been included in comparative studies. The preparations followed Samuelson and Kimbrough’s (1978), and Curry and Kimbrough’s (1983).

- **Fixation of apothecia**: 2 hours using a 2% paraformaldehyde, 2.5% glutaraldehyde, and 2mM calcium chloride in a 0.1M sodium cacodylate buffer solution; post-fixation for 45 minutes in a 1% osmium tetroxide solution in the same buffer.
- **Dehydration**: through a graded ethanol series from 10–90 % and 3×100% solution of EtOH, followed by treatment with acetone.
- **Embedding into Spurr’s resin using an infiltration resin series and acetone in 1:3, 1:1 and 3:1 proportions for ≥4 hours each.**
- **Polymerization for 10–16 hours at 70°C.**
- **Ultramicrotomy**: sections were made mostly using glass knives, except for some specimens when diamond knives were available.
- **Staining of the sections**: 2% uranyl acetate in 50% EtOH and 0.2% lead citrate solution.
- **Examination**: an electron microscope was used with magnifications from 5,000 × to 25,000×.

For more details, see the TEM methodologies in II–VI.

2.2.3. Molecular analysis

The methodological details of DNA extraction from fruitbodies of helotialean fungi, DNA amplification, and analysis of molecular data are given in I. Taxon sampling for multigene analysis was designed to cover the main Leotiomycetes lineages, with an emphasis on the three lineages that were previously members of the Helotiaceae (the *Encoelia furfuracea*, Cordierites, and Chlorociboria lineages), and the families Hemiphaciaceae, Rutstroemiaceae, and Sclerotiniaceae. For multigene phylogeny, genomic DNA was extracted from dried or fresh apothecia. In 70 specimens, selected regions of the nuclear 18S and 28S ribosomal subunits and three protein-coding genes (*tef1*, *rpb1* and *rpb2*) were amplified. The primers used in multigene (combined 18S and 28S rDNA) and ITS analysis are listed in I Table 1. For improving 18S rDNA and *rpb1* amplification, new primers were designed. DNA sequences obtained in I were submitted to the INSD (I Table S1).

Taxon sampling for ribosomal DNA (18S + 28 rDNA) focused on groups of helotialean fungi, members of which were used in the TEM analysis of this study. ITS rDNA taxon sampling included available public sequences with a certain percentage of similarity to the target species. This threshold value differed among studied families, the ITS sequences of which were analysed separately. DNA sequences used in Bayesian phylogeny of ITS rDNA
(Lachnaceae) and 18S+28S rDNA were submitted to UNITE via the PlutoF platform (https://plutof.ut.ee).

For constructing the Bayesian phylogeny of Leotiomycetes, a GTR+I+G evolutionary model was selected and constructed using MrModeltest (Nylander 2004) for most of the partitions. MrBayes v. 3.2.6 (Ronquist et al. 2012) was used to analyse the partitioned five-gene dataset for multigene analysis (I). The analyses were run for 50,000,000 generations using the CIPRES Science Gateway v. 3.3 (http://www.phylo.org), sampling each 1000th generation. By the end of the run, the average standard deviation of the split frequencies had reached 0.01. The first 25% of the trees were discarded as a burn-in, and the posterior probabilities (PP) calculated from the remaining trees.

ITS rDNA was used to test phylogenetic relationships among the members of Lachnaceae and encoelioid taxa. Due to the high variability in the ITS regions, the encoelioid sequences were aligned in separate matrices, conforming to the families studied (I). In addition to the original sequences, the most similar sequences were obtained from the INSD by applying a BLAST search for the target species, which were then added to the respective matrices. These resulting datasets were analysed using MrBayes v. 3.2.6 (Ronquist et al. 2012) in CIPRES. From 10,000,000 generations, 75% of trees were retained and used to calculate the PP. Species Hypothesis (SH) codes in the UNITE database (Kõljalg et al. 2013) were assigned to all ITS sequences generated in this study via the PlutoF platform.
3. RESULTS

3.1. Phylogenetic analyses

3.1.1. Multigene dataset

In the Bayesian phylogeny (I) based upon the analysis of five genes (Fig. 2), most of the terminal clades and many deeper branches, received strong posterior probability support (PP ≥ 0.95). The results revealed the Helotiales to be paraphyletic. While Cyttariales, Thelebolales, Rhytismatales, Phacidiales, and Erysiphales were monophyletic, their relationships with various lineages of helotialean fungi remained unresolved. *Encoelgia* (9 species) and *Encoelioideae* (28 species) appeared to be highly polyphyletic in all analyses. Members of *Encoelioideae* were dispersed among six families and three clades of unclear affiliation.

*Encoelgia fascicularis* and *E. pruinosa* belonged to the Sclerotiniaceae, and these species were transferred to a new genus (*Sclerencoelgia*), together with a new species, *S. fraxinicola*. The sister group of Sclerotiniaceae was Rutstroemiaceae, which included *Encoelgia tiliacea* and *Dencoeliopsis johnstonii*. Due to their close relationship to the type species of *Rutstroemia* (*R. firma*) both species were accepted in *Rutstroemia*. *Piceomphale bulgaroides* and *Cenangium acuum*, whose taxonomy remained unsettled, formed the sister group of the Sclerotiniaceae and Rutstroemiaceae.

The type species of *Encoelgia*, *E. furfuracea*, formed a strongly supported group with species of *Velutarina* and *Cenangiospis* (*Encoelioideae* s. str.), as well as *Trochila* spp. and an undescribed taxon. The sister group of this clade comprised species of *Chlorencelgia* and *Heyderia*, *Sarcotricha longispora* (a Hemiphacidium clade in Wang et al. 2006a), as well as *Crumenulpis sororia*, and *Cenangium ferruginosum*. Altogether the *E. furfuracea* clade, the extended Hemiphacidium clade, and *Rhabdocline laricis*, were considered to represent Cenangiaceae, which thus includes the Hemiphacidiaeae.

Eleven encoelioids (“*Encoelgia*” *fimbriata* and “*E. heteromera**, and species of *Ameghinia*, *Cordierites*, *Diplocarpa*, *Ionomidotis*, *Llimoniella*, and *Unguicularis*) formed a strongly supported clade with four non-encoelioid lichenicolous species. Altogether, the members of this clade were considered to constitute the family Cordieritidaceae Sacc. “*Encoelgia*” *fimbriata* and “*E. heteromera*” were not congeneric and *Ionomidotis* appeared to be polyphyletic.

The Chlorociboriaceae comprised *Chlorociboria* spp. and *Encoelgia glauca*, which was transferred to the former genus. Species of *Chaemomella*, *Pilidium*, and *Xeropilelum dennisii* (=*Encoelgia fucicellii*), formed a strongly supported group representing the Chaetomellaceae. However, phylogenetic relationships of this family remained unresolved.
Bayesian phylogeny of Leotiomycetes inferred from 5 genes (18S, 28S rDNA; tef1, rpb1, rpb2). Species traditionally recognised in Encelioideae are presented in bold, with those of *Encoelis* in capital letters. Species studied with TEM (in II, III, VI) are underlined. Taxa marked with "KL" are sequenced for this study. Sordariomycetes strains represent the outgroup. Branches with posterior probability scores ≥0.95 are presented in bold. Scale bar indicates substitutions per site.
3.1.2. 18S and 28S rDNA dataset

For rDNA (18S + 28S) dataset of Leotiomycetes, sequences of 90 genera, including newly sequenced *Encoelia furfuracea*, *Podophacidium xanthomelum*, the selected members of the Lachnaceae and the *Mollisia-Pyrenopeziza* complex, were merged into the matrix. Four species of Sordariomycetes were chosen to constitute an outgroup. *Mollisia revincta* and *Podophacidium xanthomelum* were represented with only 28S, and *Mollisia dilutella* with only 18S. The 18S + 28S rDNA dataset included 6797 characters; after removing ambiguously aligned nucleotids and long insertions, the matrix comprised 1480 bp from 18S and 1332 bp from 28S, of which 275 and 381 positions were, respectively, parsimony-informative. The Bayesian analysis was run using a partitioned gene dataset.

In the rDNA phylogeny the lineages of Helotiales were intermixed with those of Erysiphales, Rhytismatales, Phacidiales, and Cyttariales (Fig. 3), as in the multigene analysis (Fig. 2). Most of the Leotiomycetes lineages received low support. *Encoelia furfuracea* formed a clade with *Velutarina* spp., *Cenangiopsis quercicola*, *Trochila laurocerasi* and *Cenangium ferruginosum*. This clade formed together with the *Chlorencoelia-Sarcotrochila* clade, *Piceomphale* clade, *Rutstroemiaceae* and *Sclerotoniaeae* clades a well-supported large clade. Its sister group was formed of strongly supported *Cordieritidaceae*.

Lachnaceae was found to be monophyletic with *Phaeohelotium geogenum* as a poorly supported sister group. Within Lachnaceae, the close relationship of *Perrotia populina* to *Lachnellula willkommii* and *L. subtilissima* was strongly supported. *Incrucipulum ciliare* represented a sister group to these three species whereas *Lachnellula abietis* was not closely related to this group, but constituted a sister taxon of *Erioscyphella curvispora* and *Belonidium aeruginosum*. *Capitotricha bicolor*, *Dasycyphella nivea* and *D. cassandrae* formed a sister clade to *Lachnum virgineum* and *L. arcticum*.

The *Mollisiaceae- Loramycetaceae-Vibrisseaceae* clade included *Belonopsis* spp., *Loramycetes macrosporus*, *Mollisia cinerea*, *M. clavata*, *M. melaleuca*, *Tapesia fusca*, *Vibrissa truncorum*, and *Phialocephala fortinii*. Two analyzed strains of *Mollisia cinerea* most likely represent different species. The relationships of *Mollisia* species with other members of Mollisiaceae remained unresolved.

A clade corresponding to the family Ploettnerulaceae Kirschst. (fide Baral 2016) included three species of *Mollisia*, two *Pyrenopeziza* and *Peltigeromyces* sp. *Pyrenopeziza gentiana*, *Mitrula paludosa*, and *Encoeliopsis rhodendri* formed a separate clade. Thus, *Pyrenopeziza* was found to be not monophyletic and further studies are needed to ascertain the phylogenetic relationships of *Pyrenopeziza* species, including its type species, *P. chailletii* (Pers.) Fuckel.

The analysis confirmed the inclusion of *Pezicula carpinea*, *Dermea acerina* and *Neofabreae malicorticis* in Dermateaceae. The affinities of *Trichopezizella nidulus* and *Podophacidium xanthomelum* in the Leotimycetes remained unresolved.
Fig. 3. Bayesian phylogeny inferred from rDNA 18S and 28S sequences of Leotiomycetes. Species studied with TEM (in II–VI) are presented in bold, in capital letters. Sordariomycetes strains represent the outgroup. Taxa marked with “KL” or “AR” are sequenced for this study. Generic types are marked with a. Scale bar indicates substitutions per site.
3.1.3. ITS rDNA dataset

The high interspecific variation hampered unambiguous alignment of ITS sequences from different genera and higher level taxa. ITS phylograms, as exemplified in Fig. 4 and in I, resulted in partly/largely unresolved phylogenies with limited support to deeper nodes. In particular, Bayesian analyses of original ITS sequences of nine species of Cenangiaceae from different genera along with ≥90% similar INSD sequences for each of these, resulted in a largely unresolved tree (I Fig. S1). However, the monophyly of genera and species was strongly supported in case of Heyderia, Rhabdocline, Sarcotrophila and Trochila. Also in Sclerotiniaceae, a clade comprising three encoelioid species of newly described genus, Sclerencoelia was well supported, but the relationships among many sclerotiniaceous genera remained unsettled. The same analysis also supported the idea about the lack of extant close relatives of E. furfuracea (note: the sequence of Velutarina rufoolivacea, the most similar taxon based on morphology, was unavailable). While the sequences of E. furfuracea from Europe and North America were almost identical, these showed only 87% overlap with the most similar INSD sequence, and 88.3 % and to 88 % with Cenangiopsis quercicola and Cenangium ferruginosum, respectively.

The ITS phylogenetic tree of Lachnaceae was poorly resolved, too. INSD BLAST searches were conducted using nine sequences of putatively distinct genera of Lachnaceae. ITS sequences of ≥95% similarity to the queries were added to the matrix including sequences obtained from apothecia. The dataset contained 65 sequences and the Lachnaceae were represented by 46 species and 13 genera. In the phylogenetic tree (Fig. 4) Brunnipila and Lachnellula appeared monophyletic. Most species of Lachnum formed a strongly supported clade, including the type species, L. virgineum. Relationship of Albotricha spp. and Perrotia flammea, type species of Perrotia, remained unresolved. Belonidium aeruginosum formed a clade with “Lachnum” euterpes which was a lineage in a clade including septate-spored segregates of previous Lachnum with 4 species of Erioscypella (as resurrected by Perić & Baral 2014) and “Lachnum” pteridophyllum. Lasiobelonium spp., Trichopezizella nidulus, and Trichopeziza spp. formed a well-supported clade.

Differences in ITS rDNA sequences among members of one genus and family varied considerably. At family level, widest range of sequence variation (15–16%) was observed among Cenangiaceae as delimited in I, Chlorociboriaceae and Chaetomellaceae. ITS analyses revealed several genera not to be monophyletic, these including Ciboria, Chlorenceloa, Chlorociboria, Ruststroemia, Lanzia, Dasyscyphella, Incruophilum, Perrotia and others. In contrast, ITS data supported the distinction of a newly described species, Sclerencoelia fraxinicola, the ITS sequence of which differed from that of its closest relative, S. fascicularis, at 15 positions.
Fig. 4. Bayesian phylogeny based on rDNA ITS sequences of Lachnaceae with Hymenoscyphus spp. as the outgroup. The datamatrrix included sequences obtained from fruitbodies in this study (marked with „KL“ or „AR“), with those studied with TEM (in capital letters) and available sequences originating from fruitbody or culture of INSD (all in italics). INSD environmental sequences with capital letters) and available sequences origin fruitbodies in this study (marked with “KL” or “AR”), with those studied with TEM (in italics). Taxa marked with ♦ are generic types. Scale bar indicates substitutions per site.
Incorporation of public sequences from various biological samples (labelled as ‘uncultured Helotiales/Leotiomycetes/Ascomycota/fungus’ in the INSD) in ITS rDNA analyses allowed to identify sequenced organisms at species, genus, or family level and added information on the ecology of several taxa. For example, INSD sequences originating from needles or twigs of Pinus spp. and Viscum album parasitizing these, could be identified as belonging to Cenangium ferruginosum. Endophytic isolates were also included in Sclerencoelia fascicularis, S. fraxinicola, Xeropilidium dennisii, Heyderia abietis, Rhodocline laricis, R. parkeri. An INSD sequence obtained from the European elm bark beetle (Scolytus multistriatus), the vector of Dutch elm disease, was shown to belong to Xeropilidium dennisii. An isolate from Quercus leaf-litter was congeneric with Belonidium. The genus Lachnum comprised unnamed members sampled from soil, from roots of Pyrola, Rhododendron and Ledum, and from ectomycorrhizae.

The ITS sequences of helotialean fungi generated in this study were assigned to 41 Species Hypotheses (SH, Kõljalg et al. 2013) according to the 1.5% distance treshold. More than half of the ITS sequences generated in I (40 out of unique 73 sequences) had also no >97% similar sequences available. New SHs were generated for these sequences in the 7.1 version of UNITE SHs (https://unite.ut.ee).

In several cases ITS sequences from biological samples formed lineages devoid from, but closely related to groups including apothecia-derived sequences. For example, sequences from EcM root tips or litter of conifers were closely related to C. versiformis and C. torta. Cenangium ferruginosum clade comprised sequences from surface sterilised tissues of conifers, a forest grass, a liverwort and a lichen. Sclerotiniaceae and “Rutstroemia” calopus clade included lineages of INSD sequences originating mostly from soil samples. In addition, three strongly supported groups with unresolved relationships in Cenangiaceae comprised sequences only from endophytes, mostly originating from roots or soil.

### 3.2. Evaluation of characters

Delimitation of monophyletic lineages comprising encoelioid fungi revealed the importance of observing the complex of morphological characters of apothecia, and of avoiding the overestimation of the importance of one or a few characters when aiming at a natural classification. The members of each studied lineage could be delimited according to a typical combination of characters (I Table S3). Namely, most of the monophyletic groups observed in the multigene analysis (Fig. 2) differ with respect of the type of the ascus apical structure, the presence/absence of an ionomidotic reaction, the characteristics of the asexual state (if the anamorph was studied), and vacuolar bodies (VB) in living vegetative cells. However, in some lineages one or a few characters varied among closely related species/genera.
3.2.1. The ascus apical apparatus

The ascus apical apparatus was studied in 21 species of Lachnaceae, mollisioids, and Encoelia. As a result, five main types of ascus ultrastructure were distinguished (Table 2). In general, taxa that were closely related in the 18S and 28S rDNA phylogeny (Fig. 3) and available for TEM studies, shared the general structure of ascus apex. These could be assigned to the types distinguished by Verkley (1995b). Type VIII, (Chlorociboria-Pezizella-Calyccina ascus type) included members of the Lachnaceae except for Lachnellula (III–IV and Fig. 5b, c), and Mollisia spp., Pyrenopeziza spp., Belonopsis hydrophila (Fig. 5e and VI). However, the latter three genera sharing a morphologically similar mollisioid subtype of ascal apex, were distributed among three lineages (Fig. 3). Encoelia (Fig. 5a), Lachnellula (Fig. 5d), and Podophacidium (Fig. 5g), belonging to three lineages (Fig. 3), each represented an unique type of ascus apex ultrastructure (II, V, VI), not described in previous literature. The Pezicula type was published by Bellemère (1977), and specific ontogenesis and annulus (Fig. 5f) were described in VI. Pezicula is placed in Dermateaceae clade (Fig. 3).

Table 2. The ascus apical apparatus characteristics of the studied fungal species in comparison with Baral’s (1987b, LM) and Verkley’s (1995b, TEM) typifications. Lineages of taxa are presented according to rDNA phylogenetic analysis (Figs. 3–4).

<table>
<thead>
<tr>
<th>Species; lineage</th>
<th>Ascus shape/ apex shape (LM)</th>
<th>Annulus type/ amyloidity in LUG (LM)</th>
<th>Ascus apical apparatus (TEM) Comparative type according to Verkley in Roman numera</th>
<th>paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encoelia furfuracea; Cenangiaceae/ Encoelia</td>
<td>cyl cl with long narrow stipe/ ro–tr</td>
<td>Calycina-like/ I+, bb</td>
<td>Encoelia type. Apical thickening increases gradually, annular protrusion absent, annulus is homogenous, relatively broad, narrowing downwards.</td>
<td>II</td>
</tr>
<tr>
<td>Lachnum brevipilosum; Lachnaceae/ Lachnum</td>
<td>cyl cl/ co–subpapillate</td>
<td>Calycina-like/ I+, bb</td>
<td>t VIII, Lachnum st. Apical thickening moderal, distintct annular protrusions, tapering and becoming more electron-dense toward lower end. Apical chamber quite high.</td>
<td>III</td>
</tr>
<tr>
<td>Dasysscyphella cassandrae; Lachnaceae</td>
<td>cyl cl/ co–ro</td>
<td>Calycina-like/ I+, bb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brunnipila clandestinum; Lachnaceae/ Brunnipila</td>
<td>cyl cl/ co–tr to subpapillate</td>
<td>Calycina-like/ I+, bb</td>
<td>As in the t VIII, Lachnum st, but annular protrusion more blunt</td>
<td></td>
</tr>
<tr>
<td>Albotricha acutipila; Lachnaceae</td>
<td>cyl cl/ subpapillate</td>
<td>Calycina-like/ I+, bb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capitortricha bicolor; Lachnaceae</td>
<td>cyl cl/ co–tr</td>
<td>Calycina-like/ I+, bb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incrucipilum ciliare; Lachnaceae/ Incrucipilum</td>
<td>cyl cl/ ro</td>
<td>Calycina-like/ I+, rb</td>
<td>t VIII, similar to Lachnum, but annulus broadening upwards and electron-dense apical cap (nasse apicale). Apical chamber more rounded than those of Lachnum</td>
<td>IV</td>
</tr>
<tr>
<td>Belonidium aeruginosum; Lachnaceae/ Belonidium</td>
<td>cyl cl/ co–tr</td>
<td>Calycina-like/ I+, bb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species; lineage</td>
<td>Ascus shape/apex shape (LM)</td>
<td>Annulus type/amylolidity in LUG (LM)</td>
<td>Ascus apical apparatus (TEM) Comparative type according to Verkley in Roman numerals</td>
<td>paper</td>
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<tr>
<td>Lachnellula willkommii; Lachnaceae/ Lachnellula</td>
<td>cyl cl/ blunt, tr–ro</td>
<td>NA/ I–</td>
<td>Lachnellula t. Apical thickening abruptly becomes to annulus. Annular protrusion incurved, apical camber and central cylinder wide.</td>
<td>V</td>
</tr>
<tr>
<td>Belonopsis hydrophila; Mollisiaceae/ Belonopsis</td>
<td>cyl cl/ co–tr</td>
<td>Calycina-like/ I+, bb</td>
<td></td>
<td>VI</td>
</tr>
<tr>
<td>Mollisia clavata; Mollisiaceae</td>
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<tr>
<td>Mollisia stromaticola; NA</td>
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<tr>
<td>Mollisia revincta; Ploettnerulaceae(^1)</td>
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<tr>
<td>Mollisia dilutella; Ploettnerulaceae</td>
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<tr>
<td>Pyrenopeziza millegrana; NA</td>
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<tr>
<td>Pyrenopeziza pulveracea; Ploettnerulaceae</td>
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<tr>
<td>Pyrenopeziza rubi; Ploettnerulaceae</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mollisia melaleuca; Mollisiaceae</td>
<td>cyl cl/ co–tr,</td>
<td>Calycina-like/ I+, bb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mollisia ramealis; Cenangiaceae? (^2)</td>
<td>cyl cl/ co–tr</td>
<td>Calycina-like/ I+, rb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pezicula cinnamomea; Dermateaceae/Pezicula (Verkley 1999)</td>
<td>cyl cl/ ro</td>
<td>Pezicula-like/ I+, rr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: cylindrical = cyl; clavate = cl; conical = co; I+, bb = euamyloid; I+, rb/rr = hemiamyloid; I– inamyloid; NA = data not available; st = subtype; rounded = ro; truncate = tr; type of ascus = t

\(^1\) Based on the phylogeny in Crous & Groenewald (2003), M. revincta is a member of the Mollisiaceae.

\(^2\) Based on BLAST search of ITS rDNA, this species is likely a member of Cenangiaceae.
Fig. 5. Studied types of ascus apical apparatus, the schematic representation
a Encoelia furfuracea. b Lachnum virgineum. c Belonidium aeruginosum. d Lachnellula willkommii. e Belonopsis hydrophila. f Pezicula cinnamomea. g Podophacium xanthomelum.

3.2.2. Apothecial hair ultrastructure

Owing to the increased magnification, the micrographs of the hairs obtained using TEM, in particular complemented the characteristics of the ornamentation of walls. This allowed for better comparison with related taxa than is possible using LM. The hair ultrastructure in each lineage was characterized by a similar thickness, stratification, and ornamentation of the wall. The refracted or pigmented areas seen in LM, differed in electron density under TEM. This allowed to refine the description of hair ultrastructure in the following lineages of Leotiomycetes:

Hyaloscyphaceae, sensu Han et al. (2014)
The hair walls of Hyaloscypha aureliella (Nyl.) Huhtinen, Olla millepunctata (Lib.) Svrček and Unguiculella hamulata (Feltgen) Höhn. (Fig. 6 a–c), were thin and unclearly stratified, and the refractive parts of the hairs (under LM, Olla and Unguiculella) were electron-transparent under TEM.
Fig. 6. Hairs of helotialean species, as observed under TEM
a–c Hyaloscyphaceae s.str.: a Hyaloscypha aureliella TAAM165603. b Unguiculella hamulata TAAM165350. c Glassy apex of hair. Olla millepunctata TAAM164053. d Pezizellaceae: Phialina ulmariae TAAM165353. e Lachnaceae: Lachnellula calyciformis TAAM165524, f–g Trichopeziza lineage: f Lasiobelonium variegatum TAAM165343. g Trichopezizella nidulus TAAM165352. b, e, f–cross-sections, a, c, d, g–longitudinal sections. Bar = 1µm, except for a 5µm.
The Pezizellaceae lineage (according to Baral & Rämä 2015)
Phialina ulmariae (Lasch) Dennis, which based on morphology belongs (the DNA barcode was unavailable) to the recently resurrected Pezizellaceae Velen., showed under TEM very homogeneous electron dense regions (Fig. 6d) in the apical part of hairs, where yellow vacuolar bodies were observed under LM.

The Lasiobelonium-Trichopeziza-Trichopezizella lineage (Fig. 3)
In contrast to the Lachnaceae, in the Trichopeziza lineage the outer layer of the hair wall was very electron dense, as examplified by Lasiobelonium variegatum (Fuckel) Raitv. and Trichopezizella nidulus (J.C. Schmidt & Kunze) Raitv. (Fig. 6 f–g).

Lachnaceae (III–V)
Seven genera and nine species of warty members of the monophyletic Lachnaceae clade (Fig. 3), had more complex hair walls than members of the Hyaloscyphaceae that were available for comparison. The studied Lachnaceae members also differed in hair wall thickness, stratification, warts’ shape, electron-density, and erodibility. The hair ultrastructure of Lachnum brevipurposum and L. virgeneum, as well Brunnipila clandestinum and B. calyculiformis were highly similar in respective genera. Although sampling was restricted, a genus-specific pattern can be suggested, because the genera Albotricha, Belonidium, Brunnipila, Capitotricha, Dasyscyphella, and Incruci- pulum were all represented by their type species (Fig. 6e and Figs. in III–V).

3.2.3. Vacuolar bodies
Vacuolar bodies (VB, as introduced by Baral 1992) in the apical part of the paraphyses or outer excipulum cells occured in some helotial groups. VBs of studied taxa were either hyaline, bright yellow or greenish, globose or elongated. These were affirmed as taxonomically informative and represented the main synapomorph in the resurrected family Cenangiaceae (I). The morphology of the mostly elongated VBs were lineage-specific, e.g. pigmented in Cenangiaceae and hyaline in Mollsia spp., but in the latter group vacuolar bodies turned yellow in KOH. According to Baral (2016), cylindrical refractive VBs in the paraphyses apex occur in Mollisia but are absent in Pyrenopeziza and Pirottaea.

3.2.4. Anamorphs and stromata
The anamorphs clearly indicated the previous misplacement of following species of Encoelia (I): a) Chlorociboria glauca apothecia were observed with Dothiorina asexual morphs on the same substrate, and b) Xeropilidium dennisii synanamorphs (sporodochial conidiomata in culture and pycnidial conidiomata on bark along the apothecia).
Sclerotium-like structures of the genus *Sclerencoelia*, were described here for two previous *Encoelia* members, *E. fascicularis* and *E. pruinosa* and a new species (I). These structures were hidden under the apothecia in the substratum under the bark of trees. This fact pointed to an additional reason to accept these taxa, whose apothecia emerge from sclerotia or stromatized plant debris, into the Sclerotiniaceae. Typical for Rutstroemiaceae, the stipe base of apothecia of *Rutstroemia tiliacea* was blackish brown, and arose from indeterminate dark substratal stroma. The latter was visible as a black line in wood cross-section under the apothecia.

3.2.5. Ionomidotic reaction

Studying the genus *Ionomidotis*, Korf (1958) introduced the term ionomidotic reaction (IR) for a chemical reaction whereby aqueous potassium hydroxide solution (KOH) extracts pigments from fungal tissues. The pigments are released into the medium seconds after adding KOH to a microscope slide of fungal preparate. IR can be detected also in the dried fungal specimens of collections. However, the chemical background of this reaction has yet to be studied among the Leotiomycetes. In the current work, IR was observed in most members of the monophyletic Cordieritidaceae (I). IR can be considered as the main synapomorph in this family, where morphological characteristics are quite deviating. The colour resulting IR, however, differed among the members of Cordieritidaceae. For example, in species of *Ionomidotis irregularis* and *Diplocarpa curreyana*, the extracted pigments were purple, whereas the IR of taxa related to *Ameghiniella* was ochraceous. However, *“Encoelia” heteromera* and “*E*. fimbriata”, belonging to different lineages, had a golden-yellow reaction. It can be concluded that among the Cordieritidaceae, IR is a valuable characteristic for discriminating genera. However, solitary IR+ exceptions in generally IR- families were observed, e.g. in Lachnaceae (*Brunnipila calyciformis*, pinkish IR) and Cenangiaceae (*Cenangium ferruginosum*, peach-colored IR). The basal part of *Belonidium aeruginosum* (Lachnaceae) hairs and outer excipulum turn lilac in KOH, distinguishing it from the morphologically similar genus *Incrucipulum*. Based on references, IR is known to occur in *Godronia* spp. and members of Dermateaceae s. str. (Baral 2016).

3.2.6. Ecology

The rDNA ITS phylogeny of Leotiomycetes allowed to make the following observations (compare with Table 1):

An endophytic lifestyle is quite common in the Cenangiaceae, whereas it is almost entirely absent among its sister families Rutstroemiaceae and Sclerotiniaceae (I Figs. S1–S4). Many INS sequences of Cenangiaceae originated from the leaves and roots of coniferous trees (I Fig. S1). ITS analysis provided strong evidence for the occurrence of *Cenangium ferruginosum* as an endophyte.
in pine needles and twigs, as well as in *Viscum album* parasitizing pines. ITS phylogeny supported the distinction of the endophytic *Rhabdocline parkeri* from the pathogenic *R. pseudotsugae, R. epiphylla, R. oblonga, and R. obovata*.

In the Sclerotiniaceae, the inclusion of the newly described genus *Sclerencoelia* expanded the concept of the ecology of this family to include lignicolous members. A sequence originating from the shoots of *Fraxinus* spp. was assigned to *Sclerencoelia fraxinicola* (Fig. 2), providing additional evidence for the distinction of this supposedly *Fraxinus*-restricted species from its siblings that grow mainly on *Populus* spp. The apothecia of *Sclerencoelia fraxinicola* grew on recently dead branches. *S. pruinosa* was found to act as an intensive parasite (Anonymous 2011), whereas own observations about *S. fascicularis* pointed only saprotrophic occurrence.

Inclusion of *Rutstroemia* (=*Dencoeliopsis*) *johnstonii* expanded the Rutstroemiaceae to include a fungicolous species. Rutstroemiaceae split into two groups: a) the *Rutstroemia firma* clade comprising species growing on fallen branches and leaves of trees, or on fruits, which also includes *R. johnstonii*; and b) the clade of species related to *Rutstroemia calopus*, whose apothecia form on monocot stems; this group included many DNA sequences obtained from soil in various habitats (Fig. S4).

The Chaetomellaceae was expanded by the inclusion of desiccation-tolerant species with a xylicolous lifestyle, transferred to a new genus *Xeropilidium*. The others members of this family are desiccation-sensitive and parasitic or saprobic on leaves, stems, or fruits of dicots (Fig. S6).

Analysis of Lachnaceae revealed an INSD sequence from *Quercus deserticola* leaf-litter, closely related to *Belonidium aeruginosum*, which also inhabits oak leaves. The *Lachnum* clade included sequences from soil, roots of Ericaceae, and from ectomycorrhizae of herbaceous plants (Fig. 4).

### 3.3. Taxonomical novelties

**Resurrected families.** The phylogenetic analyses in I distinguished two monophyletic groups of helotialean fungi without a name in current use at the family rank. However, as old family names were available for some members of these groups, two names were resurrected and applied to these groups while expanding the concept of respective families.

1. **Cenangiaceae** Rehm 1888 was the sister group of Sclerotiniaceae and Rutstroemiaceae, and was emended by Baral & Pärtel, using additional information regarding neglected morphological characteristics, e.g. refractive vacuolar bodies of the vegetative cells. Beside *Velutarina, Encoelia, Cenangium* and *Cenangiopsis* (Rehm’s original genera *Cenangiaceae*), relationships with members of the family *Hemiphacidiaceae* (Korf 1962) were affirmed in the current work. The hymenium in the premature apothecia of many taxa of Cenangiaceae s. str. and former Hemiphacidiaceae is initially protected in unsuitable dry conditions by inrolled margins or by a
membraneous lid (Fig. 3). Members of the Cenangiaceae grow as endophytes, saprobes, or parasites, and inhabit wood or needles of conifers.

2. Cordieritidaceae Sacc. 1889 was originally described to include helotialean fungi with leathery, carbonaceous apothecia developing from a common or branched and often excentric stipes. The current work, however, showed more extended morphological variation. Many members of this group have an ionomidotatic reaction or change the colour of their excipulum in KOH. Cordieritidaceae species are lignicolous, lichenicolous, fungicolous on ascomycetes, or co-occur with certain fungi.

New species and genera, and new combinations

Chlorociboria glauca (Dennis) Baral & Pärtel (Chlorociboriaceae)
   Basionym: Encoelia glauca Dennis 1975

Genus Sclerencoelia Pärtel & Baral (Sclerotiniaceae)
   Sclerencoelia fraxinicolae Baral & Pärtel
   Sclerencoelia fascicularis (Alb. & Schwein.) Pärtel & Baral (neotype selected)
      Basionym: Peziza fascicularis Alb. & Schwein. 1805
   Sclerencoelia pruinosa (Ellis & Everh.) Pärtel & Baral
      Basionym Dermatea pruinosa Ellis & Everh. 1888

Genus Xeropilidium Baral & Pärtel (Chaetomellaceae)
   Xeropilidium dennisii Baral, Pärtel & G. Marson
4. DISCUSSION

4.1. Distinction of monophyletic groups of helotialean fungi

The results of this study showed that the taxonomy of studied helotialean fungi has suffered from reliance on convergent morphological characters. Evidence on this was provided by the genus *Encoelia*, members of which were distributed across major lineages of Leotiomycetes based on the multigene phylogeny. One of such lineages, representing the family Chaetomellaceae, might even not belong to the Leotiomycetes as its phylogenetic relationship remained unresolved in the multigene phylogeny. The sampling used for multigene analysis was more extended in terms of genes and taxa than in previously published phylogenies of the Leotiomycetes, despite it being uneven for various lineages due to the focus on encoelioids. Hibbett et al. (2007) commented in their fungal classification, that Leotiomycetes is one of the most undersampled higher taxa among the Ascomycota, and predicted the creation of additional orders after more extensive molecular sampling. Until now, the situation has not changed much and the polyphyletic Helotiales is used *sensu lato*.

The present work contributed to establishing a phylogeny-based taxonomy of Leotiomycetes by accumulating molecular data of the genera thus far classified in the Helotiaceae. Moreover, a distinct clade of Leotiomycetes was found that could be described as a new order, the Sclerotiniiales. This lineage includes members of the Sclerotiniaceae, Rutstroemiaceae, the Piceomphale clade), and Cenangiaceae. The Sclerotiniiales lineage was affirmed as clearly unrelated to the Helotiaceae *s.s.*, the core group of the Helotiales. However, we preferred to postpone describing the new order until experts of different taxa will contribute additional DNA sequences from well studied voucher specimens that would enable to construct a new order-level classification for the major part of Leotiomycetes.

4.2. Ultrastructural characters of helotialean fungi

Phylogenetic analyses and morphological observations, including ascus ultrastructure, enabled to re-evaluate the diagnostic characters thus far used for the delimitation of the families Helotiaceae, Hyaloscyphaceae, and Dermateaceae. It can be summarized that for completing historical taxon descriptions of helotialean fungi it is necessary to study the type of the ascus apex. Whenever possible, living specimens should be used for detecting characters that may disappear in dried vouchers and for obtaining the anamorph stage in culture.

Distinct patterns of ascus apical apparatus characters were detected in families/lineages of Helotiales, and in general these proved to be informative for the taxonomy. Some types of the ascus apical apparatus were distributed
among many lineages, whereas others were unique. Ascus apparatus type VIII (Verkely’s (1995b) appeared to be widely distributed in unrelated lineages, such as the Lachnaceae (III–IV, Verkley 1996), Chlorociboriaeae (Verkley 1993b), Pezizellaceae (Calycina, “Hymenoscyphus” herbarum, and Pezizella, Verkley 1993b), Mollisiaceae, and Ploettnerulaceae (VI). This work supported the conclusion of Verkley (1995b) that the ascus annulus amyloidity of the Helotiales observed in LM correspond to the most electron-dense structurally differentiated areas in the TEM micrographs. This enables one to compare the general apical apparatus morphology obtained by TEM and LM. However, owing to the size of the annulus (approximately 3 µm wide), light microscopy has limitations for observing details, especially in cases when the amyloid reaction is absent/very weak or very strong (overshadow). For example, the ascus apical apparatus of Encoelia furfuracea could not be distinguished from that of Calycina until using TEM. LM can be useful for characterizing the ascus apex, if illustrations are presented along indication which chemicals have been used for testing the amyloidity (see e.g. Baral 1987b). Without figures of ascus apex it is nearly impossible to compare the ascus apex characters of different helotialean taxa.

**a) Cenangiaceae**

*Encoelia furfuracea* placement in the Cenangiaceae was in accordance with the morphological similarity of related fungi, especially *Velutarina rufoolivacea*. In general, the fruitbody’s macroscopical depiction, as illustrated in Fig. 3, can vary largely among the Cenangiaceae. Under LM, the ascus apices showed different amyloidity among genera. Many Cenangiaceae members were with euamyloid annulus, but some were hemiamyloid or inamyloid. For example, *Velutarina rufoolivacea* is hemiamyloid whereas *V. bertiscensis* is inamyloid (Baral & Perić 2014). Such variation has also been observed in the genera *Sarcotrochila* and *Rhabdocline* (Stone & Gernandt 2005). *Encoelia furfuracea* had a well-developed ascus apparatus (II), whereas *Cenangium ferruginosum* has a strongly reduced apical apparatus (Verkley 1995a), with a recognizable apical chamber and annulus, but which do not function during dehiscence. According to Verkley (1995a), the ejaculation of the ascospores instead occurs via an irregular slit next to the apical apparatus, which is unique among the helotialean fungi.

In the sister families Sclerotiniaceae and Rutstroemiaceae, the characters of the ascus apex were similar in these groups under LM and TEM. The asci were mostly euamyloid, with one ascus apical type characterized thus far (Verkely 1993a). Spooner (1987) has proposed a correlation between the presence of stromatic tissues, and a long and narrow ascus pore. In support of this idea, the length of ascus apical thickening was observed as relatively short in Cenangiaceae, a closely related family, whose members are non-stromatic. However, the extent of the variation of the apical apparatus among the Cenangiaceae and its differences from those in Sclerotiniaceae and Rutstroemiaceae remain unknown.
b) Cordieritidaceae

The Cordieritidaceae lineage is one of the few monophyletic lineages (beside Ascocorticaceae, Chaetomellaceae, and Loramyces and Roesleria lineages), whose known members have inamyloid asci that lack the observable ascus apical apparatus due the absence of annulus. This is significant variation comparing to their sister clade, Sclerotiniales lineage. In this family, the asci were observed to be apically rounded, and with thickened apical wall in some taxa. Verkley (1995a) has shown unique ascus dehiscence by the lid for “Encoelia” fimbriata, the only member of the Cordieritidaceae studied with TEM. The thickened ascus wall structure is probably caused by the repeated desiccation and rehydration of the longeval apothecia in nature according to Verkley (1995a). Encoelia furfuracea shares the longevity and retracting of apothecia in unsuitable conditions with “E.” fimbriata, but has a different ascus lateral wall and opening mechanism (II), which indicates that the ascus ultrastructure of helotialean fungi apparently do not show direct adaptation to the xero-tolerance. Besides the ascus characters, the ionomiotic reaction in Cordieritidaceae was observed as unique. Baral et al. (2015) noticed that the presence of vacuolar bodies is negatively correlated with IR, and VBs are never seen together in the same taxa/lineage. Further studies could detect how Cordieritidaceae species eject spores, and whether the discharge is more passive compared to taxa with a well-developed ascal apparatus.

The family Cordieritidaceae includes genera in which apothecia vary from tiny immersed perithecioids to apothecia 10 cm in diam (Fig. 1k), and that are lignicolous, fungicolous or lichenicolous. The type genus Cordierites is comprised of tropical species with cupulate brownish apothecia that arise from branched stipes; it is lignicolous, but associated with Xylariales (Zhuang 1988). In phylogenetic analyses (I, Peterson & Pfister 2010, Suija et al. 2015), Cordieritidaceae has been distinguished as a strongly supported group. It is likely that adaptation to a possible fungicolous lifestyle has created the morphological diversity in this group.

c) Lachnaceae

In this family, the ascus apical apparatus was represented by two types, one in the Lachnellula (V) and the second in Lachnum-related taxa (III–IV, Verkley 1996), but intergeneric variation was described for hair walls using TEM (III). The ITS rDNA phylogeny of Hyaloscyphaceae s.l. (Cantrell & Hanlin, 1997) and subsequent works with extended gene-sampling (Hosoya et al. 2010, Han et al. 2014), have demonstrated different hyaloscyphaceous lineages and multiple origins of the hairs among the Helotiales. Until now, molecular sampling has been quite occasional among hairy helotialean fungi. Here (III–IV), additional evidence was offered to support the distinctness of the family Lachnaceae and Hyaloscyphaceae based on the TEM characters of hairs. All studied members of Lachnaceae formed a monophyletic group (Fig. 3), and monophyly of most of the studied genera (Lachnum, Lachnellula, Brunnipila, Incrucipulum and Albotricha) was supported by ITS phylogeny (Fig. 4, compare Hosoya et al.
2010, Perić & Baral 2014). As congruent with TEM studies, Brunnipila species with unique pigmented hair wall formed a distinct clade. More extensive sampling with molecular methods is needed for the delimitation of Albotricha, Capitotricha, and Dasyscyphella. TEM studies of the excipular hairs in Lachnaceae offered a more detailed view of the cell wall stratification and ornamentation compared with studies published based on scanning electron microscopy (Hain 1980).

Belonidium aeruginosum was not closely related to Incrucipulum according to the rDNA phylogenetic analysis (Figs. 3–4). This fact rejected the hypotheses proposed in IV, which was based on hair wall and ascus ultrastructural characters of the type species in both genera, Belonidium aeruginosum (IV) and Incrucipulum ciliare (III). Based on phylogeny, B. aeruginosum belonged to a complex of species having elongated ascospores. The apical cap (nasse apicale sensu Bellemère 1977) was present in Belonidium aeruginosum and Incrucipulum ciliare as shown under TEM. Vibrissea (Vibrisseaceae) is the only other genus that has this structure of the helotialean fungi, as illustrated in a micrograph of V. decolorans (Bellemère, 1977: 244) and the LM figure of V. truncata (Baral 1987b, Fig. 17). Our results on B. aeruginosum and I. ciliare provide new evidence of homoplasy of ultrastructural characters in Lachnaceae, complementing those of Hosoya et al. (2010: Table 4) acquired using LM. Further sampling is needed for taxa with elongated spores like Eriocystylla species, to establish monophyletic lineages and delimit genera in Lachnaceae.

d) Dermateaceae compared to Mollisiaceae and Ploettnerulaceae

Dermateaceae s. str. was monophyletic, and characterized by mostly a hemiamyloid ascus apex of a specific structure (VI). The Mollisia-like fungi, even though sharing a similar ascus apparatus among Belonopsis, Pyrenopeziza, and Mollisia (VI), appeared to belong to not closely related groups based on their rDNA (Fig. 3). Taxon sampling of mollisioid species was limited in this work, and further phylogenetic studies are needed to reveal their phylogenetic relationships. However, the species of Mollisiaceae studied in this work were distinct from those in the Ploettnerulaceae. Mollisia pro parte and two Belonopsis species were related to the Loramycetaceae-Vibrisseaceae-Mollisiaceae (Fig. 3) clade, as shown for Mollisia in other published phylogenies (Wang et al. 2006b, Grünig et al. 2009).

In the Ploettnerulaceae, some original strains of Pyrenopeziza spp., “Mollisia” dilutella and “M.” revincta, complemented the list of members of this lineage. Designation of reference sequences from well-studied voucher specimens is critical for identification of mollisioids species. At present many misidentified entries from this group occur in INSD. For example, Mollisia cinerea is represented by several deviating ITS rDNA sequences. In the mollisioid complex, epitypification and neotypification of generic types is needed to introduce meaningful names for the phylogenies at higher levels.

The diagnostic importance of the content of the paraphyses, as introduced by Baral (1992), was confirmed for certain lineages. According to Beckett et al.
(1974), the cell vacuoles may have many functions, and these organelles have been mentioned as having the most variable structure in cells (Riquelme et al. 2011). The published descriptions of helotialean fungi often miss out this characteristic, because it is only visible in living material. This can be a reason for misidentification of macroscopically similar taxa throughout the mollisioids.

### 4.3. Ecological patterns

The role of helotialean fungi in nature is complex. Different lifestyles alternate during the life of a fungus, the switches are likely determined by senescence or weakening of the host the mycelium is living in. The host range of an ascomycete can be broader in the endophytic than in the saprotrophic stage when fruitbodies are formed (Sieber 2007). This work offered some examples of this trend: a common aspen-dwelling species, *Selerencoelia fascicularis*, was found from pine needles based on an INSD sequence. Similarly, Tanney et al. (2016) described the life history of *Phialocephala* spp.: a vegetative endophytic stage occurs in *Picea* leaves, followed by a saprotrophic anamorphic stage on non-foliar substrates (angiosperm fallen branches, intact to decayed), and the formation of a teleomorph on the same substrate. A common toolbox of genes in Sclerotiniaceae, necessary for plant symbiosis, was shown to be selectively expressed during these different lifestyle stages (Andrew et al. 2012).

In case of encoelioid fungi that form tough apothecia on still attached, recently dead branches, the sequence-based discovery of mycelium in their substrates seems rather predictable. In the Cenangiaceae, INSD data revealed that its members commonly grow as endophytes or parasites in leaves and roots of coniferous trees. An endophytic lifestyle is widely distributed among the Leotiomycetes (Wang et al. 2006a), however the current study concluded that it does not define the morphology of associated apothecia, as suggested by Wang et al. (2009). We do not know whether the ability to inroll the apothecia of Cenangiaceae is an adaptation to survive in arid conditions or it has rather evolved to protect the structures, related to reproduction and dissemination, from insects.

The sequences from roots and soil, including ectomycorrhizal samples, were found in lineages related to genera *Lachnum* and *Chlorenceoelia*. Many species of these genera form apothecia on decorticated branches, that lie on the ground, in close contact with soil. It is unknown how many of these fungi lack reproductive structures or whether these have not yet been discovered or sequenced. Based on this study, the mycorrhizal symbiosis is uncommon in Cenangiaceae and *Chlorenceoelia* represents an atypical member of this family in respect of ecology.

This work detected a wider substrate range than previously known in some of the helotialean lineages. One example was the addition of the caulicolous saprobe *Chlorociboria aeruginella* to the well-known lignicolous Chlorociboriaceae (I, compare with Johnston & Park 2005). According to Johnston &
Park, *Chlorociboria* is more diverse in the southern hemisphere, but further evidence is needed regarding whether a shift to a herbicolous substrate occurred in the northern hemisphere.

The substrate spectrum of all helotialean fungi is quite wide and is yet randomly sampled with molecular methods. Most probably hyphae inside the substratum precede fruitbodies in the majority of the helotialean fungi, and that could be detected by molecular methods. It seems that sequences from living deciduous trees are currently less represented than those from conifers in public databases. The search for similar sequences for taxa forming fruitbodies on deciduous substrates (*Encoelia furfuracea*, *Dasyscyphella* spp., *Incrucipulum* spp., *Lasiobelonium* spp., *Trochila* spp.) did not result in finding close matches from endophytic organisms in INSD. However, the studied group of fungi may play an important role in the decay of plant material in natural environments, but their task in the living plant tissues could be similarly important.

### 4.4. Methodological suggestions for the future

- The incubation of substrates (mostly decaying plant material) is useful to be able to study living fungi, especially in case of ephemeral apothecia. In this way the apothecia can easily be initiated to observe their morphology. For temperate and boreal zones, the substrate should be frozen before incubation to follow natural seasonality necessary for formation of apothecia.
- Chemotaxonomy should be given more attention as biochemical differences are likely to provide additional synapomorphies for distinguishing taxa of Leotiomyceses. Determination of KOH soluble pigments should be useful for taxonomic analysis of the members of Leotiomyces, as has been done in studies of Sordariomycetes (see the review by Stadler 2011). Cell wall components should be investigated in the Cyttariales related lineages to find out whether these lack chitin like *Cyttaria* (Oliva et al. 1986).
- TEM provides informative characters for taxonomy, but its relevance is currently limited owing to a lack of comparative studies at larger taxonomic scale. It is quite unrealistic to suppose that usage of this method will increase much in the future because of the time, resource, and skill demands. Therefore, more precise LM observations, including those of the ascus apex, are recommended for refining the taxonomy of helotialean fungi.
- Due to the taxonomic value of vacuoles in paraphyses and excipular tissues, it would be very important to study their ultrastructure, chemical content, and function(s).
- Specific primers should be designed for amplification of genes containing insertions (e.g. 18S rDNA in Leotiomyceses).
- For species identification, rDNA ITS sequencing should be more extensively used among the helotialean fungi. This method is independent of specimen alteration during drying (loss of original apothecium shape and
colour, disappearance of some micromorphological characters). It also allows one to compare sequences from complex biological samples, deposited in public databases in order to complement the fruitbody-based information on the ecology lifecycle for these fungi.

- The UNITE (https://unite.ut.ee/) platform should be used to develop further ITS rDNA barcode-based Species Hypothesis in a large and intricate group as Leotiomycetes. It is critical to increase the number of reference sequences from holotypes or designated epitypes to serve as name anchors in public DNA databases.
- Isolation of pure cultures from ascospores should be increasingly used for characterizing asexual stages and obtaining pure DNA for molecular studies. This is especially important in case of rare species, or those with tiny solitary apothecia, and the vouchers of new taxa.
5. CONCLUSIONS

Revisions of classifications should first and foremost rely on the reconstruction of phylogenies. Special attention to type species is inevitable for linking phylogenies with traditional taxonomy. Integration of phylogenetic analyses with morphological and ecological observations on helotialean fungi led to the following conclusions:

1) A complex of several characters, rather than individual features, defined the studied taxa. High diagnostic value was ascribed to the ascus apex features, refractive vacuolar bodies in living paraphyses, ionomotic reaction of apothecial tissues, morphology of anamorphs, presence of stromata/sclerotia, and the features of excipulum and hymenial parts of apothecia. TEM studies alone are insufficient for drawing taxonomic conclusions, but can offer additional details for describing the morphology of specimens under LM.

2) Phylogenetic hypotheses offer a new point of view regarding the delimitation of helotialean taxa. Based on the phylogenetic analyses of multigene data, the subfamily Encoelioideae and the genus *Encoelia* appeared to be polyphyletic, with species distributed among eight major lineages of Leotiomycetes. A large extent of homoplasy of morphological characters was confirmed. The type species of *Encoelia, E. furfuracea*, was shown to belong to the Cenangiaceae. Considering its morphological uniqueness and isolated position in phylogenetic trees, it likely represents an early diverged species with no extant siblings. The ascus apparatus of *E. furfuracea* differs considerably from species previously accepted in *Encoelia*.

3) Based on ultrastructure, the wall of apothecial hairs in Lachnaceae varies at the genus level. Despite the ascus apical apparatus being largely similar among the segregates of *Lachnum*, it is unique in *Lachnellula*. *Lachnum* should be used sensu stricto, because the phylogeny and ultrastructural data endorse the distinction of the genera *Albotricha, Brunnipila, Belonidium, Capitotricha, Dasyscyphella, and Incrucipulum*, merged in *Lachnum* by some earlier authors. *Belonidium aeruginosum* is not congeneric with *Incrucipulum* as proposed based on ultrastructure.

4) To improve the taxonomy of mollisioid fungi further studies are needed to ascertain the synapomorphies characterising members of phylogenetic lineages. Based on their ascus apical apparatus, mollisioids are clearly distinct from Dermateaceae s. str.

5) For detecting helotialean fungi in diverse habitats, DNA-based methods are valuable, enabling to accumulate information about their distribution, substrata and lifestyle. Phylogenetic analyses combining ITS rDNA sequences from fruitbodies and complex biological samples enabled to provide a name to the unidentified source organisms of many INSD ITS sequences, and indicated that members of each lineage mostly share a
common lifestyle. Members of the Cenangiaceae frequently grow as endophytes in various host tissues, a feature thus far ascribed to Hemiphalciaceae, here merged in the Cenangiaceae. This study highlights the potential of DNA-based identification methods in studies on the ecology of cryptic fungi in a phylogenetic context.
REFERENCES


50
Boudier JLÉ. 1907. Histoire et classification des discomycètes d'Europe, Paris
Boudier JLÉ. 1879. On the importance that should be attached to the dehiscence of asci in the classification of the discomycetes. Grevillea 8: 45–49.


Fries, E 1822. Systema mycologicum 2(1): 137


Index Fungorum: http://www.indexfungorum.org


Korf RP. 1962. A synopsis of the Hemiphaeaciaceae, a family of the Helotiales (discomycetes) causing needle-blight of conifers. Mycologia 54:12–33
Li D, Ashby AM. and Johnstone K. 2003. Molecular evidence that the extracellular cutinase Pbc1 is required for pathogenicity of *Pyrenopeziza brassicae* on oilseed rape. Molecular Plant–Microbe Interactions, 16, 545–552.
Mycobank http://www.mycobank.org/


Nylander JAA. 2004. MRMODELTEST 2.2. Evolutionary Biology Centre, Uppsala University, Uppsala


Perić B & Baral HO. 2014. Eriscyphella curvispora sp. nov. from Montenegro. Mycol Monten 17: 89–104


Sandoval-Leiva P, Carmara’n CC, Park D, Romero AI, Johnston PR. 2014. Vibrissaceous fungi from the southern hemisphere, including *Chlorovibrissea chilensis* (Helotiales, incertae sedis) sp. nov. Mycologia 106: 1159–1167


Trail F & Seminara A. 2014. The mechanism of ascus firing – Merging biophysical and mycological viewpoints. Fungal Biology Reviews 28 70e76


KOKKUVÕTE

Ultrastruktuuri ja molekulaarse tuumuste andmete rakendused tiksikseente taksonoomias

Tänapäeval süsteemtaika järgib põhīmõtet, et ühte taksonisse kuuluvad üheist eellast pärinevad organismid. Klassifikatsiooni aluseks olevaid evolutsiooni-
hüpoteese püstitatakse geneetiliste andmete alusel. DNA-põhi meedotite kasutamine on iseäranis oluline väikesemõõtmeliste organismide taksonoomias,
uuviid nende morfoloogilised kriteeriumid on raskemini tuvastatavad. Sarnane
on olukord kottseente hulgas, kõige liigirikkamas seeenhõimkonnas, kus siiani
osutub taksonite piiritlemine paljudes rühmades keeruliseks.

Doktoritööga püütakse anda panus fülogeneesipõhise klassifikatsiooni
loomisesse ühes molekulaarselt vähe uuritud kottseente rühmas, tiksikseened.

Doktoritöö eesmärk oli 1) hinnata morfoloogiliste ja ultrastruktuuri tunnuste
sohivust taksonite eristamiseks nii liigid, perekonna kui ka sugukonna tasandil;
sugukonna Lachnaceae ja alamsugukonna Encoelioideae esindajatel ning Mollisia
ruhmaseentel; 2) selgitada välja alamsugukonna Encelioideae ja perekonna
lõhhik liikide sugulussuhted; 3) esitada perekonna lõhhik ja sellega lähisuguluses
olevate liikide fülogeneesile tuginev klassifikatsioon; 4) värskendada infot
uuritud rühmade ökolooogia kohta, kasutades ITS geenijärjestusi viljakehadest,
seenekultuurdest ja keskkonnaproovidest avalikes andmebaasides.

Põhimeetodid püstitatud ülesannete lahendamisel olid valgus- ja transmis-
sioon-elektronmikroskoopia, DNA sekkeneerimine ning fülogeneesesti rekonstr-
ueerimine molekulaarse tuumuste põhjal. Peaaegu kõigil uuritud taksonitel
määratat DNA ITS nukleotiidide järjestus – seente tripliistamine marker,
imist koostatud andmemaatriksitesse kasasati avalikes geeninmeebaisides
talletatud keskkonnaproovidest pärit sekventsid. Et hinnata ultrastruktuuri
tunnuste kasutatavust liikide ja perekondade piiritlemisel, rekonstrueeriti klas-
Leotiomyceses fülogeneesipuu rDNA ITS ja 28S põhjal, haaretas valmis-
võimalikult palju taksoneid, mille ultrastruktuur oli kirjeldatud. Multigeen-
analüüsi jaoks sekkeneeriti 5 geenilõiku (rDNA 18S ja 28S ning valge kodeeriv
geenid rpb1, rpb2 ja tefl) 70 taksonil, kasates kättesaadavaid Encelioideae
	aksonid.

Multigeenianalüüsili osutus perekond lõhkik polüfüleetiliseks, kuna selle
liigid jagunesid kui sugukonna vahel. Perekonna tüüpliik, Eestiski sarapuudel

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Sugukonda Sclerotiniaeae kuuluvad kobarlõhikik (*Encoelia fascicularis*) ja *E. pruinosa*, vastavalt saproob ja parasit vakuoolikehi elusate viljakehade parafüüsides. Cenangiaceae sisaldab puidusaproobe ning okaspude parasiite-endofüüte, hõlmates varasema sugukonna Hemiphaciidae. ITS analüüslib leidis kinnitust perekonna *Cenangium* liikide esinemine endofüütidena nii mändides kui ka männi puuvõõrikus.

Sugukonda Sclerotiniaeae kuuluvad kobarlõhikik (*Encoelia fascicularis*) ja *E. pruinosa*, vastavalt saproob ja parasit vakuoolikehi elusate viljakehade parafüüsides. Cenangiaceae sisaldab puidusaproobe ning okaspude parasiite-endofüüte, hõlmates varasema sugukonna Hemiphaciidae. ITS analüüslib leidis kinnitust perekonna *Cenangium* liikide esinemine endofüütidena, mis võib olla uus tiksiseente selts.


Lachnaceae oli rDNA fülogeneesi põhjal monofüüliitse rühm ning perekonnast Lachnum saab eristada mitmeid väiksemaid perekondi, mis erinevad üksisteid atahereoslate karvade seinte ultrastruktuuri poolest. Lachnaceae esindajate eoskoti tipustruktuur oli sugukonna piirel sellalt sarnane, v.a. perekond Lachnellula.

Mollisia rühma seintel osutus perekondade Belonopsis, Mollisia ja Pyrenopezzia eoskoti tipustruktuur vähem varieeruvaks ning perekondad selle tunnuse alusel ei eristanud. Neil seintel on taksonoomiliselt olulised anamorfide ja parafüüsidid vakuoolikehade vundused, olles kaalumärkas perekondade asetsemisega fülogeneesipul.

ACKNOWLEDGEMENTS

I am deeply indebted to my late mycological mentors. Ain Raitviri was the first to introduce the diverse world of Ascomycetes, sharing his experience and supporting my work. It often surprises me how much his intuitive opinions about taxonomy of helotialean fungi are proved by the application of modern methods. Erast Parmasto created a strong scientific environment in Tartu and still influences us via his mentorship.

Kadri Põldmaa supervised me during the last three years in finalizing my Ph.D. studies and I am very thankful for her great dedication, sharing her knowledge equally with friendship. Thank you for your valuable time, Kadri!

The grand old expert of helotialean fungi, Hans-Otto Baral alias Zotto, in Germany, is acknowledged for cooperating on the encoelioid paper, for his constructive criticism, specimens, and most of all, for infecting me with his enthusiasm for looking at the details.

Accompanying my colleagues Kuulo Kalamees, Anu Kollom, Bellis Kullman, Ilmi Parmasto, Irja Saar, Leho Tedersoo and the late Leili Järva and Mall Vaasma during the field work and in the Tartu mycologicums, I learned much about fungal diversity and many practical things. I thank Urmas Kõljalg for believing in me. I am indebted to Heidi Tamm for her contribution to the phylogenetic part of this work, and for being so helpful in solving the problems with selfish helotialean genes, and to Rasmus Puusepp for carrying out the molecular work in the lab. My colleagues in the fungarium of the Estonian University of Life Sciences, Irma Zettur, Triin Varvas and Märt Rahi, are thanked for their patience during last years.

I would never have succeeded with my TEM studies without colleagues, and am grateful to the late Jüri Kärner for his positive attitude while working in the lab of developmental biology, to Raivo Raid for assisting in managing with electron microscopy, to Dagni Krinka, Martin Kärner and others for sharing their equipment and hints. I am also indebted to the TEM teams at the University of Helsinki in Bioviikki, at Copenhagen University, and in Saint Peterburg Komarov Botanical Institute.

Sharing unpublished DNA sequences was of great help for confirming the identification of some fungal strains, and in this regard Ricardo Galán (Alcala de Henares), Peter Johnston (New Zealand), Guy Marson (Luxembourg), and Luis Quijada (Canary Islands) are acknowledged.

I thank the collectors and the curators of mycological collections for the specimens, as well as all the people who have provided images of helotialean fungi, especially Vello Liiv.

I am very much in dept to my dear family.

Financial support was received from the Estonian Science Agency (project IUT20-30), the European Regional Development Fund (Centre of Excellence EcolChange).
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Haridus
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Teadusartiklid
Pärtel K, Baral H-O, Tamm H, Põldmaa K. 2016. Evidence for the polyphyly
of Encoelia and Encoelioideae with reconsideration of respective families
in Leotiomycetes. Fungal Diversity DOI 10.1007/s13225-016-0370-0
(published online)
Pärtel K. 2014. Ultrastructure of the ascus apical apparatus of Encoelia fur-
DOI 10.1007/s11557-014-0982-2
Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R,
Villarreal Ruiz L, Vasco-Palacios AM, Thu PT, Suija A, Smith ME, Sharp
C, Saluveer E, Saitta A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Põldmaa
Global diversity and geography of soil fungi. Science 346 no. 6213. DOI:
10.1126/science.1256688


Muud publikatsioonid


Konverentsiettekanded


166


Stipendiumid
2001. märts–aprill, Centre of International Mobility (CIMO) stipendium, Helsingi Ülikooli biokeskus, TEM labor
2001. november, Copenhagen Biosystematics Centre (COBICE) stipendium, Kopenhaageni ülikool, TEM labor
2015. aprill, Maateaduste ja ökoloogia doktorikooli toetus reisitoetus, osalemine Amsterdami kottseente töötoas.

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