

# ***Phaeohelotium undulatum* comb. nov. and *Phaeoh. succineoguttulatum* sp. nov., two segregates of the *Discinella terrestris* aggregate found under *Eucalyptus* in Spain: taxonomy, molecular biology, ecology and distribution**

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**Abstract:** Two terricolous species of the Australasian *Discinella terrestris* aggregate are reported from Mediterranean eucalypt plantations on the Iberian Peninsula. The two species were recorded in Spain since 1996–97, but were possibly imported several decades earlier, perhaps already during the mid-eighteenth century. Their obvious restriction to *Eucalyptus*, presumably by mycorrhiza, is discussed. One of them (here named *Phaeohelotium undulatum*) possesses a yellow-ochraceous hymenium and amyloid asci, whereas the other (*Phaeoh. succineoguttulatum*) deviates by an ochre-brown hymenium due to abundant, refractive, yellowish-brown vacuolar guttules in the paraphyses, and by consistently inamyloid asci. Both species have asci arising from simple septa, a *Hymenoscyphus*-type of apical ring, ascospores that turn brown when overmature, and a gelatinized ectal excipulum of prismatic to hyphoid cells. Ecologically the two taxa are very similar, though *Phaeoh. succineoguttulatum* is adapted to a little cooler and more humid climate, following its occurrence in the north and northwest of Spain, though both species were sometimes recorded at the same site in the centre and south of Spain.

Our molecular analysis revealed that these two species and a specimen from New Zealand, here accepted as *Phaeohelotium confusum*, form a clade with *Phaeoh. monticola* (which is currently believed to be conspecific with the type of *Phaeohelotium*, *Phaeoh. flavum*), whereas a sequence gained by us from *Discinella boudieri* (type of *Discinella*) is quite distant from *D. terrestris*, clustering instead with *Pezoloma ciliifera*, a typical species of *Pezoloma*. The problematic generic limits around *Hymenoscyphus*, *Cudoniella* and *Phaeohelotium* are discussed. The *Discinella terrestris* aggregate is here transferred to *Phaeohelotium*, though this genus is apparently paraphyletic. Alternatively, *Cudoniella* or *Hymenoscyphus* could be extended to include the species of the *Phaeohelotium* clade. Based on morphological features as well as DNA sequences, we conclude that *Gelatinodiscus* is a synonym of *Chloroscypha*, and some species currently placed in *Bisporella* are better assigned to *Calycina*. Hence, we propose the

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following new combinations: *Calycina claroflava*, *C. drosodes*, *C. lactea*, *C. languida*, *C. scolochloae*, and *C. subcitrina*, *Cyathicula amenti*, *Chloroscypha flavida*, *Phaeohelotium fulvidulum* and *Phaeoh. tamaricis*.

A restudy of type material of the *D. terrestris* aggregate, comprising the five Australasian taxa *Helotium terrestre*, *Phaeopezia ochracea*, *Aleurina readeri*, *Pseudohelotium undulatum*, and *Discinella confusa*, shows that four of them differ from those two species recorded from Spain in possessing croziers at the ascus base, besides showing a certain tendency to narrower asci and ascospores. Only *Ps. undulatum* from Tasmania was found to have simple-septate asci and is here tentatively considered conspecific with that Spanish species having a yellow disc. *Aleurina readeri* from Victoria deviates by consistently inamyloid asci. For the latter two taxa new combinations in *Phaeohelotium* are here proposed. The earlier asserted synonymy of *Phaeopezia ochracea* from Tasmania with the older *H. terrestre* from Queensland is confirmed, though the spores of the former are slightly longer and narrower. In order to avoid a homonym, a new name *Phaeohelotium baileyianum* is here proposed for *H. terrestre*. *Discinella confusa* from New Zealand differs only insignificantly from *Phaeoh. baileyianum* in a pale bluing around the strongly amyloid apical ring and in slightly narrower asci and slightly smaller spores. However, specimens here assigned to *Phaeoh. baileyianum* partly also showed such pale bluing. Although the two taxa are not clearly separable, the combination *Phaeohelotium confusum* is here proposed, based mainly on a different host and geographical distribution, also because of the consistent absence of brown spores.

The *D. terrestris* aggregate shows a rather dense distribution in the forests along the southeast and southwest coast of Australia, and throughout Tasmania and New Zealand. A connection to a host tree is unclear in many of the known records. For *D. confusa*, a species from New Zealand, a connection to *Nothofagus* is certain. *Phaeoh. baileyianum* occurs in Australia and Tasmania, and a relation to *Eucalyptus* seems possible, while its type derives from a quite isolated site in eastern Australia. Since *Eucalyptus* is not native to New Zealand, the two Spanish species might originate from Australia or Tasmania. However, apart from the type locality in Tasmania, specimens referable to *Phaeoh. undulatum* were also discovered in New Zealand under Myrtaceae of the genera *Kunzea* and *Leptospermum*. In conclusion, the here reported heterogeneity of the *D. terrestris* aggregate demands a re-examination of all the many Australasian records under that name, specifically examination of the ascus base.

**Key words:** Ascomycetes, ectomycorrhizal, *Eucalyptus*, *Nothofagus*, exotic fungi, postfire community, *Hymenoscyphus*, *Pezoloma*, *Calycina*

## 西班牙桉树林下土小平盘菌复合群中两个独立的种——暗柔膜菌属一新组合及一新种：分类、分子生物学、生态与分布

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**摘要:** 土小平盘菌复合群原产澳大利亚, 其中两个土生种在西班牙报道, 很可能是在 18 世纪中叶引入西班牙。根据形态学特征和序列分析的结果, 建立一个新组合和一个新种, 分别定名为 *Phaeohelotium undulatum* comb. nov. 和 *Phaeoh. succineoguttulatum* sp. nov.。对其宏观和微观形态特征进行了详尽的描述和图示, ITS rDNA 序列分析的结果显示, 它们以 100% 的支持率与 *Phaeohelotium monticola* 聚类在同一个分支。文中提供了该复合群 *Phaeohelotium* 属 5 个土生种的分种检索表, 并对它们的生态习性与世界分布进行了深入讨论。

**关键词:** 子囊菌, 外生菌根, 桉属, 南青冈属, 外来真菌, 过火生境群落, 膜盘菌属, 胶鞘盘菌属, 半杯菌属

## INTRODUCTION

On January 1995, one of us (R.G.) received from the south of Spain (Córdoba) dry material of an interesting fungus with light yellow apothecia and a short white stalk growing gregariously directly on soil under *Eucalyptus globules*. The fungus was tentatively identified by the collector (Javier Gómez) as an unidentified species of *Hymenoscyphus* Gray. Subsequent microscopic study revealed two infrequent features for that genus: a gelatinized ectal excipulum, and broadly ellipsoid to subfusoid spores that contained large oil drops and become brown with age. These features, together with its characteristic habitat allowed us to identify the fungus as *Discinella terrestris* (Berk. & Broome) Dennis (Dennis 1958). During a stay of one of us (H.B.) in Spain in 1996, we had the opportunity to study in detail this collection by accepting not only the apparently first appearance of the species outside its original (Australasian) region but also the atypical combination of features, with the aim to accommodate it to some of the existing satellite genera such as *Phaeohelotium* Kanouse, *Bisporella* Sacc., or *Hymenoscyphus* Gray, or to retain it within *Discinella* Boud.

In the period of 1997–1999 the species was

again collected and then also studied in the living state (Pl. 1), also from Córdoba and Huelva. In the same period we received from the north of Spain (Castrillón, Asturias) an outstanding fungus that shared identical ecology (soil under *Eucalyptus globulus*) and macro- as well as microscopic features, but deviated by a distinctly brown hymenium due to paraphyses with yellowish-brownish instead of hyaline vacuolar bodies, and by asci with inamyloid apical thickenings (Pl. 4). Between 1996 and 2013 this deviating species was also discovered in Cataluña, Extremadura, Andalucía, and País Vasco, while the yellow species showed up between 1999 and 2013 in various localities of Andalucía, also Extremadura and Castilla La Mancha. At the areas where eucalypts have been planted in pulpwood plantations within the Iberian Peninsula, only Portugal and the regions of Galicia and Cantabria so far lack any records of the *Discinella terrestris* aggregate.

The macroscopic appearance of the brown species prompted the collector from Cataluña (Jordi Vila) to identify the fungus as “*Rutstroemia* sp.?” , while the terricolous habitat led Enrique Rubio, who collected the very same species in Asturias, to consider it a member of the Pezizales. Based on the

few deviating characters we initially believed this taxon to be an undescribed variety of *Discinella terrestris* and tentatively named it “*D. terrestris* var. *inamyloidea*” (Baral & Marson 2005).

Subsequent attempts of culturing both fungi were repeatedly unsuccessful, although different media, temperatures, and extracts of *Eucalyptus* were used. This fact was also observed by Peter McGee (pers. comm.) years ago based on Australian collections identified as *Discinella terrestris*, who told us about its supposed ectomycorrhizal mode of life and its intrinsic difficulty to obtain a pure culture.

In order to assess their phylogenetic relationships with other members of the Helotiales, we extracted DNA from fresh tissue of apothecia from both taxa and amplified their ITS1-5.8S-ITS2 ribosomal region. Our results were presented at the 7th International Mycological Congress in Oslo (Collado et al. 2002). For the clarification of their phylogenetic relationships, DNA of dry apothecia of recent collections of the type species of *Discinella* and *Phaeohelotium* was also extracted.

The two taxa here reported in detail from Spain occurred always on soil linked to reforestations with *Eucalyptus* spp., sometimes in postfire sites. Meanwhile it is noteworthy to mention that comparable records from outside Spain appear not to have been made in mycologically explored European countries such as England, France, Greece, Ireland, Italy, Malta, Portugal, where *Eucalyptus* plantations are also common, in spite of a wide search not only in the relevant mycological literature but also within the main mycological internet forums. The same appears to be true for the American continent and for Macaronesia.

According to GIT Forestry Consulting ([http://git-forestry.com/download\\_git\\_eucalyptus\\_m](http://git-forestry.com/download_git_eucalyptus_m)

ap.htm), the *Eucalyptus* forests reached in 2008 a surface area of 640.000 and 647.000ha in Spain and Portugal respectively, being after India, Brazil and China the fourth world-wide region where such reforestations are more extensive, involving *E. globulus* and *E. camaldulensis* as the two mainly planted species. The first species introduced to Spain was *E. globulus*, around 1860, while *E. camaldulensis* was imported later (without a precise date). The specific regions where *Eucalyptus* was planted comprise the autonomous communities of Galicia, Andalucía, Extremadura, Cantabria, Asturias, País Vasco, Cataluña, and Castilla La Mancha (Ciudad Real province), in the order of the total surface area of the plantations. In a parallel way, there are many fungi associated to *Eucalyptus* whose distribution area has been artificially enlarged, comprising saprophytic and parasitic (Sankaran et al. 1995) as well as ectomycorrhizal species (Galán & Moreno 1998; Giachini et al. 2000; Díez 2005; Díez et al. 2010; Pérez-Butrón et al. 2011). Among those recorded from Spain, many were already known from Australasia, as is the case with *Melanogaster tuberiformis* Corda, *Urnula rhytidia* (Berk.) Cooke, *Setchelliogaster tenuipes* var. *rheophyllus* (Bertault & Malençon) G. Moreno & M.P. Martín, *Ruhlandiella berlinensis* Henn., *R. reticulata* (P.H.B. Talbot) E. Rubio et al., *R. truncata* (P.H.B. Talbot) E. Rubio et al., *Hysterangium inflatum* Rodway, *Reddellomyces donkii* (Malençon) Trappe et al., *Descomyces albus* (Berk.) Bougher & Castellano, *Hydnangium carneum* Wallr., *Phaeohelotium undulatum* (Rodway) Baral et al. (here redescribed), etc. The contrary case is not so common, i.e., Spanish records of fungi specifically associated to *Eucalyptus* that have not been recorded yet in Australasia as, for instance, *Harknessia spermatoidea* R. Galán, G. Moreno & B. Sutton,

*Kallistoskypha incarnata* (Duvernoy & Maire) Pfister *et al.*, or *Phaeohelotium succineoguttulatum* Baral *et al.* (here described). For the latter case one could assume as a first hypothesis that such fungi should be detectable also at the regions where *Eucalyptus* is native, while another possible explanation concerns the formation of hybrids (Rhymer & Simberloff 1996) or newly formed species.

In the following we describe the two species of the *Discinella terrestris* aggregate on the basis of the Spanish specimens examined. Also the characteristics of the examined type material of five Australasian taxa are documented and discussed, and their nomenclature updated.

## 1 MATERIALS AND METHODS

### Morphology

Collections were examined either in the living state in tap water or from rehydrated herbarium material. Drawings were done free-hand using a Zeiss Standard 14 microscope. Microphotos were either taken on this microscope with a Nikon Coolpix 4500 adapted free-hand to a Kpl 10×/20 Zeiss wide field ocular (H.B.), or on an Olympus BX 41 microscope with a tripod hold Canon PowerShot A495 and adapted to a WHC10X (FN22) ocular (R.T.). Macrophotos were obtained using a Nikon F801 (R.G.), a Canon PowerShot A490 (R.T.), a Nikon D-70 adapted to a trinocular microscope Nikon E-400 (E.R.D.), some with the analogous Nikon FX-35DX mounted on a dissecting Nikon SMZ-2T microscope (R.G.). The iodine reaction was tested with Lugol's solution (IKI = ~1% I<sub>2</sub>, 2% KI, in H<sub>2</sub>O), without and with pre-treatment by potassium hydroxide (5% KOH), rarely with Melzer's reagent (MLZ). Brilliant Cresyl Blue (CRB, ~1% in H<sub>2</sub>O) was added to a water mount for testing presence of low-refractive gel, and Congo Red (CR, aqueous) to a KOH mount for staining the

ascus wall and ascogenous hyphae. Type material is deposited in AH (University of Alcalá). Additional collections are held in F (Fundación Medina's Fungal Culture collection), GDAC (Fac. Sciences, Univ. Granada), HO (Tasmanian Museum, Hobart), JA (Consejería de Medio Ambiente, Junta de Andalucía), PDD (Landcare Research, Auckland), and SESTAO (Sestao Natural Sciences Society, País Vasco), and in the private herbaria of Celestino Gelpi (C.G.), Carles Roqué (C.R.P.), Demetrio Merino (D.M.A.), Enrique Rubio Domínguez (E.R.D.), Hans-Otto Baral (H.B.), Joaquim Carbó (J.C.), Miguel Ángel Ribes (M.A.R.), P. Daniëls (P.D.), and Raúl Tena (R.T.).

Abbreviations: LBs = lipid bodies (oil drops) in living spores and paraphyses, VBs = refractive vacuolar bodies (in living paraphyses), \* = living state, † = dead state, ∅ = unpreserved, *n.v.* = no specimen studied by us (but photos were available). A number in curly brackets { } indicates how many specimens were studied, or (for host taxa) the number of collection sites. The arrow → indicates the development from immature to mature asci.

### DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from mycelia of agar cultures or from fructifications (Peláez *et al.* 1996). The ITS1-5.8S-ITS2 region was amplified using universal primers ITS5 (White *et al.* 1990) and ITS4a (Larena *et al.* 1999). PCR reactions were carried out following standard procedures (40 cycles of 30s at 93°C, 30s at 53°C and 2min at 72°C). About 0.1µg/mL of the double-stranded amplification products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Norwalk CT) following the manufacturer's recommended procedures. Purified PCR products were directly sequenced using the same primer pairs as in the PCR

reactions. Partial sequences obtained in sequencing reactions were assembled with Genestudio 2.1.1.5. (Genestudio, Inc., Suwanee, GA, USA). All sequences were deposited in GenBank (Table 1).

### Phylogenetic analysis

Initial alignment of the selected sequences was performed by Genestudio 2.1.1.5, and modified manually to improve the quality of the alignment. Bayesian analysis based on the Markov chain Monte Carlo (MCMC) approach was run in the program MrBayes 3.01 (Ronquist & Huelsenbeck 2003). To

improve mixing of the chain, four incrementally heated simultaneous MCMCs were run over 2,000,000 generations. MrModeltest 2.2 (Nylander 2004) was used to perform hierarchical likelihood ratio tests to calculate the Akaike Information Criterion (AIC) and hierarchical likelihood ratio tests (hLRTs) values of the nucleotide substitution models. The model selected by hLRT and AIC for the alignment of the ITS fragment was the GTR+I+G model. This model allowed for six classes of substitution types, a portion of invariant

**Table 1 Species, code, ecology, collection place, and GenBank code number of the specimens investigated**

| Species                                 | Code      | Ecology                                   | Collection place                      | GenBank code number |
|---|-----------|---|---------------------------------------|---------------------|
| <i>Leotia lubrica</i>                   | AH7651    | On moss in a beech forest                 | Gallinero de Rioja (La Rioja)         | KC411986            |
| <i>Microglossum viride</i>              | AH7560    | Beech forest                              | Valgañón (La Rioja)                   | KC411987            |
| <i>Phaeohelotium undulatum</i>          | AH7337    | On humus under <i>Eucalyptus globulus</i> | Puerto de Miravete (Cáceres)          | KC411988            |
| <i>Phaeohelotium succineoguttulatum</i> | AH7643    | On humus under <i>Eucalyptus globulus</i> | Llagostera (Girona)                   | KC411989            |
| <i>Phaeohelotium succineoguttulatum</i> | AH7163    | On humus under <i>Eucalyptus globulus</i> | El Monte, Camporriundo (Asturias)     | KC411990            |
| <i>Phaeohelotium monticola</i>          | H.B.8612  | <i>Fagus sylvatica</i> trunk (wood)       | Chemnitz (Sachsen)                    | KC411991            |
| <i>Phaeohelotium geogenum</i>           | H.B.7222A | <i>Fagus sylvatica</i> (seeds & cupules)  | Tübingen (Baden-Württemberg)          | KC411992            |
| <i>Hymenoscyphus imberbis</i>           | H.B.6797  | <i>Salix</i> branch (wood)                | Tübingen (Baden-Württemberg)          | KC411993            |
| <i>Hymenoscyphus kathiae</i>            | H.B.6924  | <i>Salix</i> branch (wood)                | Esch-sur-Alzette (Luxembourg)         | KC411994            |
| <i>Hymenoscyphus vernus</i>             | H.B.6915  | <i>Alnus</i> branch (bark & wood)         | Ettelbruck (Luxembourg)               | KC411995            |
| <i>Ombrophila janthina</i>              | H.B.7044  | <i>Picea abies</i> cone                   | Tübingen (Baden-Württemberg)          | KC411996            |
| <i>Neobulgaria pura</i>                 | H.B.6880  | <i>Fagus sylvatica</i> trunk              | Tübingen (Baden-Württemberg)          | KC411997            |
| <i>Bisporella subpallida</i>            | H.B.6849A | <i>Fraxinus excelsior</i> stump (wood)    | Tübingen (Baden-Württemberg)          | KC411998            |
| <i>Ascocoryne sarcoides</i>             | H.B.6789  | <i>Fagus sylvatica</i> branch (bark)      | Tübingen (Baden-Württemberg)          | KC411999            |
| <i>Pezoloma ciliifera</i>               | F265954   | On rotten vegetation (pine forest)        | Bronchales (Teruel)                   | KC412000            |
| <i>Discinella boudieri</i>              | H.B.4326  | burnt soil                                | Kaiserslautern (Rheinland-Pfalz)      | KC412001            |
| <i>Calycina languida</i>                | F116599   | On <i>Fagus sylvatica</i>                 | Puerto de la Quesera, Riaza (Segovia) | KC412002            |
| <i>Calycina languida</i>                | F116600   | On <i>Fagus sylvatica</i>                 | Puerto de la Quesera, Riaza (Segovia) | KC412003            |
| <i>Calycina citrina</i>                 | F115889   | On <i>Fagus sylvatica</i>                 | Puerto de la Quesera, Riaza (Segovia) | KC412004            |
| <i>Calycina citrina</i>                 | F118000   | On <i>Quercus robur</i> (mixed forest)    | Oseja de Sajambre (León)              | KC412005            |
| <i>Calycina claroflava</i>              | F132983   | <i>Quercus ilex</i> branches              | L'Erbossar, Pollença (Mallorca)       | KC412006            |
| <i>Calycina lactea</i>                  | H.B.7224A | <i>Acer pseudoplatanus</i> trunk (wood)   | Tübingen (Baden-Württemberg)          | KC412007            |
| <i>Torrendiella ciliata</i>             | F132996   | <i>Quercus ilex</i> leaves                | S'Estret, Valldemossa (Mallorca)      | KC412008            |

alignment positions, and mean substitution rates varying across the remaining positions according to a gamma distribution. Priors used for the MCMC process were a Dirichlet distribution for substitution rates and nucleotide frequencies and a uniform prior for the rate parameter of the gamma distribution (Ronquist *et al.* 2009). The MCMC analysis used the following parameters: sampling frequency=100; 1,000 trees were discarded for the consensus tree; the resulting consensus tree was a majority rule consensus tree including compatible groups of lower frequencies.

## 2 RESULTS

*Phaeohelotium undulatum* (Rodway) Baral, R. Galán & R. Tena, **comb. nov.** – **MycoBank MB803670**

Basionym: *Pseudohelotium undulatum* Rodway, *Pap. Proc. R. Soc. Tasm.*: 106 (1925) [1924].

**Type:** Tasmania, National Park (very probably Mount Field National Park, see [http://en.wikipedia.org/wiki/Mount\\_Field\\_National\\_Park](http://en.wikipedia.org/wiki/Mount_Field_National_Park)), “on rotten wood”, XI.1923, [L. Rodway], K(M) 179950 (isotype, examined), HO (holotype, not examined). – Spanish material only included in the below description

### Key to the accepted terrestrial, (possibly) mycorrhizal species of *Phaeohelotium* belonging to the *Discinella terrestris* aggregate

1. Asci arising from croziers; asci  $\dagger 135\text{--}225 \times (8\text{--}9\text{--}11\text{--}12)\mu\text{m}$ , ascospores  $\dagger (11\text{--}13\text{--}20\text{--}23) \times (5.5\text{--}6\text{--}8\text{--}9)\mu\text{m}$ , under Fagales (*Nothofagus*) or Myrtales (*Eucalyptus*), but host plant often unknown .. 2
1. Asci arising from simple septa; asci  $\dagger 160\text{--}250 \times (9\text{--}10\text{--}11\text{--}12)\mu\text{m}$ , ascospores  $\dagger (12\text{--}14\text{--}20\text{--}24) \times (6.2\text{--}7\text{--}9.5\text{--}10)\mu\text{m}$ , under Myrtales (*Eucalyptus*, *Kunzea*, *Leptospermum*) ..... 4
2. Apical ascus wall consistently inamyloid (IKI); asci  $\dagger 135\text{--}150\mu\text{m}$  long; ascospores  $\dagger (11\text{--}13\text{--}16\text{--}18) \times 6.8\text{--}8.3\mu\text{m}$ ; host plant unknown, S-Australia ..... *Phaeoh. readeri*
2. Apical ascus wall predominantly with euamyloid apical ring (visible in IKI as two thin lines that react faintly to strongly blue); asci  $\dagger (140\text{--}160\text{--}210\text{--}225)\mu\text{m}$  long ..... 3
3. Asci  $\dagger 8\text{--}9.5\text{--}10\mu\text{m}$  wide; ascospores  $\dagger (11.5\text{--}13\text{--}17\text{--}19) \times (5.5\text{--}6\text{--}7\text{--}7.5)\mu\text{m}$ ; brown spores never observed; under *Nothofagus*, New Zealand ..... *Phaeoh. confusum*
3. Asci  $\dagger (8\text{--}9\text{--}10\text{--}12)\mu\text{m}$  wide; ascospores  $\dagger (12\text{--}14\text{--}20\text{--}23) \times (5.5\text{--}6\text{--}8\text{--}9)\mu\text{m}$ ; brown spores often observed; under *Eucalyptus*, W- and S-Australia, Tasmania ..... *Phaeoh. baileyannum*
4. Ascus apical wall consistently inamyloid (IKI); upper part of living as well as dead paraphyses in H<sub>2</sub>O containing numerous strongly refractive, globose, bright yellowish-ochre to reddish-brown VBs (persistent in dead state); hymenium fresh ochraceous- to reddish-brown; Spain (Asturias, País Vasco, Cataluña, Andalucía and Extremadura) ..... *Phaeoh. succineoguttulatum*
4. Ascus apical wall predominantly or consistently with euamyloid apical ring (visible in IKI as two thin lines that react faintly to rather strongly blue); upper part of living paraphyses in H<sub>2</sub>O without visible contents or with slightly to strongly refractive, globose to elongate, hyaline vacuolar guttules (VBs), these in the dead state turning into very pale yellowish granules at the most; hymenium fresh (ochraceous-) yellow, rarely white (Tasmania); Spain (Andalucía and Extremadura), Tasmania, New Zealand ..... *Phaeoh. undulatum*

and diagnosis.

**Diagnosis:** Apothecia 1–7mm diam, disc yellow, stipe distinct, white, 1–3mm long. Asci \*175–260×12.3–15.5µm, 8-spored, with euamyloid apical ring of the *Hymenoscyphus*-type, arising from simple septa. Ascospores \*/†13–24×7–10.5µm, broadly fusoid, hyaline, containing very large oil drops, overmature ochre-brown. Paraphyses (\*) without or with hyaline vacuolar bodies. Ectal excipulum at flanks of gelatinized *textura prismatica-porrecta*. Medullary excipulum slightly gelatinized. On soil under *Eucalyptus*.

**Description:** **Apothecia** ± gregarious, sometimes fasciculate from a common stipe, hydrated (1–)1.5–5.5(–7)mm diam. {6}, round, non-gelatinous, disc young concave, then flat, light to bright yellow(–ochraceous), margin thin, smooth, even, externally whitish-cream or light yellow, smooth to minutely downy; stipe distinct, white or whitish-cream to the very base, short to medium long (1–2× longer than wide), 1–2.5(–3)×0.8–1.2(–1.5)mm, overall slightly scurfy-floccose or sometimes hairy, hardly immersed in soil. **Asci** \*(175–)185–250(–260)×(12.3–)13–15(–15.5)µm {4}, †(160–)170–220(–250)×(9.2–)10–12µm {7}, 8-spored, pars sporifera \*(60–)80–93µm {3}, spores (\*) obliquely uniseriate; apex (†) broadly rounded to subconical-subtruncate, apical wall \*~1µm thick, †3.5–5(–6)→1.7–3µm thick {3}, rarely subapically 3→1.5µm and apically 1.5→0.5–1µm thick {1}; with a thin apical ring that reacts very pale or usually light greyish-blue in IKI (unpretreated, euamyloid, type BB) {12}, pale to light blue in MLZ or KOH+MLZ {2}, of the *Hymenoscyphus*-type but upper part of ring mostly stronger amyloid than lower part, not extending laterally, remaining wall inamyloid, rarely complete wall of some asci inamyloid (especially when apical thickening

reduced) {2}; gradually tapered towards base, arising from simple septa {13}; upper part of asci prior to spore formation multiguttulate (high lipid content). **Ascospores** \*((11.5–)(13.5–)15–21(–24)×((7–)(7.5–)8–10(–10.5)((–11))µm {10}, †((12–)(13–)14–20(–22)((–26))×((6.5–)(7–)7.5–9(–9.5)((–10))µm {6}, broadly fusoid, rarely ovoid with one end rounded, consistently hyaline when inside living asci; (\*) with 1–3 very large (4–9µm diam) and several smaller **LBs** (lipid content very high), (†) **LBs** often confluent to one ellipsoid body; wall †0.15–0.2µm thick, surface in CRB (very) pale lilac, sometimes surrounded by irregular sheath-like remnants when recently ejected; overmature with a pale to dark ochre- to reddish-brown, in KOH (olivaceous-)brown, \*/†0.4–0.8µm thick wall, smooth or sometimes very finely punctate, aseptate, size and lipid pattern unchanged, no visible germ pores, germination never observed. **Paraphyses** cylindrical, terminal cell \*(42–)48–75(–88){2}×2.5–4(–5)µm {3}, †(1.7–)2–3(–4.2)µm wide {5}, lower cells \*14–29(–36)×1.8–3.5µm {2}; 0–15µm longer than dead asci, while mature living asci projecting (2–)15–25µm beyond paraphyses; upper 1(–2) cells at a length of 42–88µm containing many globose to elongate, hyaline, slightly to medium, sometimes strongly refractive **VBs** 0.5–3µm diam. {4} or without visible contents {2}, disappearing in KOH; VBs in dead cells persistent when mounted in H<sub>2</sub>O, hyaline to pale yellowish, slightly to strongly refractive, KOH-soluble; adding IKI to living or dead cells causes a deep yellowish- or red-brown stain to the VBs while red-brown granules sometimes pass into the surrounding medium; a few minute yellowish LBs present near septa, more abundant in lower part. **Ectal excipulum** hyaline, (\*) slightly or (†) rather strongly gelatinized, common walls †1–3µm thick; 60(–100)µm thick at

stipe and flanks, in stipe of strongly undulating *textura intricata*; at lower flanks of *t. prismatica-porrecta* somewhat irregularly oriented at a 0–10(–70)° angle to the surface, individual cells  $*(15-20-50(-62))\{2\} \times (4-7-12(-15))\mu\text{m}\{3\}$ ,  $\dagger(18-20-40(-45)) \times 4-7\mu\text{m}\{2\}$ ; 30–40 $\mu\text{m}$  thick at mid flanks, oriented at 0–30°, 10–20 $\mu\text{m}$  thick at upper margin, cells  $*16-31 \times 6-9\mu\text{m}$ , oriented at 0–10°; externally covered from base to lower flanks by an up to 10–20 $\mu\text{m}$  thick layer of parallel, medium gelatinized hyphae (cells  $*20-25 \times 4-8\mu\text{m}$ ); cortical cells containing very low-refractive VBs, surface cells forming 5–10 up to 30–50 $\mu\text{m}$  long, somewhat undulating,  $\pm$  appressed hairs 3–4(–5) $\mu\text{m}$  wide, with a smooth,  $\dagger 0.3-0.8\mu\text{m}$  thick wall. **Medullary excipulum** hyaline, upper part 200–300 $\mu\text{m}$  thick at base of receptacle, of rather loose to mostly dense *textura intricata*, towards ectal excipulum of a 200 $\mu\text{m}$  thick *t. porrecta-intricata*, individual cells  $*27-80 \times (3.5-5-12(-14))\mu\text{m}\{3\}$ ,  $\dagger 16-45 \times 2-4\mu\text{m}\{1\}$ , smooth, thin-walled or slightly gelatinized (common walls 0.8–1.2 $\mu\text{m}$  thick), very indistinctly differentiated from ectal excipulum. **Subhymenium**: 30–40(–80) $\mu\text{m}$  thick, of dense, pale yellow *textura intricata*, slightly to medium gelatinized, some cells containing groups of yellow LBs  $\sim 0.5-1\mu\text{m}$  diam. **Anchoring hyphae** 2.5–5.5 $\mu\text{m}$  wide, wall 0.3–1 $\mu\text{m}$  thick, smooth, hyaline. – All tissues IKI–, without crystals, gel pale lilac in CRB.

**Habitat**: in Mediterranean eucalypt plantations, either pure or mixed with *Quercus ilex*, *Cistus monspeliensis*, *C. ladanifer*, under *Eucalyptus* sp. {2}, *E. camaldulensis* {3}, *E. fruticetorum* {1}, *E. globulus* {6}, on mostly acid soil (granite, quartzite, phyllite, slate, sandstone *etc.*), rarely calcareous (dolomite, but possibly acidified), not rarely with remains of charcoal, closely associated with a high amount of fine roots (very probably of *Eucalyptus*),

naked or more or less densely covered by acro- and pleurocarpous mosses (?*Hypnum etc.*), *Cladonia* sp., closely associated with *Kallistoscypha incarnata* {1}, *Pseudoplectania ericae* {1}, 40–660m a.s.l., (Nov.–)Jan.–March(–April). **Desiccation tolerance**: asci and paraphyses were all dead after 4–5 days in the air-dried state while many hyaline spores were still alive; all spores (hyaline and brown) were dead when examined after 16 months.

**Specimens examined** (all under *Eucalyptus* spp.): **SPAIN: ANDALUCÍA, Córdoba**, 11km NNW of Córdoba, Los Villares Periurban Country Park, 600m, I.1995, J. Gómez (H.B. 5556, AH 7084); – *ibid.*, 26.I.1997, J. Gómez (H.B. 5709, AH 7083); – 9km NNW of Córdoba, 1.5km N of Santo Domingo, Los Villares Periurban Country Park, Camping area, 575m, 15.III.2003, P. Daniëls (P.D. 1564, JA-CUSSTA 6184, Moreno-Arroyo 2004 as *Hymenoscyphus* sp., *n.v.*); – *ibid.*, 15.II.2009, P. Daniëls (P.D. 2634, JA, *n.v.*); – **Huelva**, 50km NW of Huelva, 0.7km SE of Sanlúcar de Guadiana, 40m, 5.I.2011, Á. Martín ( $\emptyset$ , *n.v.*); – *ibid.*, 10.I.2011, Á. Martín (R.T. 11011201); – 4km N of Aracena, 1.5km SE of Castañuelo, NE of Sierra de Aracena, W of Finca Los Barrancos, 565m, 12.XI.1999, G. Moreno, A. Altés & L. Romero de la Osa (AH 7260, H.B. 6537); – *ibid.*, 31.XII.1999, R. Galán (AH 7262); – 9km ENE of Aracena, 2.5km NE of Puerto Moral, 340m, 31.I.2011, J.F. Moreno ( $\emptyset$ , *n.v.*); – *ibid.*, 325m, 22.XII.2012, J.F. Moreno (R.T. 12122702); – 3km ENE of Puerto Moral, Zufre, 308m, 29.XII.2012, L. Romero de la Osa (AH 7646); – 23km ESE of Huelva, 10km S of Bonares, Arboreto de El Villar, 82m, 11.I.2011, D. Estrada & D. Merino (D.M.A. 20110111, *n.v.*); – *ibid.*, 26.III.2011, M.Á. Ribes, D. Merino, T. Illescas *et al.* (M.A.R. 260311-47  $\emptyset$ , *n.v.*); – *ibid.*, 3.I.2013, D. Estrada & D. Merino (R.T. 13011101); – *ibid.*, 13.I.2013, M.Á. Ribes *et al.*

(M.A.R. 130113-04, *n.v.*) – **Sevilla**, 50km NW of Sevilla, 7.5km ENE of El Madroño, 460m, 29.XI.2002, L. Alcoba & A. Ortega (GDAC 59119,

*n.v.*); – 48km NW of Sevilla, 0.4km SW of Castillo de las Guardas, entrance to Biological Reserve, 370m, 30.I.2001, L. Alcoba & A. Ortega

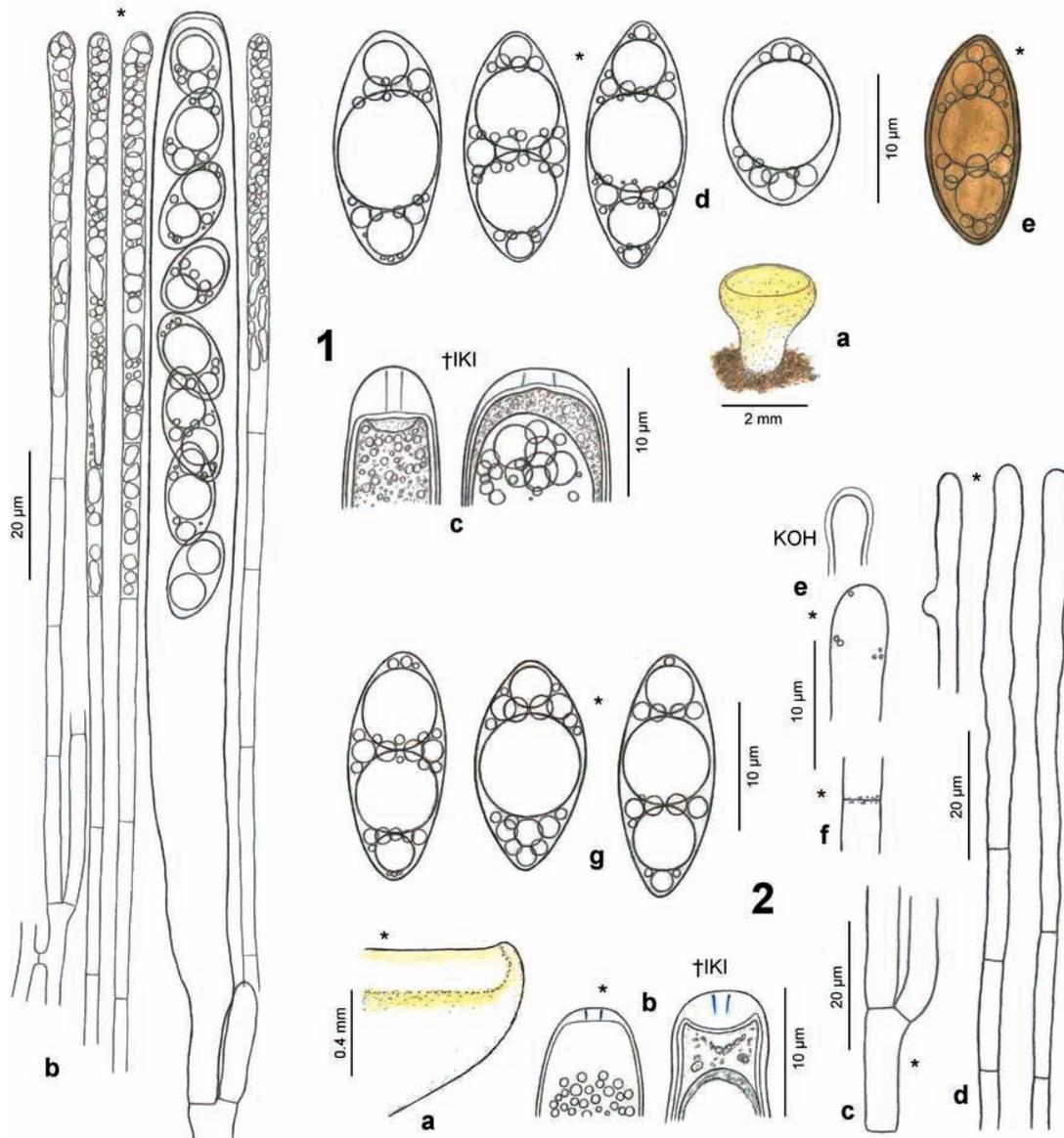


Plate 1 *Phaeohelotium undulatum*. Fig. 1a: Fresh apothecium; Fig. 2a: Median section of receptacle; Figs 1b, 2d: Ascus and paraphyses (in 2b containing VBs in terminal cell); Fig. 2c: Simple-septate ascus base; Figs 1c, 2b: Apex of immature (left, with many small LBs) and mature ascus (right); Fig. 2e: Apices of paraphyses with minute LBs (in KOH with a gel sheath); Fig. 2f: Lower part of paraphysis with septum and minute LBs; Figs 1d, 2g: Mature hyaline ascospores containing large and small LBs; Fig. 1e: Overmature brown ascospore. Living state except for Figs 1c, 2b (right), Fig. 1e (top). — Fig. 1a–e: Huelva, Aracena (H.B. 6537, AH 7260); Fig. 2a–g: Córdoba, Los Villares (H.B. 5709, AH 7083); del. H.O. Baral.



Plate 2 *Phaeohelotium undulatum*. Apothecia on soil under *Eucalyptus* (fresh state). a, d: Huelva, Aracena (phot. R. Galán, AH 7262); b, f: Córdoba, Los Villares (phot. P. Daniëls, P.D. 2634); c: Huelva, Aracena (phot. R. Galán, H.B. 6537, AH 7260); e: Cáceres, Las Veguillas (phot. C. Gelpi, I.I.2002); g: Huelva, Sanlúcar de Guadiana (phot. Á. Martín, 5.I.2011); h: *ibid.* (phot. R. Tena, R.T. 11011201); i: Cáceres, Puerto de Miravete (phot. R. Galán, AH 7337); j: Cáceres, Las Veguillas (phot. C. Gelpi, R.T. 12122701); k, l: Huelva, Aracena (phot. J.F. Moreno, R.T. 12122702).

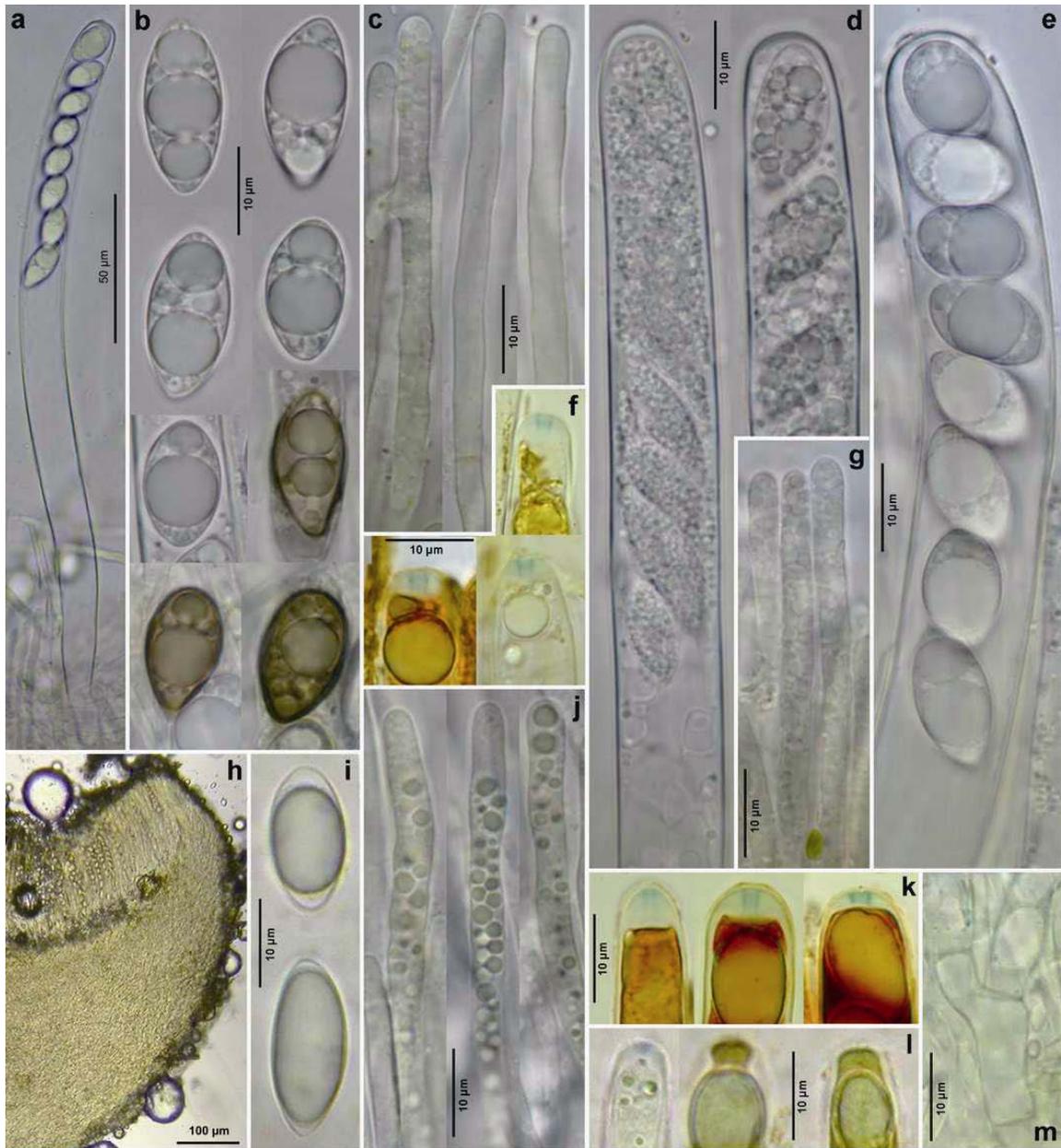


Plate 3 *Phaeohelotium undulatum*. a, e: Mature asci with hyaline spores; b, i: Mature (hyaline) and overmature (brown) ascospores; d: Immature asci, young spores filled with and surrounded by oil drops; c, g, j: Paraphyses (c, g: Without or with low-refractive vacuolar guttules, j: With high-refractive vacuolar guttules); f, k: Ascus apices with apical thickening and amyloid apical ring; l: Ascus apices with reduced apical thickening, only sometimes faintly amyloid (left); h: Median section of apothecium; m: Simple-septate ascus base. Living state except for i (in H<sub>2</sub>O), f, k (in IKI), h (in KOH), l (in KOH+IKI). — a–g, m: Huelva, Sanlúcar de Gadiana (phot. R. Tena, R.T. 11011201); j: Cáceres, Torreseca (phot. R. Tena, R.T. 13021302); h: Huelva, Aracena (phot. H.O. Baral, H.B. 6537, AH 7260); k: *Ibid.* (phot. R. Tena, R.T. 12122702); i, l: Sevilla, Los Cerrillares (phot. H.O. Baral, H.B. 9743, M.A.R. 081208-09).

(GDAC 59118, *n.v.*); – 7km N of La Puebla de los Infantes, Los Cerrillares, 240m, 8.XII.2008, T. Illescas, D. Merino, M.Á. Ribes *et al.* (M.A.R. 081208-09, H.B. 9743); – **Málaga**: 9km SSW of Cortes de la Frontera, 0.5km N of El Colmenar, arboretum, 320m, 5.XII.2011, M.Á. Pérez de Gregorio (C.R.P. 001220111, *n.v.*); – **Jaén**, 4.5km WSW of Santa Elena, La Aliseda, 660m, 18.IV.2010, T. Illescas & M.Á. Ribes (M.A.R. 180410-08  $\emptyset$ , *n.v.*); **EXTREMADURA, Cáceres**, 25km SW of Navalmoral de la Mata, S of Casas de Miravete, Puerto de Miravete climb, ~500m, 15.II.2001, G. Moreno (AH 7337); – *ibid.*, 10.III.2001 (AH 7337 bis); – 25km SW of Navalmoral de la Mata, NW of Casas de Miravete, Puerto de Miravete climb, 455m, 15.II.2013, R. Galán (AH 7652, R.T. 13022701); – 2km SSW of Jarandilla de la Vera, 575m, 24.IV.2010, J. Muñoz, M.Á. Ribes *et al.* (M.A.R. 240410-18  $\emptyset$ , *n.v.*); – 9km SSE of Jarandilla de la Vera, 1km NW of Torreseca, Las Veguillas, 290m, 1.I.2002, C. Gelpi ( $\emptyset$ , *n.v.*); – *ibid.*, 12.II.2005 ( $\emptyset$ , *n.v.*); – *ibid.*, 4.II.2006 ( $\emptyset$ , *n.v.*); – *ibid.*, 22.III.2010 ( $\emptyset$ , *n.v.*); – *ibid.*, 30.I.2011 (R.T. 12122701); – *ibid.*, 4.II.2011 ( $\emptyset$ , *n.v.*); – *ibid.*, 27.I.2012 ( $\emptyset$ , *n.v.*); – *ibid.*, 11.II.2012 ( $\emptyset$ , *n.v.*); – *ibid.*, 2.II.2013 (E.R.D. 5768, *n.v.*); – *ibid.*, 8.II.2013, J.A. Suárez & C. Gelpi (R.T. 13021301, C.G. 822013V); – 9km SE of Jarandilla de la Vera, 2km NNE of Torreseca, 360m, 27.II.2011, C. Gelpi ( $\emptyset$ , *n.v.*); – *ibid.*, 8.II.2013, J.A. Suárez & C. Gelpi (R.T. 13021302, C.G. 822013T).

***Phaeohelotium succineoguttulatum*** Baral, R. Galán, R. Tena & E. Rubio, **sp. nov.** – MycoBank MB 803811

**Type:** Spain, Asturias, Castrillón, Avilés, La Candaliega, Quiloño, under *Eucalyptus globulus*, 7.I.1998, E. Rubio (AH 7157 holotype, H.B. 6011 isotype).

**Diagnosis:** Apothecia 1.5–11mm diam, disc ochre-brown, stipe distinct, whitish-cream. Asci \*197–263 $\times$ 12–15 $\mu$ m, 8-spored, with inamyloid apical thickening, arising from simple septa. Ascospores \*/†13–21 $\times$ 6.3–10 $\mu$ m, broadly fusoid, hyaline, containing very large oil drops, overmature brown. Paraphyses (\*) consistently containing bright yellowish-brownish vacuolar bodies. Ectal excipulum at flanks of gelatinized *textura prismatica-porrecta*. Medullary excipulum non-gelatinized. On soil under *Eucalyptus*.

**Description:** Apothecia scattered or gregarious in small groups, sometimes fasciculate from a common stipe, hydrated (1.5–)2–5(–7) or (4–)5–8(–11)mm diam, round or with somewhat undulating margin, non-gelatinous but cartilaginous in the ectal excipulum, disc young concave, soon flat, bright dirty ochraceous-brownish(-greyish) to chestnut-brown, turning blackish-brown when overmature, margin thin, smooth, externally pale to bright ochraceous, smooth to minutely downy; stipe distinct, whitish-cream to the very base, short to medium long (1/2–3 $\times$  longer than wide), 0.7–1.3 $\times$ 0.9–2 or 2–3(–4) $\times$ 1.3–2.6mm, short-haired near base, hardly immersed in soil. **Asci** \*(197–)210–250(–263) $\times$ 12–15 $\mu$ m {6}, †(170–)180–207(–228) $\times$ (9–)10–11(–12) $\mu$ m {5}, 8-spored, pars sporifera \*78–97(–104) $\mu$ m {2}, spores (\*) obliquely uniseriate; apex (†) broadly rounded to subconical-subtruncate, apical wall \*0.8–1 $\mu$ m thick, †2–5  $\rightarrow$  1.7–2.8 $\mu$ m thick {2}, inamyloid in IKI or MLZ {10} (without or with KOH-pretreatment); gradually tapered towards base, arising from simple septa {9}; upper part of asci prior to spore formation multiguttulate (high lipid content). **Ascospores** \*((12–))(13–)14–20(–21)((–23.5)) $\times$ (6.3–)7–9.5(–10) $\mu$ m {9}, †(13–)14–19(–21.5) $\times$ (6.2–)7–9.5(–10) $\mu$ m {6}, broadly fusoid(-limoniform), rarely ovoid with

one end rounded, consistently hyaline when inside living asci; (\*) with (1–)2–3(–4) very large (4–8 $\mu$ m diam) and several smaller **LBs** (lipid content very high), (†) **LBs** mostly confluent to one ellipsoid body; wall †0.2–0.3 $\mu$ m thick, surface in CRB pale to light lilac; overmature spores with a light to dark (reddish-)brown, in KOH (olivaceous-)brown, \*/†0.3–0.7 $\mu$ m thick wall, smooth or sometimes very finely punctate, aseptate, size and lipid content unchanged, no visible germ pores, germination rarely observed {2}, terminal or lateral, forming microconidia \*3–5 $\times$ 1–1.5 $\mu$ m on short germ tubes, probably phialidic. **Paraphyses** cylindrical, terminal cell \*(25–)37–55 $\times$ (2.2–)3–4.5(–6.2) $\mu$ m {2}, †(2–)2.5–3.5(–4) $\mu$ m wide {2}, lower cells \*14–37 $\times$ 1.8–2.8(–3.5) $\mu$ m {2}; exceeding the dead asci for 0–15 $\mu$ m, while mature living asci projecting 10–25(–40) $\mu$ m beyond paraphyses; (\*) upper 1–2(–3) cells at a length of 40–100 $\mu$ m containing many globose, bright yellowish-brownish (golden-ochraceous-amber), strongly refractive **VBs** 0.5–1.5 $\mu$ m diam. {9}, these persistent in dead cells when mounted in H<sub>2</sub>O, bright to dark yellowish- or reddish-brown, very conspicuous, in KOH refractivity and pigmentation strongly or only slightly diminished; **LBs** in lower part of paraphyses and in subhymenial cells hyaline. **Ectal excipulum** hyaline to very pale ochraceous, (\*) slightly, (†) rather strongly gelatinized, common walls \*1(–2) $\mu$ m thick (†1–3 $\mu$ m); 80–100 $\mu$ m thick in stipe, of a strongly undulating *textura prismatica* oriented at a 45–80° angle to the surface, individual cells \*10–20 $\times$ 6–14 $\mu$ m {1}; 80–130 $\mu$ m thick at lower flanks, of  $\pm$  undulating *t. prismatica*, cells \*30–50 $\times$ 8.5–11(–13) $\mu$ m {2}, †(16–)20–35 $\times$ 5–8(–12) $\mu$ m {1}, oriented at a (0–)30–80° angle; 40–50 $\mu$ m thick near margin, of *t. prismatica-porrecta* oriented at a 0–10° angle;

externally covered at base and lower flanks by a ~15–20 $\mu$ m thick layer of parallel, gelatinized, \*3–6(–7) $\mu$ m wide hyphae; **hairs** on surface absent or present. **Medullary excipulum** hyaline to pale brownish, 200–500 $\mu$ m thick at base of receptacle, upper part of dense or loose *textura porrecta-intricata*, individual cells \*(30–)40–70(–96) $\times$ (3–)4–8(–9) $\mu$ m {2}, smooth, thin-walled, rather sharply delimited from ectal excipulum by a 50–70 $\mu$ m thick layer of *t. porrecta*. **Subhymenium**: 30–50 $\mu$ m thick, of pale yellowish-ochraceous to ochre-brown *textura intricata*, non-gelatinized. **Anchoring hyphae** \*2.5–5.5 $\mu$ m wide, wall 0.3–0.6 $\mu$ m wide, smooth, hyaline. – All tissues IKI–, without crystals, without conspicuous oil drops, gel in CRB pale lilac.

**Habitat**: in Mediterranean, pure eucalypt plantations, under *Eucalyptus globulus* {4}, *E. camaldulensis* {2}, *Eucalyptus* sp. {2}, on acid soil (granite, quartzite, slate, sandstone etc.), rarely slightly basic (sandstone or marlite, but possibly acidified), often with remains of charcoal, partly around burnt stump, closely associated with a high amount of fine roots (very probably of *Eucalyptus*), naked or loosely to densely covered by small acrocarpous (*Funaria hygrometrica*, ?*Dicranella*, *Pogonatum*) or pleurocarpous mosses, *Cladonia* sp., further associated with *Descomyces albus*, *Hydnangium carneum*, *Ruhlandiella berolinensis*, *Setchelliogaster tenuipes*, *Urnula rhytidia*, 60–575m a.s.l., Dec.–March(–June). **Desiccation tolerance**: asci and paraphyses were all dead after 1 day in the air-dried state; all hyaline and brown spores were dead after 15 months.

**Specimens examined** (all under *Eucalyptus* spp.): **SPAIN: ASTURIAS, Castrillón**, 3km W of Avilés, 1.3km SE of Piedras Blancas, La Candaliega, Quiloño, 80m, 7.I.1998, E. Rubio (AH 7157, H.B.

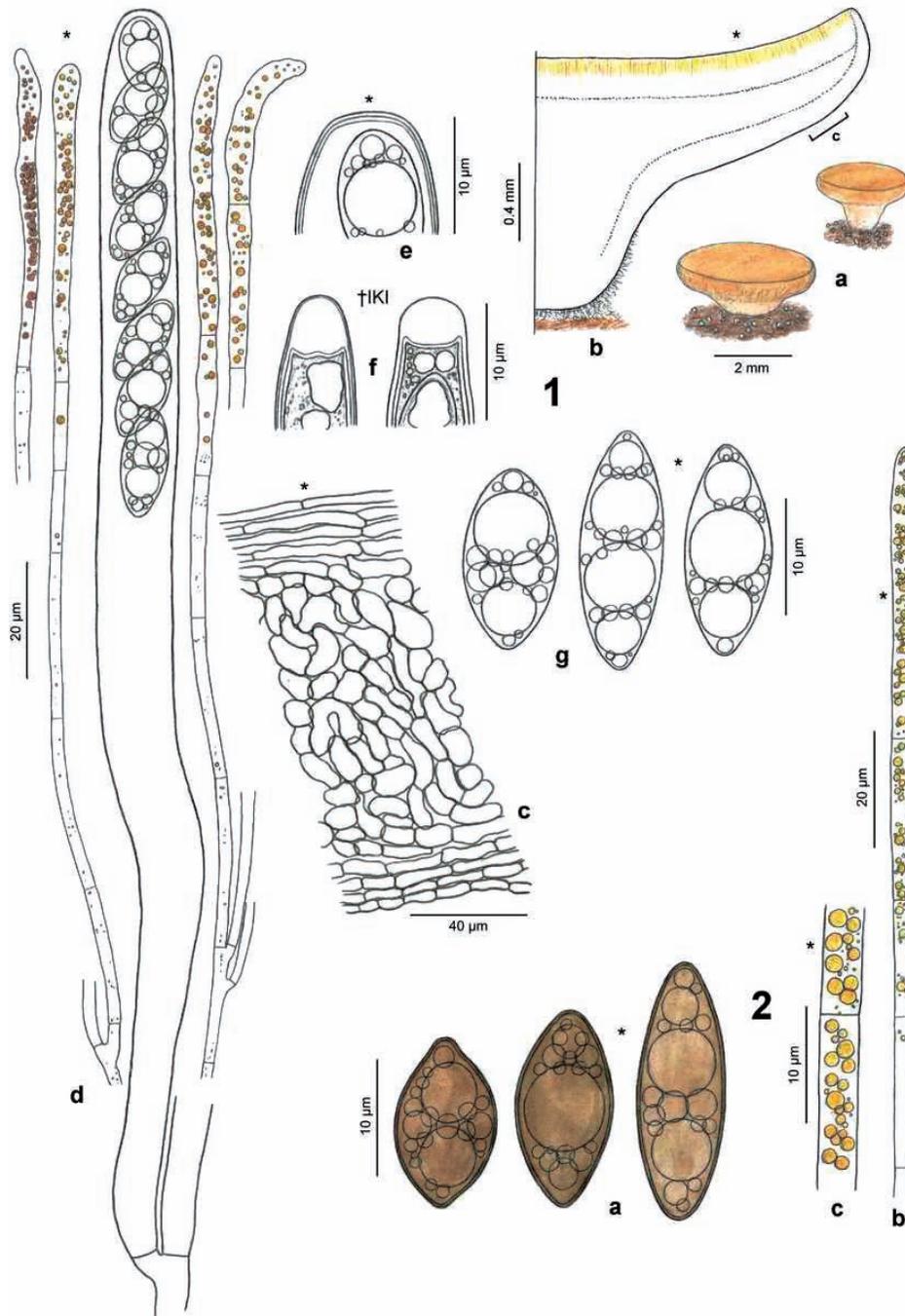


Plate 4 *Phaeohelotium succineoguttulatum*. Fig. 1a: Fresh apothecia; 1b: Median section of apothecium; Fig. 1c: Median section of ectal excipulum (lower flanks near margin), with covering layer below and parts of medullary excipulum; Figs 1d, 2b: Ascus and paraphyses; Fig. 1e: Apex of mature living ascus; Fig. 1f: Apices of immature (left) and submature (right) dead asci (left); Fig. 2c: Lower part of paraphysis, with septum and yellow-ochre VBs; Fig. 1g: Mature hyaline ascospores containing large and small LBs; Fig. 2a: Overmature brown ascospore. Living state except for 1f. — Figs 1a–g: Castrillón, Avilés (H.B. 6011). Figs 2a–c: *Ibid.* (H.B. 6549); del. H.O. Baral.



Plate 5 *Phaeohelotium succineoguttulatum*. Apothecia on soil under *Eucalyptus* (fresh state). a, c: Asturias, La Candaliega (AH 7157 = H.B. 6011, phot. a: E. Rubio, c: R. Galán); b: Córdoba, Los Villares (15.II.2009, phot. T. Illescas); d: *Ibid.* (R.T. 12120601, phot. T. Illescas); e: Cáceres, Torreseca (R.T. 11030401, phot. C. Gelpi); g, h: Asturias, La Candaliega (AH 7256, phot. R. Galán); i, j: *Ibid.* (AH 7263, phot. R. Galán); f: Cataluña, Girona (AH 7643, phot. M.À. Pérez-De Gregorio); – k, l: *Ibid.* (AH 7283, k, l: phot. J. Vila, scale applies to k, l); m: Cáceres, Torreseca (R.T. 13021201, phot. C. Gelpi).

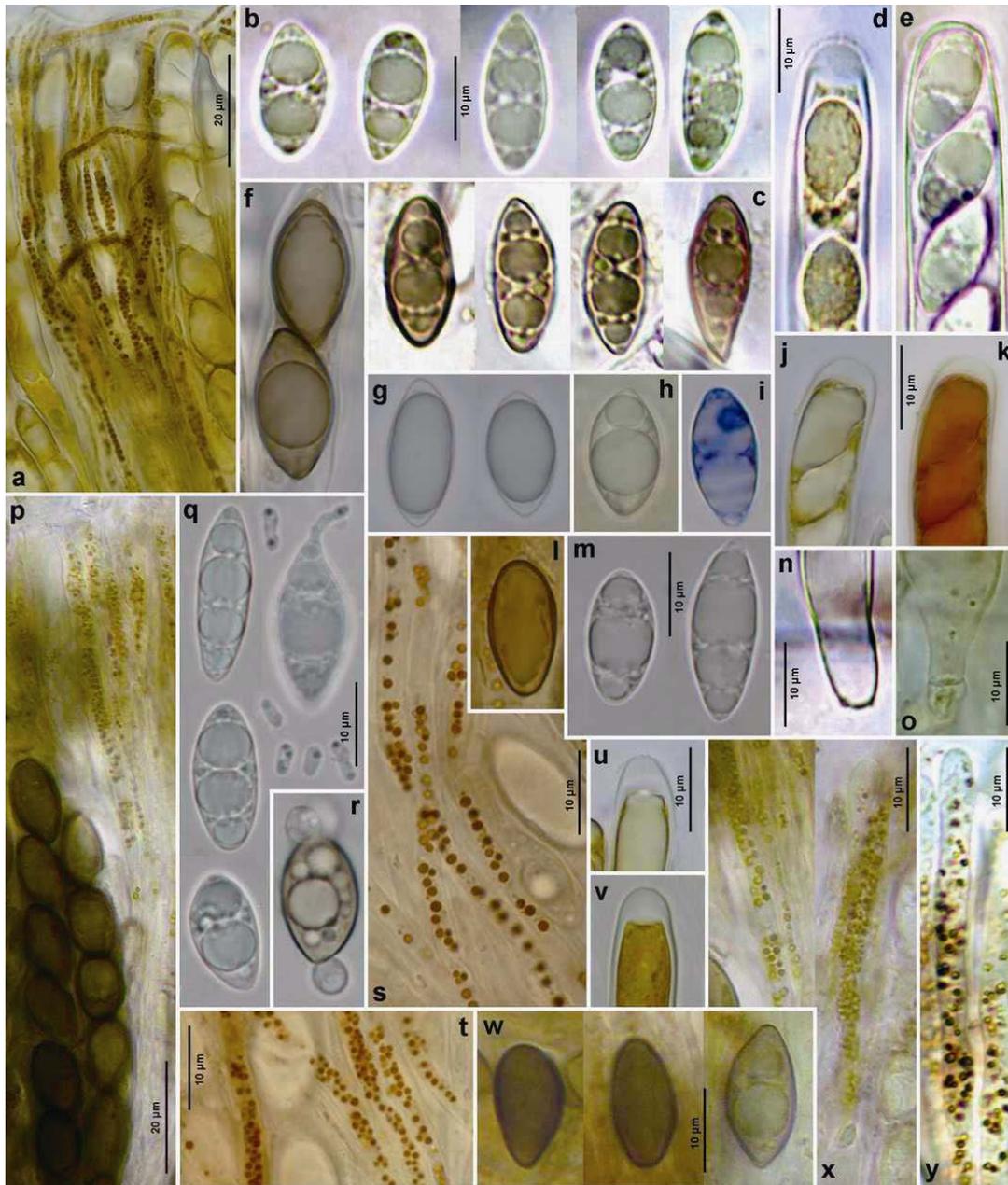


Plate 6 *Phaeohelotium succineoguttulatum*. a, p, s–t, x–y: Paraphyses containing yellowish-brown vacuolar guttules (and asci with hyaline or brown spores); b, c, f–i, l–m, q, r, w: Mature (hyaline) and overmature (brown) ascospores (q, r: Two germinating spores and five conidia); d, e, j, k: Apices of mature asci; u, v: Apices of immature asci; n, o: Simple-septate ascus bases. Living state (b, c, e, h, n, q, r, y); dead state in H<sub>2</sub>O (a, d, f, g, l, m, p, s, t, w, x), IKI low concentration (j, u), IKI high concentration (k, v), or CRB (i). – a, f–h, j–k, o, t–u: Cáceres, Torreseca (R.T. 11030401, phot. R. Tena); q: *Ibid.* (R.T. 13021302, phot. R. Tena); b–e, n, y: Asturias, Núñez (21.II.2009, phot. E. Rubio); i, l, m, s: Córdoba, Los Villares (R.T. 12120601, phot. R. Tena); p, w: Asturias, El Monte (AH 7163, H.B. 6023, phot. H.O. Baral); r, v: Cataluña, Solius (R.T. 13011001, phot. R. Tena).

6011); – *ibid.*, 3.XII.1999, E. Rubio (H.B. 6549); – *ibid.*, 6.XII.1998, E. Rubio (AH 7256); – *ibid.*, 12.I.2000, E. Rubio (AH 7263); – *ibid.*, 31.XII.1997, E. Rubio (E.R.D. 1037, Rubio *et al.* 2006 as *Discinella terrestris*, *n.v.*); – 11km NE of Avilés, 1km SW of El Monte, Camporriundo, 60m, 28.I.1998, E. Rubio (H.B. 6023, AH 7163); – 5.5km SE of Avilés, 1.3km NW of Corvera de Asturias, NE of Núñez, 200m, 21.II.2009, Á. Suárez ( $\emptyset$ , *n.v.*); – **PAÍS VASCO, Vizcaya**, 17km WNW of 2km S of Muskiz, Rebornun recreational area, 100m, 9.III.2000, J. Fernández & J.L. Pérez-Butrón (SESTAO 00030901, *n.v.*). – **CATALUÑA, Girona**, 19km NNE of Girona, 1.8km E of Llagostera, Mas Roure, 130m, 10.II.1996, J. Vila (AH 7283); – *ibid.*, 9.II.2003, F. Rodríguez & J.M. Vidal (AH 7643); – 7km ESE of Llagostera, 1.5km WSW of Solius, ENE of Cueva de la Tuna, 85m, 16.I.2007, Roqué (C.R.P. 160120072); – *ibid.*, 11.II.2009, C. Roqué (C.R.P. 110220091); – *ibid.*, 17.II.2010, C. Roqué (C.R.P. 170220102); – *ibid.*, 5.I.2013, J. Carbó, M.À. Pérez-De-Gregorio & C. Roqué (J.C. 20130105). – *ibid.*, 9.I.2013, C. Roqué (A.H. 7647, R.T. 13011001). – **EXTREMADURA, Cáceres**, 9km SE of Jarandilla de la Vera, 1.6km NNE of Torreseca, 360m, 27.II.2011, C. Gelpi (R.T. 11030401); – 9km SSE of Jarandilla de la Vera, 1km NW of Torreseca, Las Veguillas, 290m, 8.II.2013, C. Gelpi (R.T. 13021201). – **ANDALUCÍA, Córdoba**, 8.5km NNW of Córdoba, Los Villares Periurban Country Park, 575m, 3.VI.2007, T. Illescas ( $\emptyset$ , *n.v.*); – *ibid.*, 15.II.2009, T. Illescas ( $\emptyset$ , *n.v.*); – *ibid.*, 5.IV.2009, T. Illescas (R.T. 12120601).

**Discussion of the morphological characters:** *Phaeohelotium succineoguttulatum* (Pl. 4–6) differs from *Phaeoh. undulatum* (Pl. 1–3) in two consistent characteristics: (1) the asci are inamyloid in the former species but amyloid in the latter, and (2) the

paraphyses in the former species contain in their upper part abundant, conspicuous, globose, yellowish-brown organelles (vacuolar bodies=VBs), which are either hyaline or absent in the latter species. Instead, the latter species possesses yellow lipid droplets (LBs) in the lower part of the paraphyses and especially in the subhymenium, which are hyaline in the former species.

A few further features are more or less correlated with these key characteristics. In *Phaeoh. succineoguttulatum* a tendency to shorter terminal cells of the paraphyses and larger apothecia with thicker stipes was noted in comparison to *Phaeoh. undulatum*, also the apothecia tend to grow less gregariously. The ectal excipulum at the flanks was frequently oriented under a moderate to high angle to the surface in *Phaeoh. succineoguttulatum*, whereas in *Phaeoh. undulatum* it was predominantly oriented at a low angle and, therefore, only indistinctly delimited from the medullary excipulum. A further difference seems to lie in a slight gelatinization of the medullary excipulum and particularly the subhymenium in *Phaeoh. undulatum*, while both of them were non-gelatinized in *Phaeoh. succineoguttulatum*.

Due to the difference in the contents of the paraphyses, *Phaeoh. succineoguttulatum* sharply differs from *Phaeoh. undulatum* by ochraceous-brown vs. yellow apothecial discs, at least at an optimum stage of development. At an advanced stage when brown spores become abundant inside dead asci, the colour of the hymenium may get brownish also in *Phaeoh. undulatum* (Pl. 2 Fig. a), though never as intense and homogeneous as in *Phaeoh. succineoguttulatum*. In herbarium material the yellow lipidic pigment fades with the years, whereas the brown vacuolar pigment is still very well seen after at least 15 years. Also

*Phaeoh. undulatum* often possesses VBs in the paraphyses, but these are always hyaline and less refractive, and do not attain a distinct pigmentation in the dead state.

Ascus and ascospore size are quite the same in the two taxa, also the spore contents do not differ at all. The brown pigmentation of the overmature ascospores was often more intense in *Phaeoh. succineoguttulatum*, and the number of brown spores was usually higher in that species. However, the brown pigmentation varies in strength among the collections of *Phaeoh. succineoguttulatum*: in those specimens from Andalucía and Extremadura the pigmented spores were rather sparse and only pale ochre-brown, and the brown pigment stained also the surroundings of the spores within the asci when examined in the dead state (in H<sub>2</sub>O). However, spore colour depends on the pH of the mounting medium, being more reddish-brown in H<sub>2</sub>O while more olivaceous-brown when mounted in KOH (see also Pl. 10 Figs s–t).

Brown ascospores were completely absent in some of the collections studied, though the apothecia appeared to be fully mature. In fact, brown spores have never been observed inside living asci, at least in collections from Spain, which allows us to conclude that only hyaline spores are forcibly ejected. According to the definition of maturity by Baral (1992), the brown spores must be considered as overmature, and their presence and abundance strongly depends on the developmental stage of the population. Of course, in both species the presence of brown spores inside dead asci contributes to the colour of the apothecial disc, which turns ochraceous in *Phaeoh. undulatum* and blackish-brown in *Phaeoh. succineoguttulatum* with age.

Ascospores being initially hyaline but becoming pigmented with age have frequently been

described within some helotialean genera, such as *Lambertella*, *Martininia*, *Rutstroemia*, *Phaeohelotium*, and *Hymenoscyphus*, or also within some hemiphacidiacean genera, such as *Rhabdocline* and *Sarcotrochila* (Korf 1962: 16). Anyway, the prevalence of brown spores within some genera or their erratic presence in others does not seem to have much taxonomical relevance (see Galán & Baral 1997: 61, Hengstmengel 2009: 271). It could be merely considered as an ecological advantage for some species growing at more xeric conditions to enable survival over perhaps several years. While in *Phaeohelotium* no species is known to us that ejects brown spores, in *Lambertella*, a genus which has been defined by its brown spores, we observed both cases: brown spores are ejected in *L. palmeri* Raitv. & R. Galán, whereas *L. tetrica* (Qué.) Dumont ejects hyaline spores (R.T. ined.); also an unidentified species of *Lambertella* on *Olea* leaves with eventually abundant dark brown spores in the hymenium ejects only hyaline spores (Pablo Chacón pers. comm.). Likewise, Korf (*l.c.*) observed in at least three species of *Lambertella* invariably hyaline spores to be ejected on agar.

The lipid bodies inside the mature ascospores occupy the maximum possible volume, therefore, in water mounts it was only possible to locate the nucleus in immature, multiguttulate spores, while when adding IKI the nucleus is stained though hardly better recognizable. All species of the *D. terrestris* aggregate follow the strategy to store as much oil in the spores as possible (as a general matter of fact, lipid bodies are always globose as long as the spores are alive). A similar spore guttulation is observed in other soil-inhabiting helotialean fungi, such as *Discinella boudieri* (Qué.) Boud., *Leotia lubrica* (Scop.) Pers., or members of *Microglossum* Gill. This strategy seems

to indicate that the amount of energy stored in the spore as lipid is the more critical value for survival in these fungi. In the young spores numerous small LBs occupy the sporoplasm (Pl. 3 Fig. d). Other ascomycetes with high lipid content differ in having multiguttulate spores also when mature. Here, the amount of lipid in relation to the spore volume is smaller, whereas the rapidness of consumption of the lipid during germination is probably higher due to a higher total surface area of the lipid bodies.

Differences in spore size and shape often occurred in both species when comparing apical, middle, and basal spores within an ascus. The spore closest to the apical ring is usually shorter because it has a rounded apical end, while the remaining spores have both ends more or less attenuated. One or two of the lowermost spores are sometimes a bit longer and narrower compared to the middle spores. Spores of an extraordinary length were found to originate inside rarely observed 4-spored asci. The living hyaline spores of the two species did not noticeably shrink when killed by KOH, therefore, spore size hardly depends on the living vs. dead state. This is because the spore wall is rather rigid, in contrast to the elastic wall of the asci. Because of this rigidity the spore wall tends to show longitudinal wrinkling when rehydrating dead spores (Pl. 10 Fig. o). The spore wall distinctly increases in thickness when the spores turn brown. Very faint minute warts were sometimes seen on the spore wall of such overmature spores in both Spanish species.

Apothecial size of *Phaeoh. succineoguttulatum* was smaller in the specimens from Asturias and País Vasco [1.5–5(–7)mm] compared to those from Cataluña, Extremadura, and Andalucía [mainly 3–8.5(–11)mm]. The apothecia of *Phaeoh. undulatum* correspond with (1–)1.5–5.5(–7)mm to the smaller ones of *Phaeoh. succineoguttulatum*.

Intraspecific variation within *Phaeoh. undulatum* was noted, e.g., in the thickness of the apical ascus wall, being much thinner and partly inamyloid in a specimen from Sevilla (Pl. 3 Fig. 1, with the central wall region only  $\pm$ 0.5–1.5 $\mu$ m thick), compared to the other records examined.

Although only a few clear and consistent morphological differences could be found, we prefer to separate the two taxa at the species level, until more fresh collections, especially from other countries, are studied and compared. Their separation is supported by our molecular data which yielded 93% similarity in the ITS of the rDNA, while the two available sequences of *Phaeoh. succineoguttulatum* are 100% identical in the ITS.

**Identity of the five Australasian types.** When Dennis (1958: 342) transferred *Helotium terrestre* Berk. & Broome to *Discinella*, he re-examined not only the type material, but included in his description also four specimens from New South Wales, five from South Australia, and one from Tasmania (type of *Phaeopezia ochracea* Masee & Rodway). Furthermore, he compared *Aleurina readeri* Rehm from Victoria as possibly conspecific, but did not see material of it. Dennis considered the species to be common in Australia, and characterized it by up to 10mm large cups with a pale ochraceous to orange disc, a prominent, crenate margin, a white, minutely downy exterior, and a short, cylindrical stalk. The white flesh he reported as homogeneous, without segregation into ectal and medullary excipulum, composed throughout of  $\sim$ 4 $\mu$ m wide undulating hyphae lying almost parallel to the surface and protruding as up to  $\sim$ 30 $\times$ 4 $\mu$ m large flexuous hairs. Dennis measured the long-stalked asci as 200–225 $\times$ 10–14 $\mu$ m, with a truncate tip and a broad and thin apical ring staining blue in MLZ. The ascospores he found

ellipsoid-fusiform, their ends usually slightly apiculate (limoniform),  $14\text{--}25 \times 7.5\text{--}9 \mu\text{m}$ , with two

large central and partly two small terminal oil drops, their wall hyaline but ultimately becoming brown.

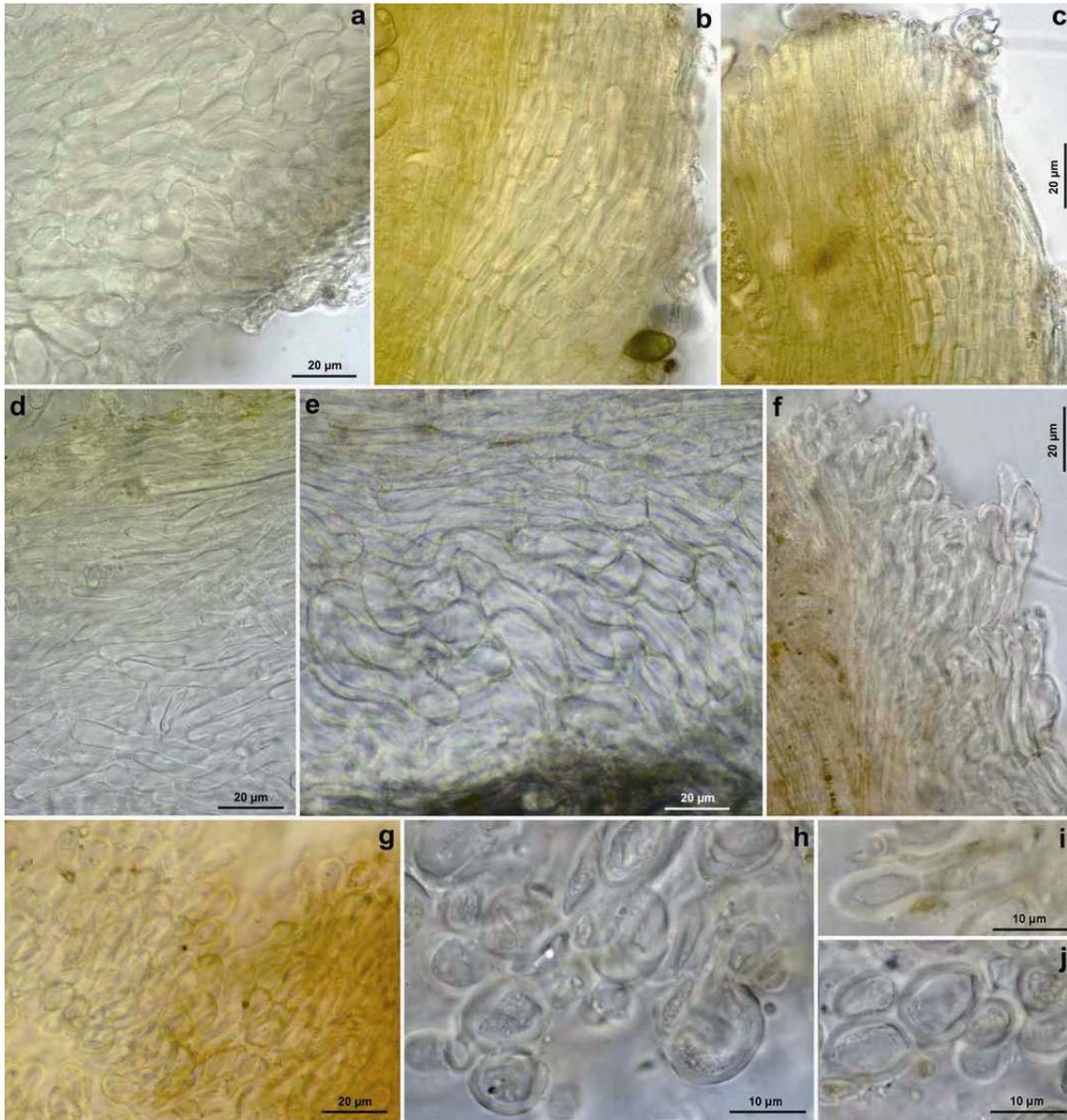


Plate 7 *Phaeohelotium undulatum* (Figs a–d) and *Phaeoh. succineoguttulatum* (Figs e–j). a–c, e–f: Ectal excipulum (median section; a, e: At flanks, b–c, f: At margin); d: Medullary excipulum near subhymenium (yellowish, median section); g–j: Ectal excipulum at margin (g: Surface view; h–j: Squash mount). Living state (a–f); dead state in  $\text{H}_2\text{O}$  (g–j). – Figs a–c: Cáceres, Miravete (AH 7652, R.T. 13022701, phot. R. Tena); d: Huelva, Sanlúcar de Guadiana (phot. R. Tena, R.T. 11011201); e–f: Cataluña, Solius (A.H. 7647, R.T. 13011001, phot. R. Tena); g–j: Cáceres, Torreseca (R.T. 11030401, phot. R. Tena).

Dennis (l.c.) did not comment on the spore size error in the protologue of *Helotium terrestre* (see below). Neither did he mention the spore size in the protologue of *Phaeopezia ochracea* which is given distinctly narrower (6–7µm) than by himself. Regrettably, Dennis did not specify the origin of his description and drawing. Based on the present re-examination of type specimens, his drawing probably shows the type of *Phaeop. ochracea*, regarding the depicted conspicuous LBs in the hyaline spores, the elongate-fusiform brown spores, and the apothecium showing a crenulate margin. A sketch was not included by Dennis in the type convolutes of both *H. terrestre* and *Phaeop. ochracea*. Later, Dennis (1961) described *Discinella confusa* Dennis from New Zealand without stating the differences to *D. terrestris*. He apparently erected the taxon by means of smaller, especially narrower spores and smaller asci, perhaps also by weighting sessile vs. stipitate apothecia. Finally, Dennis (1964: 79) stated that also *Pseudohelotium undulatum* Rodway is scarcely separable from *Discinella terrestris* though growing on rotten wood.

The present re-examination of type specimens of these five taxa revealed that all of them are undoubtedly very closely related to each other and also to the two species here reported from Spain. Except for *Phaeoh. undulatum*, however, none of them is to be considered conspecific with the two Spanish taxa. The most convincing distinguishing character concerns a frequently neglected detail of the ascus base, which also Dennis appears never to have taken into account: the asci in four of the Australasian types arise from croziers (Pl. 8 Figs 1b, 2b, 3b, Pl. 9 Fig. b), whereas in the type of *Phaeoh. undulatum* (Pl. 8 Fig. 4b) and in the Spanish specimens (Pl. 1 Fig. 2c, Pl. 3 Fig. m, Pl. 4 Fig. 1d,

Pl. 6 Figs n–o) they arise from simple septa. Besides this consistent and unambiguous feature, the asci and spores in those specimens with croziers tend to be slightly though insignificantly narrower compared to those without croziers. As a further distinguishing character, the type of *Aleurina readeri* has inamyloid asci and resembles herein *Phaeohelotium succineoguttulatum*.

Apart from the absence of a yellow colour in the fresh state, the type of *Pseudohelotium undulatum* fits quite well the Spanish specimens with a yellow disc, for which we have here adopted this name. Pigmented contents in the paraphyses comparable to those of *Phaeoh. succineoguttulatum* could not be seen in any of the Australasian specimens studied, either when viewed in water or in KOH. Because of this and the consistent presence of croziers, none of them can be conspecific with *Phaeoh. succineoguttulatum*.

The medullary excipulum of the examined Australasian specimens varies in gelatinization between strongly and very slightly, the gel being sometimes only obvious when trying to squeeze the tissue, depending on the specimen. When strongly gelatinized, the common walls are 1–3µm thick similar as in the ectal excipulum. This variable situation corresponds to that of the Spanish specimens. Possibly, a difference in the gelatinization of the medullary excipulum is characteristic at the species level.

*Helotium terrestre* was described by Berkeley & Broome (1883) based on a specimen growing on damp earth at Brisbane (SE-Queensland, Australia), with cups 1/6 inch (=4.25mm) diam, a flat, dirty yellow horn-coloured disc, externally hairy, the stem 1/24 inch (1mm) high, asci elongate, 8-spored, spores uniseriate, 0.0008×0.0004 inch (=20×10µm), elliptic, shortly appendiculate at both ends, with a

little globule at each extremity, paraphyses linear, slightly clavate at the tips. The provided sketch (Pl. 8 Fig. 1d–e) shows an apothecium with a slender stipe about  $3 \times 0.7\text{--}1\text{mm}$ , according to the disc diameter, and two spores that contain a large

elongate structure that occupies almost the entire spore volume. The above spore size is taken from Berkeley's handwritten note on the envelope of the holotype, while the protologue erroneously states the spores to be "0.0004 inch long".

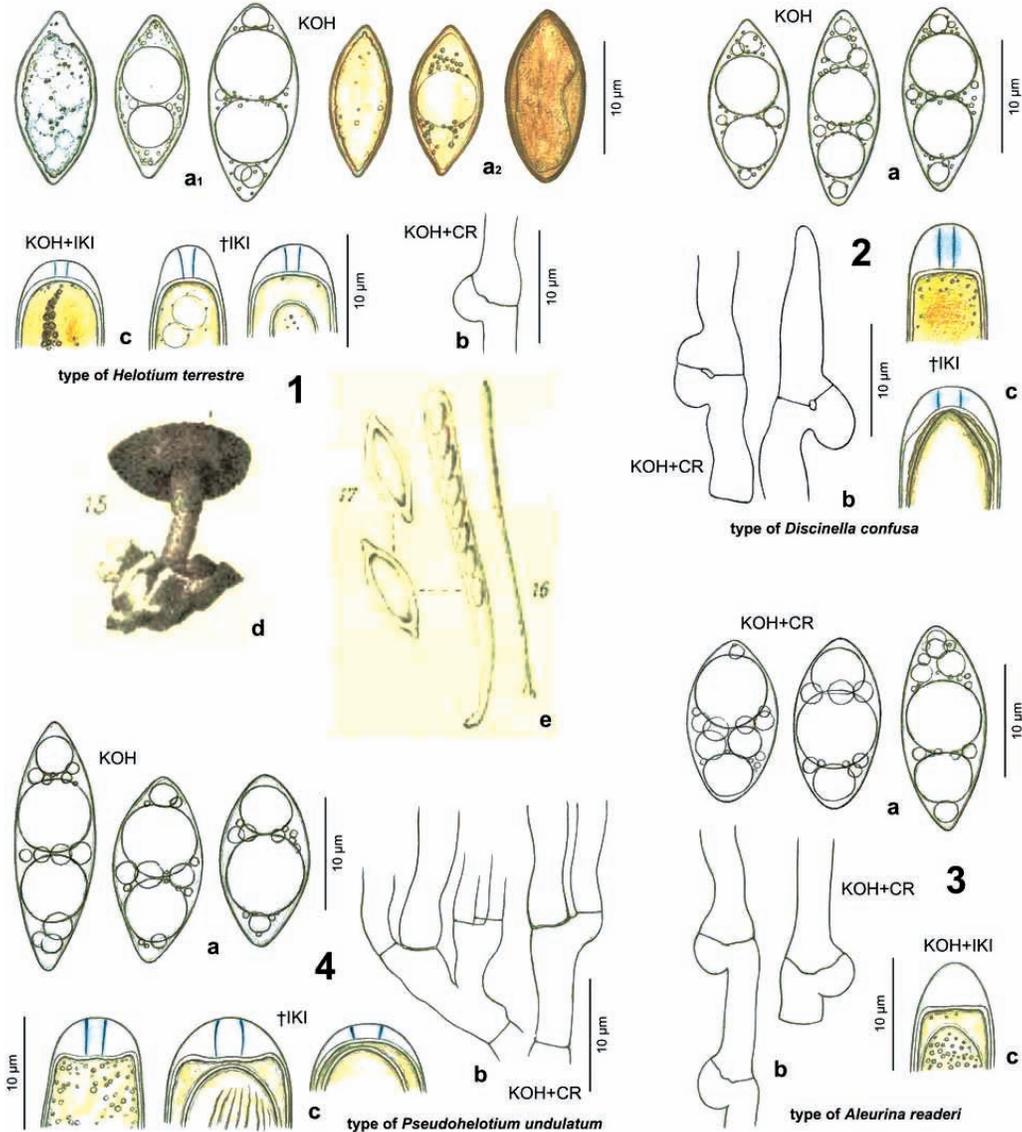


Plate 8 *Helotium terrestre* and related taxa described from Australasia. Fig. 1: Holotype of *Helotium terrestre* (K(M) 179947); Fig. 2: Holotype of *Discinella confusa* (K(M) 179945); Fig. 3: Isotype of *Aleurina readeri* (S-F 11757); Fig. 4: Isotype of *Pseudohelotium undulatum* (K(M) 179950); del. H.O. Baral. – a: Ascospores (in dead state, oil drops somewhat reconstructed from selected spores); b: Ascus apices, partly with amyloid ring (dead state); c: Ascus bases with croziers, in 4b without croziers; d, e: Original drawing by M.J. Berkeley (from Berkeley & Broome 1883).

The present re-examination of the holotype [Pl. 8 Fig. 1, Pl. 9 Figs i–q, K(M) 179947, fragment of a single apothecium, microtome sections provided by K.P. Dumont in 1970] revealed apothecia rehydrated ~3.5–4mm diam, with a stipe 1–1.2×1–1.8mm, the disc now bright ochre-brown, the margin hardly crenulate. The ascospores are broadly fusiform to limoniform, with a size of (12–)13–19(–20)((–23.3))×(5.8–)6.2–8(–9)µm. When limoniform, the acuminate ends give the spores an apiculate appearance. However, this feature might be an artifact of the dead spores, as is with certainty the elongate internal structure illustrated by Berkeley. This originates from the sporoplasm being detached from the spore wall in the dead state. Due to cytoplasm dehydration and contraction, the included large oil drops are not or only faintly recognizable (compare also Pl. 3 Fig. i, Pl. 6 Fig. g). Large globose lipid bodies could only faintly be seen in KOH in a few spores (Pl. 8 Fig. 1a<sub>1</sub>), but were undoubtedly present, the large ones having a size of 4–6.5µm. Pigmented spores were not encountered in the microtome sections, but do occur infrequently in the apothecial fragment. They were often only very pale ochraceous, but also bright (olivaceous-)brown (in KOH). The asci measure 140–205×(8–)9–10(–11)µm, with an apical wall 1–3µm thick and a consistently present, distinct though thin, medium strongly euamyloid apical ring that extends through the entire thickening (Pl. 8 Fig. 1c). It must be noted, however, that in the microtome sections the asci were found to be smaller [140–165×8–10µm vs. 180–210×9–10(–11)µm], also the spores were a little smaller, and the apical rings could only exceptionally be seen there (Pl. 9 Fig. k). The medullary excipulum is rather non-gelatinized, and the ectal excipulum only slightly so.

*Phaeopezia ochracea* was described by Masee

& Rodway (in Masee 1901) on the basis of three specimens (Rodway 125, 650, 650a) growing on the ground at Cascades near Hobart, Tasmania, with a pale ochraceous disc 5–10mm diam, an undulating margin, a short stipe, and a smooth, white exterior, asci 200×10µm, and brown, fusiform (“elliptic with both ends subacute”) spores 15–18×6–7µm.

The present re-examination of a syntype specimen [Rodway 650, K(M) 179951, 7 apothecia, microtome sections by K.P. Dumont, see Pl. 9] revealed rehydrated bright red-brown apothecia with a disc diameter of only 2–3.3mm, a crenulate margin, and a stout stipe 1.5–3×1–1.7mm. The fusiform-limoniform ascospores are very similar to the type of *H. terrestre* but more frequently bright (olivaceous-)brown and with a tendency to being longer and narrower [15–21(–23)((–25.7))×(5.7–)6–7(–7.5)µm], partly with acuminate ends, the hyaline ones in KOH sometimes with faintly visible LBs 4–6.5µm diam. The asci are rather disintegrated in their upper part, but from the hymenial height (200–250µm) a length of at least 200µm can be assumed, their width being ~9–11µm. In the few intact apices a prominent, 3–4.5→1.2–2µm tall apical thickening could be seen that includes a medium strongly euamyloid apical ring which is most reactive at the top. The medullary excipulum is composed of rather thick-walled hyphae (0.3–0.8µm), similar to the ectal excipulum. A few details in Dennis’ treatment differ from the present results: (1) Dennis did not report thick-walled ectal excipular cells, but instead illustrated them thin-walled; (2) the ectal and medullary excipulum are quite clearly segregated, the ectal excipulum at the lower flanks being oriented at a moderate to steep angle to the outside. The envelope bears parts of the protologue in original handwriting, e.g., “.5–1cm high & broad”. Dumont (in sched.) named

the specimen Rodway 650 as “lectotype”. However, no published designation of a lectotype for this taxon is known to us, hence specimen Rodway 650 is here designated as **lectotype** of *Phaeopezia ochracea*.

*Aleurina readeri* Rehm (1908) was based on a collection on soil from the County of Follett, Victoria (1907, leg. F.M. Reader), Australia, with apothecia with a dirty whitish to whitish-ochraceous disc 7–10mm diam and a very thick stipe 5–7×3–4mm, asci 150×9–10µm, inamyloid, spores oblong-ellipsoid, eguttulate, hyaline, finally brownish, 12–14×6µm, paraphyses filiform, internally granular, up to 2µm wide at the apex. Rehm’s handwritten notes on the label of the “isotype” show a sketch of an apothecium with a stipe length about 3.5× its width when neglecting the bulbous base, also a perfectly ellipsoid, eguttulate spore measuring 12×6µm. In his handwritten notes the paraphysis content is described as “with oil droplets”, that of the spores as “without oil drops”, and the asci as “opening with a pore”.

The present re-examination of the “holotype” [Pl. 8 Fig. 3, Pl. 10 Figs a–b, d–g, j–l, S–F 11757, five apothecia and microtome sections by K.P. Dumont] and “isotype” [Pl. 10 Figs c, h–i, S–F 11758, one apothecium and microtome sections] revealed rehydrated deep umbra brown apothecia with a disc diameter of 2–7mm, an even margin, and a comparatively slender stipe 3–7×0.7–1mm. The microscopic data presented here were gained mainly from the “holotype”: the ascospores are distinctly fusiform and closely resemble those of *H. terrestris*, though they are with (11–)13–16.5(–18)×6.8–8.3µm slightly shorter. They contain distinctly visible LBs (in KOH), the large ones 3.5–7µm diam. Some bright olivaceous-brown spores (in KOH) could be found in preparations taken from two apothecia from

both specimens, while the examined microtome section was devoid of them. The asci arise from croziers in both specimens, and are with (135–)150–180(–190)×9–10(–11)µm only slightly shorter than in the preceding taxa. In contrast to those, however, the 3–4(–5) → 0.5–1.5µm tall apical thickening is consistently inamyloid (IKI, KOH+IKI, KOH+MLZ, tested in both specimens). Dumont applied the terms holo- and isotype on his labels, but never appears to have published on this taxon. The two envelopes undoubtedly belong to the same collection. Because the “isotype” (containing one apothecium) bears Rehm’s original sketch, we believe this should in fact be considered as the holotype (in the legends the words holo- and isotype refer to our opinion).

Rehm compared *Aleurina substipitata* Henn. & E. Nyman from Java, which he separated from *A. readeri* by shorter-stalked apothecia and brownish paraphyses. *A. substipitata* might in fact be another candidate of the *D. terrestris* aggregate, rather than a member of the Pezizales, though the anchoring hyphae are described as fuscous-brown in the protologue.

*Pseudohelotium undulatum* Rodway (1925) from Tasmania ([Mount Field] National Park, XI.1923, on unidentified wood) was briefly described with white apothecia becoming ochre when dry, being externally slightly downy and sessile, spores ellipsoid but acutely pointed at both ends, hyaline, 12–14×6µm.

The present examination of the isotype in K [K(M) 179950; Pl. 8 Fig. 4, Pl. 10 Figs p–t] shows particles of charcoal at the apothecial base, while wood fragments could not be found. The single apothecium measures rehydrated 7mm diam, with a stout stipe 1×1.5mm. The asci have a size of 190–220×10–11µm and arise from simple septa. The

ascus apex shows a 2–3.8→1–1.5µm thick wall with an apical ring that reacts moderately to strongly blue. The spores are with (12–)14–20(–22)×(6.5–)7–8(–9)µm much larger than given by Rodway, and many of them are dark brown. The medullary excipulum is medium gelatinized, and the ectal excipulum strongly so.

*Discinella confusa* was described by Dennis (1961: 314) based on a specimen on plant debris from New Zealand (Orwel Creek, Ahaura, Westland, on litter of *Nothofagus*). The protologue includes ochraceous, 5mm large apothecia, amyloid asci 170×8–9µm, with a basal protuberance (?crozier), and fusoid spores 13–15×5.5–6µm, with two large and many small oil drops.

The present examination of the type (Pl. 8 Fig. 2) revealed that ascus as well as spore size overlap with the type of *Helotium terrestre*, with a recognizeable difference only in the width of both organs. The rehydrated apothecia measure 2–4mm diam, with an indistinct stipe 0.5×1mm, the asci measure 165–180×8–9(–10)µm and arise from croziers, the apex with a 3–4→1.5–2.5(–4)µm thick wall containing a strongly amyloid apical ring that also shows faintly amyloid lateral regions. The hyaline spores measure (11.5–)13–17(–19)×(5.5–)6–7.5µm, while brown spores were not seen. The medullary excipulum is medium gelatinized, the ectal excipulum strongly so.

#### Further Australasian specimens investigated.

A specimen from Tasmania [Newtown Track near Hobart, VI.1925, ?L. Rodway, K(M) 181009, identified by Dennis as *Discinella terrestris*] shows the same kind of strong iodine reaction with surrounding faintly amyloid regions as observed in the type of *Discinella confusa*. However, the asci (183–225×9–10µm) and spores [14–19(–20)×(5.5–)6.2–8.2µm] are distinctly larger, hence it

might be conspecific with the type of *Helotium terrestre*. This specimen was labelled *Humaria stipitata* Rod[way] by Rodway. It probably does not represent the type of that species which was published in the same year, because the manuscript was finished already in November 1924. Judging from this material, the type of *Humaria stipitata* might as well belong in the *D. terrestris* aggregate, though it was described as dark orange. A specimen from South Australia [Meningie, VI.1956, L.D. Williams, K(M) 181008, identified by Dennis as *Discinella terrestris*] appears to be conspecific. In both samples the asci arise from croziers.

A recently collected specimen from New South Wales (Katoomba, 2km SW of Wentworth Falls, between Lillian's Glen and Edinburgh Castle Rock, 1.IV.2007, G. Koller, H.B. 9746) likewise appears to fit the type of *Helotium terrestre*. The apothecia were light yellow, ~1.5–5mm diam, and grew under *Eucalyptus* (but also *Banksia*, *Ceratopetalum* and other trees were present). The asci measure †(160–)185–200×(9.5–)10–11(–12)µm and arise from croziers, and their rather strongly amyloid apical rings are surrounded by very faint amyloid regions. The hyaline spores measure †(13–)14–18(–20)×(5.5–)6–7.5µm, while brown spores were not seen. From the type of *D. confusa* this specimen deviates merely in larger asci. Similar as in the type of *H. terrestre*, the medullary excipulum is non-gelatinized here.

Macro- and micrographs of three records from New Zealand (Auckland and Coromandel Peninsula, North Island) under *Kunzea* and *Leptospermum* (PDD 77821, 78309, 83306), kindly provided by P.R. Johnston, and one under *Kunzea* (South Island, near Christchurch, PDD 96554) by J. Cooper (pers. comm.) appear to fit *Phaeoh. undulatum* very well. All of them show asci with simple-septate bases and

amyloid rings, and paraphyses without visible contents (dead state, in MLZ). The colour of the apothecial disc was (pale to) light yellow-ochraceous when fresh, hence these specimens might be part of an area of distribution from which the Spanish records have originated.

A sequence in GenBank (GU222294) under the name *Discinella terrestris* (PDD 89062, see [http://scd.landcareresearch.co.nz/specimen/PDD\\_89062](http://scd.landcareresearch.co.nz/specimen/PDD_89062)) concerns a specimen from Punakaiki (South Island, New Zealand) that grew on soil under *Nothofagus menziesii* and *N. fusca* (8.V.2006, leg. I. Dickie). P.R. Johnston (pers. comm.) kindly re-examined this specimen following our request, and supplied detailed data and micrographs which enable us to conclude that it is possibly not conspecific with the type of *Helotium terrestre* because of slightly smaller spores ( $\dagger 13.5\text{--}16 \times 6\text{--}6.5\mu\text{m}$ ). The asci arise from croziers, and their apical rings are strongly amyloid and fit quite well those here illustrated for the type of *Discinella confusa*. Also spore size fits well, and distinctly brown spores could not be found. A further specimen under *Nothofagus* from Reefton (South Island, PDD 83768) was documented by J. Cooper (pers. comm.) and likewise showed croziers, amyloid asci, light yellow apothecia, and no brown spores. Further four records from New Zealand under *Nothofagus* (PDD 93096, 93097, 103173, 103172) likewise show croziers at the ascus base, and also here brown spores could never be seen (P.R. Johnston pers. comm.).

P.R. Johnston drew our attention to GenBank records (ITS) from *Nothofagus* and *Eucalyptus* roots in Tasmania which are close to the sequence of PDD 89062. Therefore, we have included besides PDD 89062 one of these Tasmanian sequences (FN298679, *Nothofagus*, at Warra) in our

phylogenetic analysis. Considering the fact that also the type of *D. confusa* grew in association with *Nothofagus*, it might be concluded that this clade represents *D. confusa*. However, apart from another isolate from *Nothofagus* at Warra (FN298677), also three from *Eucalyptus* (JF960619, JF960769, at Mount Maurice; FN298678, at Mount Field) are more or less identical in the ITS, with a deviation of only 0–3 nucleotides among each other. That sequence from New Zealand (GU222294), however, differs from these in 16–18 nucleotides. We assume that only GU222294 represents *D. confusa*, while the remaining five sequences belong to another species, which is less adapted to a host tree, given that the roots were properly identified. This might concern either *Phaeoh. baileyianum* or *Phaeoh. readeri* (but note that the type of *Phaeoh. undulatum* was collected at Mount Field National Park).

Judging from their high genetic difference among each other, four further Tasmanian sequences (JF960780, JF960772, from *Eucalyptus* at Mount Maurice; FN298750, from *Nothofagus* at Warra; FN298689, from *Pomaderris* at Mount Field), which also belong in the *D. terrestris* aggregate, appear to concern four further species different from the former two. None of all these sequences are close enough to Spanish *Phaeoh. undulatum* and *Phaeoh. succineoguttulatum* in order to be considered conspecific with either of them.

The presently known records of fruiting *D. confusa* are restricted to New Zealand and situated in the mountain range along the west coast of the South Island and in the central and western part of the North Island. Based on the morphological and genetic distance to *Phaeohelotium undulatum*, the apparent connection to *Nothofagus*, and the restricted geographical distribution, the following new combination is here proposed:

***Phaeohelotium confusum*** (Dennis) Baral & P.R. Johnst., **comb. nov.** – **MycoBank MB803812**

Basionym: *Discinella confusa* Dennis, Kew Bulletin 15: 314 (1961)

According to the present examination of the five Australasian types, four of them – except for *Pseudohelotium undulatum* – represent taxa which are specifically distinct from those recorded in Spain. The close morphological similarity between *Helotium terrestre* and *Phaeopezia ochracea* as evaluated from the dead material supports Dennis' opinion that the two are conspecific, despite of slight differences in spore morphology. In order to avoid homonymy with the name *Phaeohelotium terrestre* (Velen.) Svrček, Česká Mykol. 41(1): 23 (1987), a species that appears to be a later synonym of *Phaeohelotium epiphyllum* (Pers.) Hengstm., the following new combination is proposed:

***Phaeohelotium baileyanaum*** Baral & R. Galán, **nom. nov.** [non *Phaeohelotium terrestre* (Velen.) Svrček, Česká Mykol. 41(1): 23 (1987)] – **MycoBank MB803814**

Basionym: *Helotium terrestre* Berk. & Broome, Trans. Linn. Soc. London, sér. 2, 2: 69, pl. XV fig. 15–17 (1883) [non *H. terrestre* Feltg., Recueil Me. Trav. Soc. G.D. Bot. Luxembourg 15: 63 (1901), ?=*Phaeohelotium geogenum* (Cooke) Svrček & Matheis]

≡*Dasyscyphus terrestris* (Berk. & Broome) Sacc., Syll. Fung. 8: 468 (1889, as *Dasyscypha*)

≡*Discinella terrestris* (Berk. & Broome) Dennis, Kew Bull. 13: 342 (1958)

=*Phaeopezia ochracea* Masee & Rodway in Masee, Bull. Misc. Inf., Kew: 159 (1901)

≡*Aleurina ochracea* (Masee & Rodway) Sacc. & D. Sacc., Syll. Fung. 18: 89 (1906)

Based on inamyloid asci and a tendency to shorter asci and ascospores, it is concluded that *Aleurina readeri* is not conspecific with *Phaeoh. baileyanaum*. Therefore, a new combination is proposed for it:

***Phaeohelotium readeri*** (Rehm) Baral, **comb. nov.** – **MycoBank MB803813**

Basionym: *Aleurina readeri* Rehm, Ann. Mycol. 6: 324 (1908)

The European *Hymenoscyphus epigaeus* (P. Karst.) Kuntze resembles members of the *Discinella terrestris* aggregate in its yellow, 1–3mm large, short-stipitate apothecia growing on soil in beech forests (*Fagus sylvatica*). It differs, according to recent collections made in southern Germany and Switzerland, by rather small, paramecium-like (heteropolar, ciborioid) ascospores  $*(11-12-15(-17) \times (3.5-4-4.5(-4.7)) \mu\text{m}$  with a comparatively low lipid content composed of many small LBs with a maximum diameter of 1–1.7 $\mu\text{m}$  (see Baral & Marson 2005). This species might belong in *Phaeohelotium*, but we hesitate to propose a new combination until the type material is studied.

Some further Southern Hemisphere taxa present similarities with the *Discinella terrestris* aggregate though being lignicolous. The Australian *Ciboria fusispora* Spooner (1987: 312) shows fusiform, guttulate, rather long spores, and similar apical rings surrounded by pale amyloid regions, and at least its placement in the Sclerotiniaceae seems us doubtful. The spores of the South American *Rutstroemia megalospora* (Speg.) Gamundí (1962: 403) are remarkably similar to *Phaeoh. baileyanaum* and allies, but the apical ring might indeed be sclerotiniaceous rather than helotiaceous.

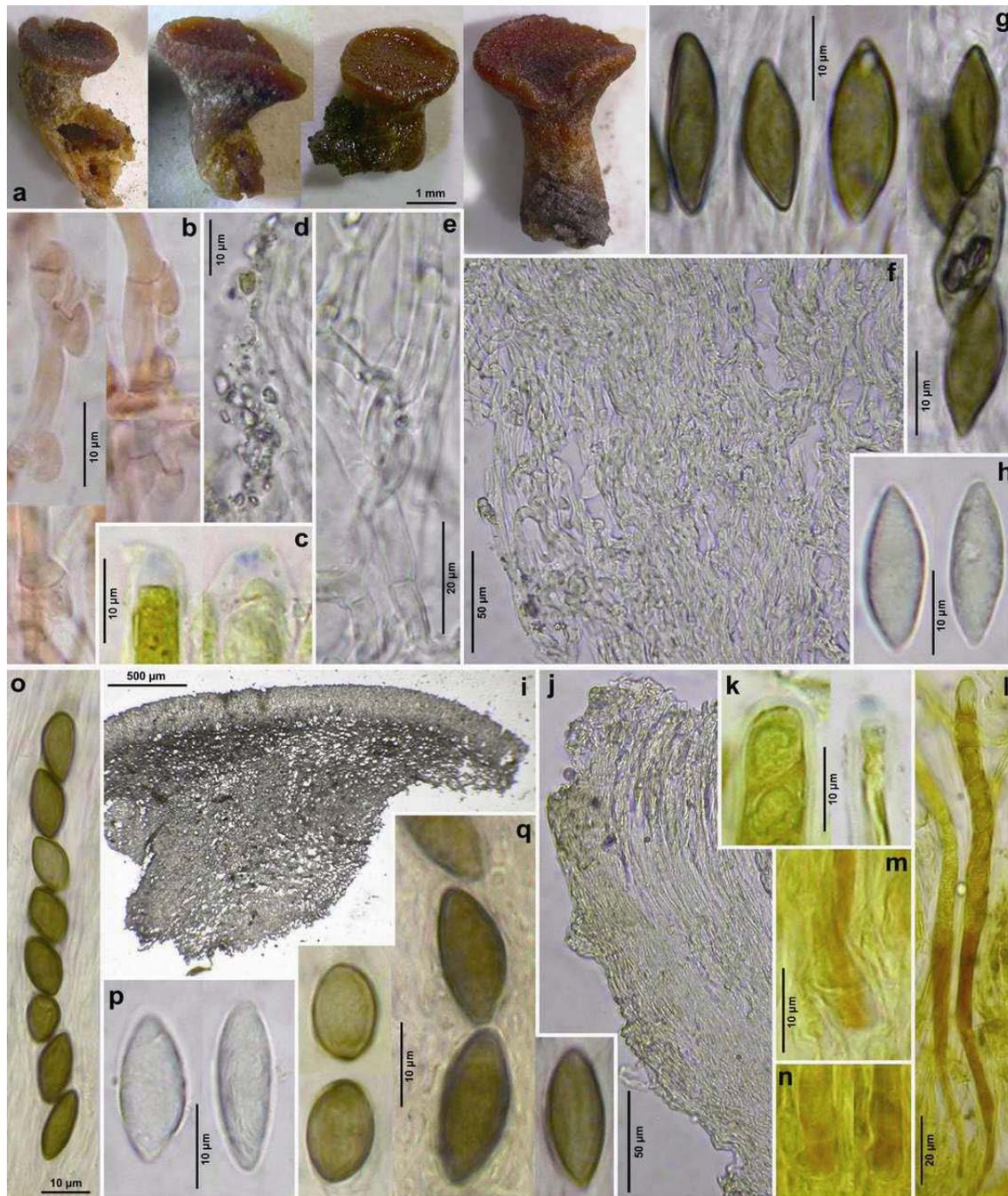


Plate 9 a–h: Holotype of *Phaeopezia ochracea*, K(M) 179951; i–q: Holotype of *Helotium terrestre*, K(M) 179947; phot. H.O. Baral. – a: Rehydrated apothecia; b, m, n: Croziers; c, k: Ascus apices with amyloid ring (in k partly inamyloid); i: Median microtome section of apothecium; d, j: Margin in median section (in d with external crystals); e: Thick-walled cortical hyphae at mid flanks; f: Section at mid flanks showing cortical layer, ectal and medullary excipulum; g, o, q: Brown ascospores; h, p: Hyaline ascospores with hardly visible large oil drops. Dead state in IKI (c); in KOH d–j, o–q; in KOH+IKI (k–n); in KOH+CR (b).

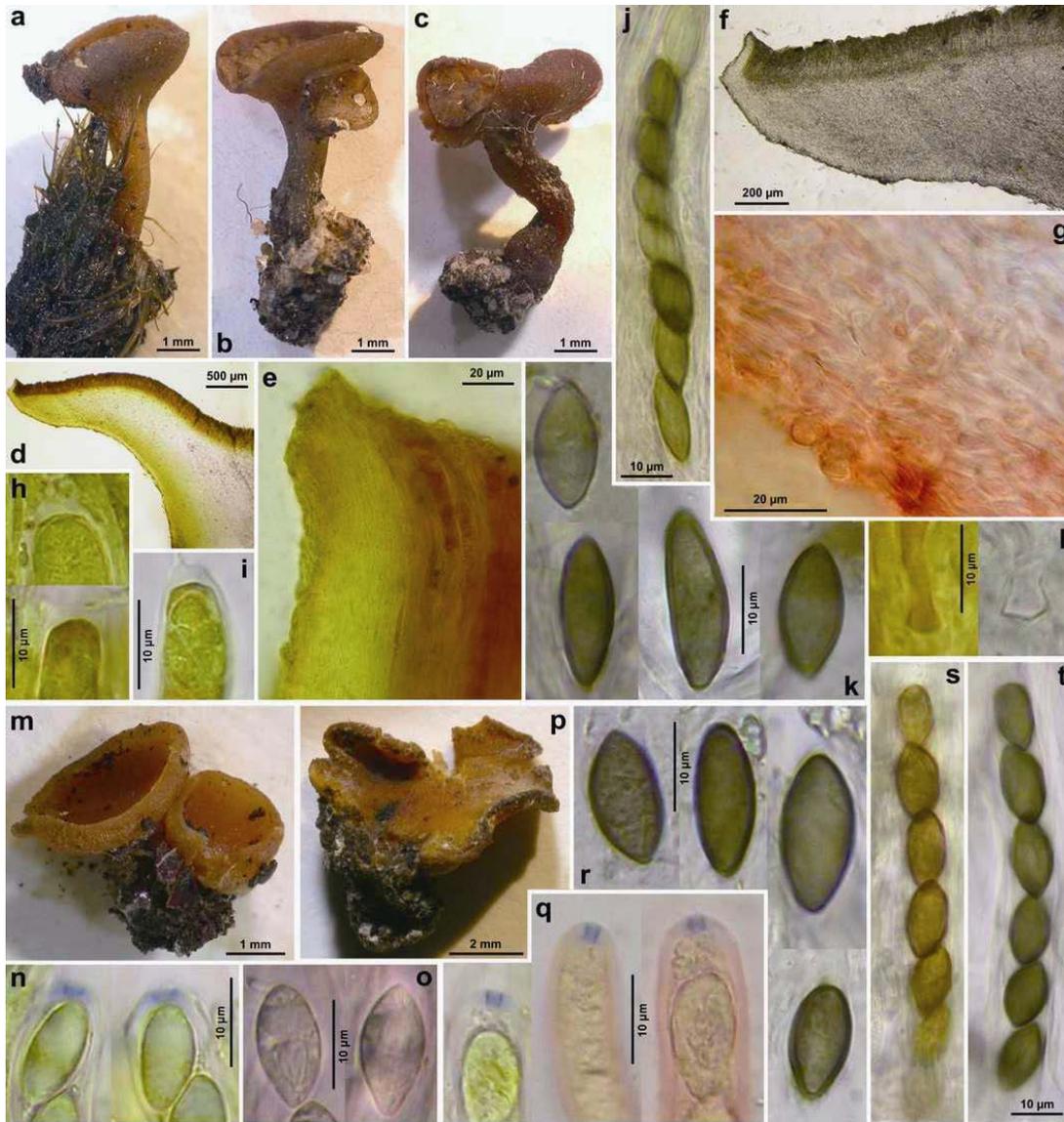


Plate 10 a–l: Type of *Aleurina readeri* (a, b, d–g, j–l: Isotype, S-F 11757, c, h, i: Holotype, S-F 11758); m–o: Holotype of *Discinella confusa*, K(M) 179945; p–t: Isotype of *Pseudohelotium undulatum* (K(M) 179950); phot. H.O. Baral. – a–c, m, p: Rehydrated apothecia; d–f: Median microtome section of apothecium; g: Section at mid flanks; h–i: Inamyloid ascus apices; l: Croziers; n, q: Ascus apices with amyloid ring; j, k, r–t: Brown ascospores; n–o, q: Hyaline ascospores with hardly visible large oil drops. Dead state: in H<sub>2</sub>O (s); in KOH (f, l right, r, t); in KOH+CR (g, j–k); in KOH+IKI (d–e, h–i, l left, q left); in KOH+IKI+CR (q right).

**Generic placement.** In our phylogenetic analysis, the sequenced members of the *Discinella terrestris* aggregate clustered in a well supported clade, which is situated sister to a clade comprising

*Phaeohelotium geogenum* (Cooke) Svrček & Matheis and *Phaeoh. monticola* (Berk.) Dennis. The latter species is currently considered as earlier synonym of *Phaeoh. flavum* Kanouse, the type

species of *Phaeohelotium*.

Members of the *Discinella terrestris* aggregate actually share many characters with *Phaeohelotium monticola*: more or less short-stalked, yellowish apothecia, an ectal excipulum at the lower flanks partly oriented at a high angle, an apical apparatus of the *Hymenoscyphus*-type, and homopolar ascospores that turn brown with age (the name *Phaeohelotium* was created in reference to this feature). The absence of croziers is not a rare case in *Phaeohelotium* as currently accepted. *Phaeohelotium* and *Cudoniella* Sacc. were considered by Baral (in Baral & Krieglsteiner 1985) to represent only subgroups within a large concept of *Hymenoscyphus*, for which precise delimitations are difficult to find. The here favoured narrower concept of *Hymenoscyphus* [type *H. fructigenus* (Bull.) Gray], concerns species with mainly heteropolar, scutuloid spores, and asci which predominantly lack croziers, features which both can be considered as apomorphic, while croziers and homopolar spores should represent plesiomorphic, ancestral features.

Our molecular analysis supports separation of different groups within *Hymenoscyphus s.l.* (Pl. 11). *Phaeohelotium undulatum*, *Phaeoh. succineoguttulatum*, and *Phaeoh. confusum* clustered with high support in a clade with *Phaeoh. monticola* and *Phaeoh. geogenum*. Together with morphological data which include several of the above-stated similarities, we here propose to accept *Phaeohelotium* as distinct from *Hymenoscyphus*.

However, the members of the *Discinella terrestris* aggregate also resemble macroscopically *Hymenoscyphus tamaricis* and *Phaeohelotium epiphyllum* quite well. These two species are closely related to each other according to our molecular analysis, but quite distant from *Phaeoh. monticola* and the *D. terrestris* aggregate, even though *Phaeoh.*

*epiphyllum* and *Phaeoh. monticola* are easily confused by morphology. There is a difference in the ectal excipulum at the lower and mid flanks, being *textura prismatica* in *Phaeoh. epiphyllum* (and *H. tamaricis*) but more of *t. angularis* in *Phaeoh. monticola* and *Phaeoh. geogenum*. The types of excipular texture are rather variable and not clear-cut, however, and consequently not well suited for generic delimitation. Also the *Discinella terrestris* aggregate shows such variation in the ectal excipulum, apart from the fact that its gelatinized tissue of rather elongate cells strongly contrasts with that in *Phaeoh. monticola* and *Phaeoh. geogenum*.

The apical ring of the *D. terrestris* aggregate is somewhat unusual within *Hymenoscyphus s.l.*, being amyloid throughout the entire or sometimes only the upper part of the apical thickening. Yet, such ring types occur also in some other members of that group, for instance in *Pachydisca fulvidula* Boud, a taxon which appears to be closely related to both *Phaeohelotium epiphyllum* and *Phaeoh. monticola*, based on its general morphology.

Our concept of *Phaeoh. monticola* is based on a rather large number of fresh collections (for a documentation see Baral & Marson 2005), and from one of them the present sequence was gained. This concept appears to be in concordance with the redescription of the type of *Phaeoh. monticola* by Dennis (1964). However, members of *Phaeohelotium* have repeatedly been confused in the past with those of the genus *Bisporella*. For instance, the illustration under the name *Phaeoh. monticola* given by Gamundí & Romero (1998) shows thick-walled excipular cells reminiscent of *Bisporella*. Particularly the apical ring, which is drawn Y-shaped in side view, matches the *Calycina*-type and thus excludes a species of *Phaeohelotium*, and ascospore size suggests a possible

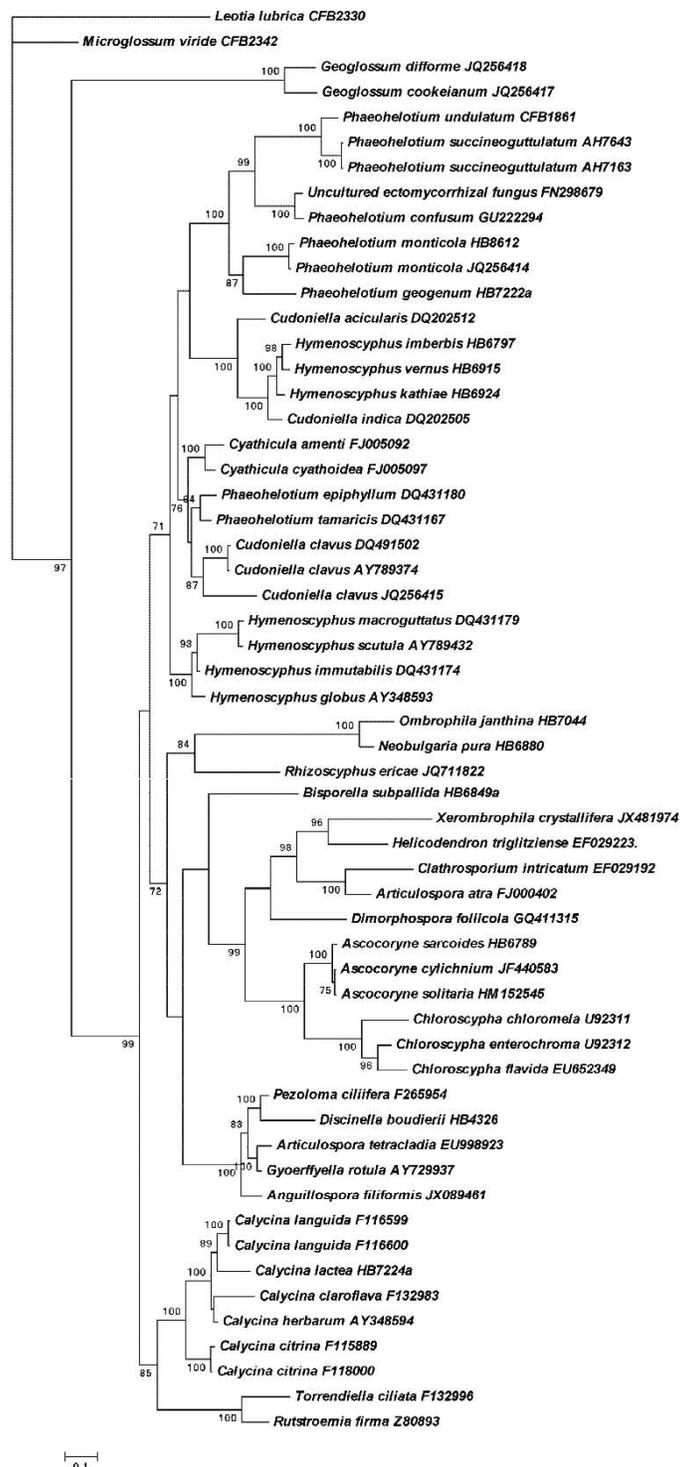


Plate 11 Bayesian analysis of selected members of Helotiales based on ITS1-5.8S-ITS2 region of rDNA showing the position of the *Phaeohelotium* clade in comparison with the genera *Hymenoscyphus*, *Cudoniella*, *Discinella*, *Calycina* and others. *Microglossum* and *Leotia* were used as outgroup. Bootstrap values below 70 were not considered.

identity with *Bisporella confluens* (Sacc.) Korf & Bujac. However, Gamundí & Romero (*l.c.*) found the spores to be smoky grey at maturity, a feature unknown to us in that genus.

Confusion at the generic level happened also concerning a sequence in GenBank under the name *Bisporella citrina* (Batsch) Korf & S.E. Carp. (JQ256414), which clustered in our analysis with *Phaeohelotium monticola* (H.B. 8612) with 98% similarity, hence appears to represent the same species. This result is supported by microphotos of JQ256414 (U.S.A., Illinois, Champaign County, Brownfield Woods, on hardwood, 3.X.2005, ILLS 61033) kindly supplied by the finder Vincent Hustad (*pers. comm.*), showing fusoid ascospores and inamyloid asci which both clearly exclude *B. citrina*. The asci in H.B. 8612 are amyloid, but *Phaeoh. monticola* appears to vary in this respect.

This perplexing situation raises the question whether the genus *Phaeohelotium* is typified in the sense as we have adopted it here, *i.e.*, whether the type species *Phaeoh. flavum* is actually conspecific with *Phaeoh. monticola*. Masee (1896: 474) and Dennis (1964: 54) redescribed the type of *Phaeoh. monticola*. Masee stated the asci to be inamyloid, while Dennis (with MLZ) found a slightly blue apical ring, the shape of which, however is not clearly drawn, however. Dennis found the spores “long remaining hyaline and nonseptate but eventually becoming 1-septate and brown-walled, 12–18×4–5µm”, and the thin-walled excipular cells oriented at a high angle. Although Masee compared the fungus with *Bisporella citrina* (as *Helotium citrinum*), its true relation seems more probably to be with *Phaeohelotium* as applied by us, particularly because of the thin-walled excipulum and faint iodine reaction.

The type specimen of *Phaeoh. flavum* was

re-examined and illustrated by Dennis (1958: 344, fig. 24 upper right), but not described. Kanouse (1935: 75) reported the ectal excipulum as “large-celled, thin-walled”, in full concordance with Dennis’ drawing. The amyloid ring in Dennis’ drawing is restricted to the innermost part of the apical thickening as two rather thick dots, and the spores are drawn with a distinctly heteropolar, clavate shape (contrary to Kanouse who described them as fusoid). All these features exclude *Bisporella citrina* and allies. It is astonishing, however, that Dennis (1964) reduced *Phaeoh. flavum* to synonymy with the earlier *Phaeoh. monticola*, which he consequently combined in *Phaeohelotium*, and which he illustrated with ± homopolar spores. A more detailed redescription of the types of both taxa would be desirable. For the time being, we rely in the interpretation of the genus *Phaeohelotium* on the identity of *Phaeoh. monticola* as redescribed by Dennis (*l.c.*).

Dennis (1958) placed *Helotium terrestre* in the genus *Discinella* Boud. [type species *D. boudieri* (Qué.) Boud.], because he saw a close similarity to “European species of *Discinella*” concerning both excipular structure and hymenial characters. The presence of brown spores was accepted by him as a new character in that genus. Later, Dennis (1964: 79) placed *D. terrestris* in a subgroup “Series B” with “Ascospores ultimately brown”, as opposed to “Ascospores permanently colourless” for *Discinella boudieri* and allies (Series A). However, according to studies published in Wieschollek *et al.* (2011), the excipular structure in *D. boudieri* as well as the very similar *D. menziesii* Boud. is quite different from that in the members of the *D. terrestris* aggregate. The ectal excipulum in *Discinella s.str.* consists of a thin-walled *textura prismatica-porrecta* which is externally covered by a thick layer of

narrow and very long-celled [up to  $160 \times 2.5\text{--}5(-7.5)\mu\text{m}$ ], interwoven hyphae being embedded in an abundant, non-refractive gel matrix (similar as the gelatinized tissue of *Ombrophila*, named *textura gelatinosa* by Dougoud 1994). At the margin the ectal excipulum is light brown and much increased in thickness. The apical rings react red at low and high concentration of IKI and strongly extend apically (*Pezicula*-type). The paraphyses tend apically to be flexuous or even hooked, also branched. Our phylogenetic analysis (Pl. 11) supports that *Discinella* is not closely related to *Phaeohelotium* in the here circumscribed sense.

Dennis (1958) indeed compared *Discinella terrestris* with the genus *Phaeohelotium*, but also with *Phaeociboria*, which he both illustrated with their respective type species *Phaeohelotium flavum* and *Phaeociboria sejournei* (Boud.) Höhn. As being diagnostic he stressed in the former species the thin ectal excipulum of short-celled *textura prismatica* being sharply delimited from a *textura intricata* of the flesh. In the latter species he did not see much difference to *D. terrestris* in excipular texture and brown spores, but stressed the softer flesh of the apothecia that grew on plant tissue instead of soil. *Phaeociboria sejournei* is today regarded as a member of the genus *Pycnopeziza*, and not as an earlier synonym of *Mollisia tetrica* Quél. as Höhnel (1918: 593) believed. The collection that Dennis (1958) depicted as "*Pycnopeziza sejournei*" (Jaap F. sel. exs. 501) was declared as neotype of *M. tetrica* by Dumont (1971: 144), who also clarified the unrelatedness of that species and *Pycnopeziza sejournei*. Dumont pointed out that *M. tetrica* is the factual type of the genus *Phaeociboria* which was placed in synonymy with *Lambertella* by him. In any case, both *Pycnopeziza* and *Lambertella* have an apical apparatus of the *Sclerotinia*-type and belong

today in the Sclerotiniaceae and Rutstroemiaceae, respectively.

The genus *Cudoniella* is currently defined by apothecia with a convex disc and usually rather long stipe, but such macroscopical features are probably not useful to distinguish a genus. In our phylogenetic analysis the genus is found in different clades. The lectotype species *C. queletii* (Fr.) Sacc. is a probable synonym of *C. acicularis* (Bull.) J. Schröt. (see Dennis 1964: 72). The latter taxon clustered with high support with *C. indica* Webster *et al.* and species around *Hymenoscyphus imberbis* (Bull.) Dennis and *H. vernus* (Boud.) Dennis. *H. imberbis* is also known as *Phaeohelotium imberbe* (Bull.) Svrček, while the very similar *H. kathiae* (Korf) Baral was originally described as *Pezoloma kathiae* Korf. However, a typical species of *Pezoloma* Clem., *P. ciliifera* (P. Karst.) Korf clustered with *Discinella boudieri* in a very distant clade.

Another species of *Cudoniella*, *C. clavus* (Alb. & Schwein.) Dennis, may be confused with *Hymenoscyphus vernus* because of similar microscopical features. Surprisingly, *C. clavus* is found in a clade with *Phaeohelotium epiphyllum* and *Hymenoscyphus tamaricis*, two species showing the general features of the genus *Phaeohelotium*, but also with species of *Cyathicula* De Not. Typical species of *Hymenoscyphus* are placed sister to this clade, but here also *H. immutabilis* (Fuckel) Dennis and *H. globus* W.Y. Zhuang & Yan H. Zhang are found which show some morphological similarities to *H. imberbis* and *H. kathiae*, e.g., isodiametrical excipular cells. *H. globus* was rather inadequately described and illustrated. Its yellow apothecia grew on wet hard wood in Jiangxi (China), and an identical sequence in GenBank (JF449743) suggests that it occurs also in Europe (Austria, on leaves of *Fagus*).

We conclude that a separation between *Cudoniella* and *Phaeohelotium* is problematic. Since *Cudoniella* has priority over *Phaeohelotium*, more research is required to clarify the relationships of these genera. A white apothecial colour is typical of the two *Cudoniella* clades, in contrast to the typical yellow colour in *Phaeohelotium*, but *Phaeoh. geogenum* has also consistently colourless apothecia and shows similarities to, e.g., *H. imberbis*.

The present adoption of the genus *Phaeohelotium* is supported by our molecular analysis only for that clade which includes *Phaeoh. monticola* and the *Discinella terrestris* aggregate. Since we see little morphological reason to separate from *Phaeohelotium* the clade with *Hymenoscyphus tamaricis* and *Phaeoh. epiphyllum*, the following new combinations are proposed:

***Phaeohelotium tamaricis*** (R. Galán, Baral & A. Ortega) Baral, R. Galán & G. Platas, **comb. nov.** – **MycoBank MB803815**

Basionym: *Hymenoscyphus tamaricis* R. Galán, Baral & A. Ortega, in Galán & Baral, Beitr. Kenntn. Pilze Mitteleur. 11: 58 (1997)

***Phaeohelotium fulvidulum*** (Boud.) Baral & Declercq, **comb. nov.** – **MycoBank MB803816**

Basionym: *Pachydisca fulvidula* Boud., Hist. Class. Discom. Eur. (Paris): 94 (1907)

Likewise we see little reason from a morphological point of view to separate the clade with *Cudoniella acicularis* from that of *C. clavus* and from *H. immutabilis*. Both *Phaeohelotium* and *Cudoniella* would then comprise a paraphyletic basal group from which the genera *Cyathicula* and *Hymenoscyphus* have emerged. An alternative solution with a wide generic concept of *Hymenoscyphus* as proposed in Baral & Krieglsteiner (1985) would likewise result in a

paraphyletic group from which *Cyathicula* has emerged. This wide concept was followed in Dimitrova & Baral (2005) when transferring *Phaeoh. monticola* to *Hymenoscyphus*. Since paraphyletic groups reflect phylogenetic processes and are, therefore, appropriate for taxonomic purposes (Gams *et al.* 2012), we think that one of these solutions may be adopted in the future.

**Taxonomic remarks on genera included in the phylogenetic analysis but excluded from *Hymenoscyphus s.l.*:**

The taxonomic position of *Peziza amenti* Batsch has been rather controversial, being previously placed in seven different genera. Carpenter (1981) transferred it to *Crocicreas* Fr., an assessment which is confirmed in the present phylogenetic analysis (Pl. 11), at least when considering *Crocicreas* and the later *Cyathicula* as congeneric. This synonymy was questioned by Baral (1994), however, because it is mainly based on a single character, the gelatinized excipulum, while other characters differ, such as the shape of the apical rings and paraphyses. A recent study of a fresh specimen of *Crocicreas gramineum* (Fr.) Fr. (Nina Filippova pers. comm.) support a separation of the two genera: *Crocicreas* differs from *Cyathicula* in the consistent absence vs. presence of refractive vacuolar bodies (VBs) in the paraphyses and excipular cells. Since *Peziza amenti* concurs with the type species of *Cyathicula*, *C. coronata* (Bull.) Rehm in the ascus apical apparatus (*Hymenoscyphus*-type) and in paraphysis shape (cylindrical), and molecular data support such relation (Peláez *et al.* 2011, present study), we here propose the following new combination:

***Cyathicula amenti*** (Batsch) Baral & R. Galán, **comb. nov.** – **MycoBank MB803817**

Basionym: *Peziza amenti* Batsch, Elench. fung.,

cont. prim. (Halle): 211 (1786)

≡*Helotium amenti* (Batsch) Fuckel, Jb. nassau. Ver. Naturk. 23-24: 313 (1870)

≡*Hymenoscyphus amenti* (Batsch) W. Phillips, Man. Brit. Discomyc. (London): 143 (1887)

≡*Phialea amenti* (Batsch) Sacc., Syll. fung. (Abellini) 8: 257 (1889)

≡*Ciboria amenti* (Batsch) Whetzel, Mycologia 37: 675 (1945)

≡*Pezizella amenti* (Batsch) Dennis, Mycol. Pap. 62: 55 (1956)

≡*Crocicreas amenti* (Batsch) S.E. Carp., Brittonia 32: 269 (1980)

The recently described genus *Xerombrophila* Baral (Baral et al. 2013) bears some macroscopical resemblance with the genus *Phaeohelotium*. It differs in many microscopical features, however, such as the *Calycina*-type of apical ring and the presence of abundant crystals in and on the excipulum. In our phylogenetic analysis it clustered with some anamorphic fungi such as *Helicodendron triglitzense* (Jaap) Linder, very distant from *Hymenoscyphus s.l.* The type species of *Helicodendron*, *H. paradoxum* Peyr., is connected to the teleomorph *Hymenoscyphus paradoxus* Fisher & Webster (1983). The genetic relation between *Xerombrophila* and *Helicodendron* was overlooked by Baral et al. (l.c.), because the phylogenetic analysis was based on 28S rDNA which did not exist for such taxa of *Helicodendron* in GenBank.

The clade comprising the genera *Ascocoryne* J.W. Groves & D.E. Wilson, *Chloroscypha* Seaver, and *Gelatinodiscus* Kanouse & A.H. Sm., was also achieved in a phylogenetic analysis of the 28S rDNA (Baral et al. 2013), though only with a moderate bootstrap support. In both analyses it became clear that *Gelatinodiscus* can be considered as congeneric with *Chloroscypha*, which is the earlier of these two

genera. Since also the morphology of *G. flavidus*, the only species ever combined in *Gelatinodiscus*, and members of *Chloroscypha* is very similar (stipitate apothecia with a bright yellow pigment, growing on coniferous substrate, partly very similar hymenial characters), a new combination is here proposed. For *G. flavidus* the family name Gelatinodiscaceae was erected by Carpenter (1976), and Baral et al. (l.c.) considered the adoption of that name for the present clade. The following new combination is here proposed:

***Chloroscypha flavida* (Kanouse & A.H. Sm.) Baral, comb. nov. – MycoBank MB803818**

Basionym: *Gelatinodiscus flavidus* Kanouse & A.H. Sm., Mycologia 32: 756 (1940)

*Discinella boudieri* (type of *Discinella*) is placed in our phylogenetic analysis in a clade with *Pezoloma ciliifera*. Such a relation was already supposed by Dennis (1964) who compared *Discinella* species with *Sphagnicola* Velen. (= *Pezoloma*). This is supported by some morphological features, such as an external layer of hyphae embedded in abundant gel (*textura gelatinosa*), hemiamyloid apical rings, paraphyses without refractive vacuoles and showing a tendency to being apically irregularly bent and branched, and a terrestrial habitat, possibly with a mycorrhizal mode of life. It remains to be clarified whether the two genera should be merged in the future under the older name *Discinella*. Surprisingly, the ITS sequences of *Pezoloma cilifera* and *Discinella boudieri* cluster in a clade with analogous sequences from several ingoldian fungi, suggesting that the telomorph of *Articulospora tetracladia* Ingold, *Hymenoscyphus tetracladius* Abdullah et al., could be closely related to *Pezoloma* or *Discinella*. We already discussed above the morphological features that permit to exclude *Phaeohelotium undulatum*

and allied taxa from *Discinella*.

Another mycorrhizal fungus, *Rhizoscyphus ericae*, was transferred to *Pezoloma* by Baral (in Baral & Krieglsteiner 2006). In our phylogenetic analysis it clustered in the clade with *Ombrophila* and *Neobulgaria*, though with moderate support.

A well supported clade in our phylogenetic analysis concerns the genus *Calycina*. Its members are characterized, e.g., by the *Calycina*-type of apical ring and elongate VBs in the paraphyses. They typically possess whitish apothecia and a slightly gelatinized ectal excipulum, though near the base of the receptacle and in the stipe the cells may be strongly gelatinized as in *Bisporella* (see Baral & Krieglsteiner 1985: 55, Baral 1994: 122). Our analysis shows that three taxa with a strongly gelatinized ectal excipulum, currently assigned to the genus *Bisporella* (*B. lactea* with white apothecia, *B. citrina* and *B. claroflava* with bright yellow apothecia), cluster in a clade with the type species of *Calycina*, *C. herbarum*. Such a relation was already assumed based on the strong similarity in the above-mentioned hymenial characters (Baral *et al.* 2013). *B. lactea* (as *Hymenoscyphus ellisii*) was compared by Dennis (1964: 48) with *C. parilis* (as *Helotium parile*). *B. confluens* is only doubtfully distinct from *B. citrina* and, therefore, not recombined here; it requires a detailed study including molecular methods.

Sequences of two Spanish records of a further species, *Pezizella languida* (P. Karst.) Svrček & Kubička, clustered also in the *Calycina* clade. Morphologically, this species closely matches *C. herbarum* except for having smaller, non-septate, biguttulate spores. The examined holotype of *P. languida* matches these records very well, including the absence of croziers (see Baral & Marson 2005).

For two further species a new combination is here proposed based merely on their morphological similarity with *B. citrina* or *C. herbarum*.

*Bisporella* in its restricted sense is characterized by a *Hymenoscyphus*-type of apical ring and multiguttulate paraphyses (see Baral & Marson 2005). In our phylogenetic analysis a sequence of *B. subpallida* (Rehm) Dennis is found quite distant from the *Calycina* clade, while a sequence of the type species, *B. pallescens*, was not available.

***Calycina citrina*** (Hedw.) Gray, Nat. Arr. Brit. Pl. (London) 1: 670 (1821)

≡ *Octospora citrina* Hedw., Descr. micr.-anal. musc. frond. 2: 28, tab. 8B, figs. 1–7 (1789)

= *Bisporella citrina* (Batsch: Fr.) Korf & S.E. Carp., Mycotaxon 1: 58 (1974)

≡ *Peziza citrina* Batsch, Elench. fung. contin. 2: 95, pl. 39, f. 218 (1789)

? = *Bisporella confluens* (Sacc.) Korf & Bujak., Agarica 6: 306 (1985)

***Calycina claroflava*** (Grev.) Baral, R. Galán & G. Platas, **comb. nov.** – MycoBank MB803819

Basionym: *Peziza claroflava* Grev., Fl. Edin.: 424 (1824)

≡ *Bisporella claroflava* (Grev.) Lizoň & Korf, Mycotaxon 54: 474 (1995)

= *Helotium citrinicolor* P. Crouan & H. Crouan, Florule Finistère (Paris): 114 (1867)

= *Bisporella sulfurina* (Quél.) S. E. Carp. in Korf & Carpenter, Mycotaxon 1: 59 (1974)

≡ *Helotium sulfurinum* Quél., Grevillea 8: 116 (1880)

= *Bisporella discedens* (P. Karst.) S. E. Carp., Mycotaxon 2: 124 (1975)

≡ *Helotium discedens* P. Karst., Hedwigia 28: 191 (1889)

***Calycina drosodes* (Rehm) Baral & Declercq, comb. nov. – MycoBank MB803820**

Basionym: *Helotium drosodes* Rehm, Hedwigia 3: 37 (1881)

≡ *Bisporella drosodes* (Rehm) S.E. Carp., Mem. N. Y. bot. Gdn 33: 209 (1981)

***Calycina lactea* (Ellis & Everh.) Baral, R. Galán & G. Platas, comb. nov. – MycoBank MB803821**

Basionym: *Helotium lacteum* Ellis & Everh., Proc. Acad. nat. Sci. Philad. 45: 145 (1893)

≡ *Hymenoscyphus ellisii* Dennis, Persoonia 3: 48 (1964) [non *H. lacteus* (Cooke) Kuntze]

≡ *Bisporella lactea* (Sacc.) Stadelmann, Nova Hedwigia 30: 830 (1979) [1978]

***Calycina languida* (P. Karst.) Baral, R. Galán & G. Platas, comb. nov. – Mycobank MB803822**

Basionym: *Peziza languida* P. Karst. in Not. Sällsk. Faun. Flora fenn. Förh. X, p. 145 (1869)

≡ *Pezizella languida* (P. Karst.) Svrček & Kubička, Česká Mykol. 18: 176 (1964)

***Calycina scolochloae* (De Not.) Baral, comb. nov. – MycoBank MB803823**

Basionym: *Helotium scolochloae* De Not., Comm. Soc. crittog. Ital. 1: 379 (1863)

≡ *Bisporella scolochloae* (De Not.) Spooner, in Kirk & Spooner, Kew Bull. 38: 557 (1984)

***Calycina subcitrina* (Velen.) Baral, comb. nov. – MycoBank MB803824**

Basionym: *Helotium subcitrinum* Velen., Novitates Mycologicae Novissimae: 120 (1947)

**Ecology and distribution:** An ericoid mycorrhizal or ectomycorrhizal mode of life within helotialean fungi has rarely been reported in the literature for some species belonging to the genera *Leotia*, *Geoglossum* and *Trichoglossum* (Bougher 1995), being also expected for some other genera

such as *Microglossum*, *Pezoloma* and *Discinella*. It has been also noticed for some unrelated species such as *Rhizoscyphus* spp., *Meliniomyces* spp. (Hambleton & Sigler 2005), *Neocudoniella radiceola* (Kohn et al. 1986), *Moserella radiceicola* (Pöder & Scheuer 1994), *Lachnum pygmaeum*, *Mollisia minutella* (Walker et al. 2011), etc. Those exceptional findings together with additional, more recent reports of formally unidentified mycelia of ascomycetes associated with roots (Rubini et al 2011, Tedersso et al. 2009) suggest that many other ascomycetes (including Helotiales) that form ectomycorrhizae remain to be identified.

Eucalypts predominantly form ectomycorrhizal (EM) associations in native forests and reforestation. The consistent occurrence of *Phaeohelotium undulatum* and *Phaeoh. succineoguttulatum* on soil under *Eucalyptus* trees (sometimes mixed with trees native to Spain) suggests a strict connection to this Australasian tree genus. As already stated in the introduction, an ectomycorrhizal mode of life is supposed for both *Phaeohelotium undulatum* and *Phaeoh. succineoguttulatum*. In several of the collections we have observed a close association of the apothecia to fine roots ~120–250µm diam, probably of *Eucalyptus*. Moreover, from these roots mycelium emerges that perfectly matches the anchoring hyphae of the apothecia and wall thickness. Supported is this hypothesis by unpublished and somewhat uncertain observations by P. McGee (see introduction), and by ectomycorrhizal isolates in GenBank which belong in the *Discinella terrestris* aggregate (P.R. Johnston pers. comm.). Also the difficulty to obtain germinating spores would be typical of mycorrhizal fungi. Warcup (1990) listed *Discinella terrestris* as occurring after a wild fire in a eucalypt forest, and it is the only one reported as unable to be cultured. The

present field observations within Spain include a partial occurrence on burnt soil for both species of *Phaeohelotium*. Fire is possibly a prerequisite for spore germination. Undoubtedly, the ejected hyaline spores turn brown in the course of time, and the pigment protects them from ultraviolet radiation during summer drought. Wild fires might eventually deactivate the inhibition of germination, and rainfalls during winter will serve as the basis for the formation of new populations. The mycelia would then attain a considerable age: in southern Australia, *D. terrestris* is reported to fruit not only in very young sites less than 1 year after a fire, but also in mature forests 7–57 years after a fire (McMullan-Fisher *et al.* 2002).

The Spanish species reported here constitute the only records of the *Discinella terrestris* aggregate outside Australasia known to us. The present distribution on the Iberian Peninsula includes all Spanish regions where *Eucalyptus* was planted in monocultures, except for Galicia and Cantabria. Apart from the collections listed by us, a record of “*Discinella terrestris*” from Castilla La Mancha (Ciudad Real, Parque Nacional de Cabañeros) was published by Díez *et al.* (2010: 164) without detailed collection data, although we think that it represents *Phaeoh. undulatum*. No record from Portugal came to our notice, but we expect at least *Phaeohelotium undulatum* also in that country: the collection site near Sanlúcar de Guadiana, Huelva, is only about 500m from the border between Spain and Portugal (see Pl. 12).

According to the present state of knowledge, *Phaeohelotium undulatum* is adapted within Spain to a warmer and drier climate than *Phaeoh. succineoguttulatum*, though the distribution areas of both species overlap (Pl. 9). At three sites (Jarandilla, Las Veguillas; Jarandilla, Torreseca;

Córdoba, Los Villares) the two species were found to grow sympatric, with a distance of ~15–70m between the populations at Jarandilla, while their distance at the site of Córdoba is unknown.

Within Australasia, the *Discinella terrestris* aggregate was reported from semihumid (Mediterranean) to semiarid sclerophyllous eucalypt forests not far from the coastline of eastern and southeastern Australia (southeast of Queensland, New South Wales, Victoria, South Australia, and southwest of West Australia), and in Tasmania (*e.g.*, Gates *et al.* 2005). These reports often lack a statement on a possible host tree, which is due to the more difficult situation in natural forests away from monocultures concerning the binding to a host tree. Perhaps therefore we rarely read about the occurrence of *D. terrestris* under *Eucalyptus* in Australia.

In New Zealand the genus *Eucalyptus* is not native, and the *D. terrestris* aggregate is reported under *Kunzea* and *Leptospermum* (Myrtaceae), and *Nothofagus* (Fagales, Anon. 2002–2013). These three plant genera are the only common and widespread native trees known to form ectomycorrhiza in New Zealand. According to the here presented results, the records under *Nothofagus* represent *Phaeoh. confusum*, and those under *Leptospermum* and *Kunzea* *Phaeoh. undulatum*. The origin of Spanish *Phaeoh. undulatum* might, therefore, be sought in New Zealand. Yet, its introduction to Spain should be expected to have happened by export of eucalypt seedlings. Regrettably, genetic data of *Phaeoh. undulatum* from New Zealand was not available for comparison. *Phaeoh. baileyianum* was twice recorded under *Eucalyptus* in Australia (H.B. 9746 and PDD 10375) and once under *Eucalyptus* in Tasmania (PDD 10375). The absence of this species in New Zealand suggests that it might be confined to

that tree genus (P.R. Johnston pers. comm.).

Although *Discinella terrestris* was frequently recorded in southern Australia and Tasmania, being also described and illustrated in regional field guides for mushrooms (e.g., Fuhrer 1985, 2005: 315; Syme 2011), all these reports require re-examination due to the lack of crucial microscopic data such as croziers and ascus amyloidity. All reports known to us from the Internet show light to bright yellow to yellow-ochraceous or yellow-orange hymenia. Australasian records referable by hymenial colour to

*Phaeoh. succineoguttulatum* did not come to our notice.

Apothecia are formed almost exclusively during the winter months (in Spain mainly from December to March, in Australia mainly from June to August, Syme 2011), though in Australia also records from January and March exist ([http://mushroomobserver.org/name/show\\_name/15219?\\_js=on&\\_new=true&q=mMwG](http://mushroomobserver.org/name/show_name/15219?_js=on&_new=true&q=mMwG)), and Gates *et al.* (2005) frequently recorded it in southern Tasmania continuously during February till November.

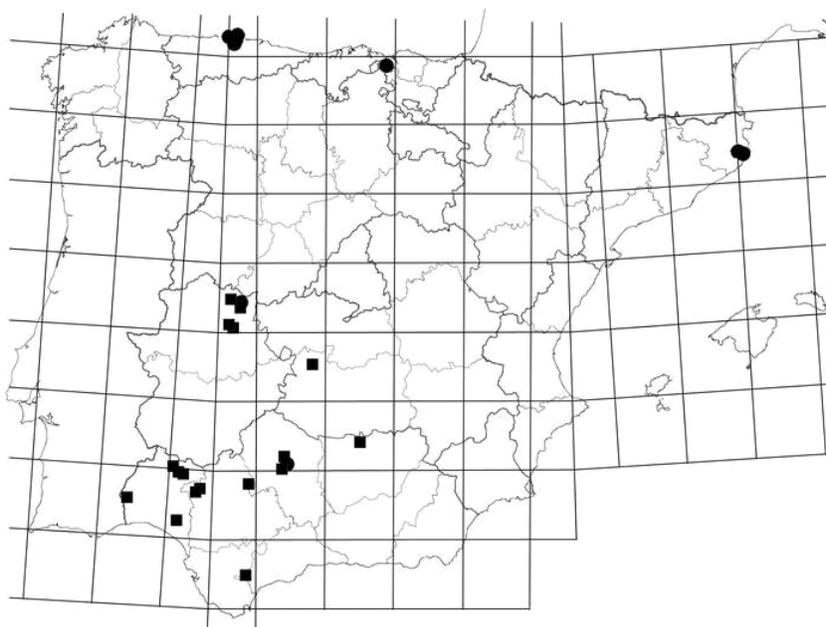


Plate 12 Known distribution of *Phaeohelotium undulatum* (■) and *Phaeoh. succineoguttulatum* (●) on the Iberian Peninsula. Note current restriction of *Phaeoh. undulatum* to the south and southwest, and sympatric occurrence of both species at two sites (Jarandilla and Córdoba).

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