Introduction

The genus Hymenoscyphus S.F. Gray in a restricted sense, with the exclusion of Phaeohelotium Kanouse and Cudoniella Sacc. as was accepted in Baral et al. (2013), comprises a large number of taxa which often exhibit only slight differences in their micromorphological characters, particularly when comparing treatments based on herbarium material. For this reason, taxonomic subgroups or dichotomous keys were often constructed based on the type of substrate (e.g., Dennis, 1956: 67, 1964, Thind & Sharma, 1980). A synoptic key by Lízoh (1992) includes various microscopic characters but neglects ascospore guttulation and croziers. A recently compiled checklist (Lízoh & Kucera, 2014) briefly mentions a total of 1180 taxa which have ever been combined in Helotium or Hymenoscyphus, and illustrates the excessive need for taxonomic work and careful type studies in this group of fungi.

The two taxa are treated in detail here, Hymenoscyphus menthae (W. Phillips) Baral (= H. consobrinus (Boud.) Hengstm.), a species very common in Central Europe, and H. macroguttatus Baral, Declercq & Hengstm. (= H. menthae s. auct.), a less common species with a very similar distribution. Whenever fresh samples were available, spore guttulation was observed to be an absolutely constant character which allowed a rapid and unambiguous determination. H. menthae sharply differs by its multiguttulate spores (Figs 1–17, 26–30) from H. macroguttatus which has oligoguttulate spores with a few large guttules and some small ones (Figs 32–42, 47–50). Over the past 40 years, 57 collections of H. menthae and 18 of H. macroguttatus were studied and often also documented by me in the fresh, living state, and some further ones from herbarium material. Various other workers have made numerous collections of these two species and examined them mostly in the living state, and in an extraordinarily high number by B. Declercq in Belgium.

This striking difference in spore guttulation can often also be recognized in old herbarium material, if mature spores, preferably not yet released from the asci, are found which show the undistorted lipid pattern. In this way, the original pattern of spore guttulation could be verified in all of the type specimens examined in the present study. The frequent neglect of Lbs in the literature as a result of studying herbarium material, together with the disregard of the ascus base, are the most important causes of confusion in this group. Spore guttulation and croziers were found to have a high diagnostic value in the genus, whereas a correlation with the substrate, even within the categories wood and bark, herbaceous stems, dicots vs. monocots, and stems of pteridophytes, was not observed in the species treated here.

Hymenoscyphus menthae and H. macroguttatus have oblong, homopolar spores of a very similar size, and are plurivorous though mainly caulicolous. Various earlier as well as more recent authors have confused them, and merged them even with the common, also plurivorous and somewhat variable H. scutula (Pers.: Fr.) W. Phillips, which is characterized by distinctly heteropolar spores which often possess prominent setulae at both ends (currently referred to as "cilia", but see HengstMEngel, 1996). Despite their homopolar spore shape, the two species treated here have actually been included in the scope of H. scutula as more or less doubtful forms or varieties (e.g., by Dennis, 1956), whereas HengstMEngel (1996) confirmed their independence in his careful study of herbarium material. Molecular data gained recently shows that the two species are not closely related to each other, and that H. menthae is not even related to Hymenoscyphus s. str. as represented by H. scutula and the type species H. fructigenus (Bull.) Gray. The latter two species are characterized by strongly heteropolar spores with a rounded to obtuse, more or less distinctly asymmetrical apex with a minute acute, oblique or lateral protrusion, and a tapered, more or less acute base. If setulae are present, they are usually inserted at the subapical protrusion of the spore apex. Hymenoscyphus menthae clearly differs by its multiguttulate spores (Figs 1–17, 26–30) from H. scutula which usually have heteropolar spores; possibly neither of them are synonyms with Hymenoscyphus vitellinus, a species described with homopolar spores and requiring restudy of the type.

Molecular data supports a close relationship between H. menthae and H. repandum, both having a yellow disc and homopolar ascospores, but the data suggests a strong separation from the bulk of Hymenoscyphus s. str., which usually have heteropolar (scutuloid) but exceptionally also homopolar (H. macroguttatus) spores, and often whitish though also yellow apothecia.

Keywords: Ascomycota, Helotiales, vital taxonomy, lipid bodies, pleuroerychous.

Summary: The taxonomic value of spore guttulation (lipid pattern inside of mature ascospores) studied with the light microscope from fresh but also dried collections is illustrated mainly for the two species of Hymenoscyphus extensively treated here. Also, the value of croziers at the ascus base is emphasized. The high intraspecific consistency of these characters permits rapid recognition of these species in the living state: H. menthae with multiguttulate ascospores and simple-septate asci, H. macroguttatus (= H. menthae s. auct.) with spores containing a few large oil drops and asci arising from croziers. Further valuable characteristics in the genus Hymenoscyphus concern the shape of ascospores (homo- versus heteropolar), the presence of polar setulae on them, and the vascular guttules in the living paraphyses. The neglect of spore guttulation due to the current dominance of herbarium studies, and also the neglect of croziers resulted in much confusion and name changes in this group of fungi. The traditional delimitation of taxa within Hymenoscyphus according to the substrate (lignicolous, herbicolous, pteridicolous) is questioned because quite a few species turned out to be highly plurivorous. The present type studies led to the following conclusions: (1) Hymenoscyphus menthae is an earlier synonym of H. consobrinus. (2) H. pteridicola Thind & Sharma is conspecific with H. menthae s. auct. and is replaced by the name H. macroguttatus because of the homonym H. pteridicola (Crouan) O. Kuntze. (3) Helotium repandum var. rumicis, H. julianum, and H. stramineum are later synonyms of Hymenoscyphus menthae. (4) Helotium geliphium and Hymenoscyphus vitellinus are conspecific and hardly separable from the older H. scutula, whilst Světěk’s interpretation of H. vitellinus mainly concerns H. menthae. (5) Helotium scutula var. solani might also be a synonym of Hymenoscyphus scutula, but type material could not be located. (6) The new species H. sharkei was described for collections from India (Himalaya) issued under the name Helotium scutula var. solani; it resembles H. trichosporus but differs in 4-spored asci. (7) A syntype of Helotium hyalopes in M contains a mixture of two similar species with scutuloid spores; possibly neither of them are synonymous with Hymenoscyphus vitellinus. (8) Hymenoscyphus solani has yellow apothecia and simple-septate spores, and is not even related to H. menthae.

Hymenoscyphus menthae, H. macroguttatus and H. scutula, a comparative taxonomic study emphasizing the value of spore guttulation and croziers

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and the acute base. Often the spores are slightly curved, which causes a unilateral flattening or slightly concave outline at the side of the protrusion. This remarkable spore shape was termed "scutloid" by me (in BARAL & KRIEGELSTEINER, 1985: 120), and is a rather unique character of the genus Hymenoscyphus s. str. (see also BARAL & BERMANN, 2014). More or less scutloid spores are illustrated in the present study in Figs 56–63.

To find a proper name for H. menthae s. auct. was a major problem. Although helpful, the revision of Velenovsky’s taxa of Helotium by SVRČEK (1985) neglects croziers, and reports eguttulate spores even though they were originally described as guttulate. The same kind of problem applies to the “Revision of the British Helotiaeae” by DENIS (1956), which includes many descriptions of types. Consequently, I have reexamined type material of W. Phillips, H. Rehm, J. Velenovsky, and K.S. Thind & M.P. Sharma concerning taxa which appeared from the available descriptions to be similar to my two species with homopolar spores. All these types are figured in the present paper.

**Abbreviations:** CB = cotton blue in lactophenol, CR = congo red (in NH₄OH), CRB = cresyl blue (ca. 0.5% aqueous), H₂O = tap water, KOH = potassium hydroxide (5%), IKI = Lugol’s solution (1% I₂, 3% KI in tap water), MLZ = Melzer’s reagent, NH₄OH = ammonium hydroxide (10%), BB = blue at low (~0.2%) and high (0.5–1%) iodine concentration (IKI), LB = lipid body (oil drop), VB = refractive vacuolar body, * = living state of a cell, † = dead state, → = from immature to mature; d.v. = document seen (usually macro- and microllestration), n.v. = no illustration or material seen, σ = unsuppressed, sq. = DNA sequence, vs. = versus (as opposed to), MTB = German grid system (Mestitschblatt), MB = MycoBank, MBT = Mycobank typification number. The numbers of examined samples in which the reported character was tested and observed are indicated between () (numbers after the slash refer to uncertain identifications).

**Mentioned official herbaria:** GENT = Laboratory of Plant Systematics, Gent; HMAS = Institute of Microbiology, Academia Sinica, Beijing; K = Royal Botanic Gardens, Kew; KR = Staatliches Museum für Naturkunde, Karlsruhe; L = Naturalis Biodiversity Center, Leiden; M = Botanische Staatsammlung, München; PAN = Punjab university, Chandigarh; PRM = National Museum, Prague; REG = Regensburgsche Botanische Gesellschaft; STU = Staatliches Museum für Naturkunde, Stuttgart; TAAM = Institute of Zoology and Botany, Tartu; TFC = Herbario Dept. de Biologia Vegetal, Universidad de La Laguna, Tenerife, Spain; Z = Universität Zürich.


**Taxa with more or less homopolar, ellipsoid-fusoid ascospores (H. menthae, H. macroguttatus, H. sharmae)**

Fig. 1. Hymenoscyphus menthae. – a. ascospores (a1 mature, a2 overmature), containing refractive guttules (LBs); b. mature ascus and paraphyses, the latter containing refractive vacuolar guttules (VBs); c. apex of nearly mature ascus in IKI, with euamyloid apical ring; d. median section of receptacle; e. do., ectal excipulum at lower flanks, with cortical hyphae containing refractive guttules (VBs); f. fresh apothecia. – Living state (except for c).

Hymenoscyphus-type, ring also visible in KOH without iodine; base with ± long stalk, arising from simple septa (43), immature asci during meiosis densely filled with 0.3–0.5 μm large LBs which fuse in dead ascus to form one large pale yellowish-chlorinaceous body. **Ascospores** *(13–)(15–)17–22(–26) × (2.8–)3.5–4.2(–4.5) μm* (26), *(14–)16–22(–26.5) × (2.6–3.2–4(–4.5) μm* (19), always non-septate within living mature asci, cylindric-fusoid-naviculate, without median constriction, consistently homopolar (>110); both ends distinctly tapered to an obtuse or acute tip, nev scutuloid, ± straight but mostly some slightly curved at centre or towards one end, recently discharged living spores ensheathed in a thin membrane that slips off the spore (1) (Fig. 29, not seen in other fresh collections), entirely without polar setulae (>110); living mature spores consistently multiguttulate: densely filled with small (0.7–1.3(–1.5) μm) and minute (0.3–0.5 μm) refractive LBs except for the globose central nucleus (very high lipid content) (>90), old herbarium specimens still with some or most spores multiguttulate (13), or all spores with 1–4 large refractive aggregations (5), wall surface lilac in CRB (1) or unstained (1); aged spores (free or in dead asci) often with one median septum (7) or up to 3 septa (3), remaining hyaline and smooth, rarely turning pale to light brown, scarcely increasing in size *(18.5–24 × 3.7–4.5 μm).** **Paraphyses** cylindrical, straight, rounded at apex, terminal cell *(40–84 (2) × (2.3–)2.5–3.5(–4) μm* (5), *(20–)29–65 × 1.7–2.7 μm* (3), lower cells *(10–24 × 2.5–3.5 μm* (1), *(14–28 × 1.7–2.5 μm* (2); VBs multiguttulate, rather strongly refractive (>60), hyaline, ± restricted to terminal cell, in upper part small guttules in 2–3 rows, downwards larger, globose to short-cylindrical, uniseriate, extending (22–)30–50 μm (3) or 50–95 μm (2) from tip, slowly staining turquoise in CRB, IKI–, disappearing in dead cells, plasma then sometimes pale amber (†H2O); abundant minute yellow-orange LBs near septa; rarely dichotomously branched near base or upper part but frequently anastomosing below. **Medullary excipulum** hyaline, of rather loose textura intricata, hyphae *2–8 μm wide* (1), eguttulate, delimited from ectal excipulum by a ca. 100 μm thick parallel layer of *t. porrecta*, individual cells *(80–140 × 2–5 μm* (1), **Subhymenium** ca. 50–120 μm thick, of upwards oriented loose *t. porrecta*, cells with abundant anastomoses, with or without yellow-orange LBs. **Ectal excipulum** hyaline, of *(*) rather thin-walled textura pris-
Tab. 1 – Phenology of Hymenoscyphus menthae, depending on the geographical region (atlantic: England, Benelux, western parts of France, northern parts of Germany). Exact collection data for Denmark were not available.

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*matica-porrecta* from base to margin, oriented at an angle of ca. 0–20°–40° to the surface, ca. 30–50 μm thick near base of receptacle, cells at flanks *[13]–18–40–60(–85)] [5] (x 5 (x 3–7–10–12(–18)) μm, 110–25 × 4–8 μm (2), slightly gelatinized in KOH (common walls of medullary excipulum; cortical hyphae one-layered, 4–6.5–(–8) μm wide (1), undulating, filled with refractive VBs (multiguttulate), forming a network in surface view, frequent at margin and flanks, also present on stipe; receptacle hairless, stipe with short guttulate forming a network in surface view, frequent at margin and flanks, also present on stipe; receptacle hairless, stipe with short guttulate cylindrical hairs.

**Cultural characteristics:** The ascospores showed a high rate of germination on malt extract agar, producing an always hyaline mycelium with somewhat mealy appearance (Wiese, 1992: 61).

**Habitat:** On previous year’s herbaceous or woody substrates lying on the moist ground (hygric), predominantly in damp places (swamps, ditches or ruts in woods, bank-communities along rivulets and brooks, small lakes, reed and sedge dominated marshes, moist meadows or forb communities), also in gardens and horticultures remote from water (but substrate lying on moist ground); mainly on herbaceous dicotyledons: on rather rotten stems (sometimes roots, rarely inflorescences) of Agrimonia eupatoria (1), Angelica sylvestris (1/1), Anthemis nobilis (1), Apiaecae indet. (3), Calthula palustris (5), Chamaenerion angustifolium (1), Cirsium sp. (4), Coreopsis verticillata (1), Epilobium sp. (1), E. hirsutum (2), Eupatorium cannabinum (1), Fallopia japonica (4), Filippendula ulmaria (1), ?Galeopsis bifida (1), Impatiens glandulifera (4), I. noli-tangere (3), ?Lamaeaeae, Lamium galeobolon (2), Lycopus europaeus (4), Lysimachia vulgaris (4), Lythrum salicaria (1), Mentha sp. (2/1), M. aquatica (1), M. × verticillata (1), Peucedanum palustre (1), Polygonum sp. (?), Potentilla palustris (1), Ranunculus aconitifolius (1), Rubus fruticosus (1), R. ideus (3/1), Rumex sp. (4/2), R. acetosa (1), R. crispus (1), Sambucus ebulus (3), Sapona-ria officinalis (1), Senecio fuchsii (1), Solanum dulcamara (4/1), Solidago ?canadensis (1), Thalictrum dicrocarpum (1), Urtica dioica (2/1), indet. plants (18); *monocotyledons:* on rotten stems, culms or leaves of Allium plantago-aquatica (1), ?Cyperaceae indet. (1), Iris pseudacorus (2), Poaceae indet. (2), Scirpus silvaticus (1), Triticum aestivum (1), Typha latifolia (1), Zea mays (2); *pdis:** petioles of Pterium aquilinum (1), *woody plants:* on rather undecayed to very rotten bark (1) and wood (6) of twigs and branches, 5–9 mm (4) or 40 mm (1) thick, of Alnus sp. (2), *Eucalyptus europaeus* (1), Populus sp. (1), Sambucus nigra (1), indet. angiosperm (4/1), cupule of Aesculus hippocastanum (1), fruit of Acer (1), main vein of strongly skeletonized leaf of indet. angiosperm (1). Associated with Calycina discret (1), C. herbarum (1), Cistella greggii (1), Cyathula cyathoidea (2), C. pulchra (1), *Hymenoscyphus macroguttatus* (1), H. repandus (2), H. scutula (1), Leptosphaeria acuta (1), Trichophyton sulphureum (1), Pyrenopeziza atrata (1), but often not associated with other ascomycetes. **Altitude:** 1–2800 m (temperate to subalpine, atlantic to sub-continental). **Desiccation tolerance:** not tested, probably intolerant concerning the asc and paraphyses.

**Remarks:** Hymenoscyphus menthae is well characterized in the living state by a more or less yellow receptacle due to carotenoids, a much longer than wide, whitish stipe, rather large, homopolar, multi-guttulate ascospores, and asci arising from simple septa. The epithecium species is rather common in temperate Europe. The macroscopically indistinguishable and closely related *H. repandus* (W. Phil- lip) Dennis differs in much smaller spores with a rather low lipid content.

Before I studied the type material of *H. menthae* in 1997, I treated the present species under its later synonym *H. consobrinus* (Baral & Krieglstein, 1985: 124), while I misapplied the name *H. menthae* for a fungus which is now named *H. macroguttatus* (loc. cit.: 131), based on the spore contents in the protologue. Other authors like Hengst-Mengel (1996) followed my misinterpretation of the name *H. menthae*. In 1985 I separated these two species mainly by spore guttulation (see also my figure in Baral, 1986: 8). Additional characters were only seen in the colour of the disc, being usually yellow-brown to brown in *H. menthae* while more whitish in *H. macroguttatus*, and in the spores which are slightly shorter and never distinctly curved in *H. macroguttatus*. Without knowing Hengst-Mengel’s (1984) unpublished study, I later detected the sharp difference in the ascus base between the two species. Quite early, however, I became aware that the protologue of Helotium menthae W. Phillips as cited in Dennis (1956: 78) deviates from my early concept of *H. menthae* in the bright yellow apothecial colour (see Baral & Krieglstein, 1985: 132).

**Dennis** (1956: 79) mentioned a British collection on Epilobium under the name Helotium consobrinum, with spores 16–21 × 3–3.5 μm (without drawing), but considered this to be only a yellow form of *H. scutula var. solani*. Since he did not take much care on spore contents and ascus croziers, *H. menthae* was misinterpreted by me until I restudied the type myself (see also the brief report in Baral et al., 2006: 157 and below under *H. macroguttatus*). Three synonyms and one paratype deposited in K and M were examined (Figs 11–13), showing multiguttulate spores and the absence of croziers. The material was found to be homogeneous and conspecific with the later *H. consobrinus*. Therefore, the name *H. menthae* must be adopted to replace *H. consobrinus* (see Krieglstein, 1993: 65).

**Svérček** (1962: 100) reported only a single collection under the name Helotium consobrinum, from Lower Tatra on stems of *Gentiana asclepiades*, with spores *20–23 × 3.5–4 μm* with a “granular content” when observed in water but with two drops when mounted in 10% KOH. Without referring to this name or collection, he later (Svérček, 1985: 150, 153, 172–3, 178) believed that Hymenoscyphus vitellinus (Rehm) O. Kuntze is the correct name for a “commonly occurring” hericobical species, “easily recognizable” already by its external appearance. Svérček recorded this species mainly on dicotyledonous, seldom on monocotyledonous herbs, and I feel that his concept of *H. vitellinus* is largely congruent with *H. menthae* in the present circumscription. Examination of an isotype of *H. vitellinus* in M (Rehm Ascomyc. Exs. 513) showed, however, that Rehm’s taxon is a member of the difficult *H. scutula* complex and needs further study to evaluate its taxonomic identity (see Figs 56–57).

Also **Mathes** (1976: 19) misinterpreted Hymenoscyphus vitellinus, based on two collections on Solanum dulcamara from Switzerland (Thurgau, Barchetsee) which R.W.G. Dennis identified as belonging to this taxon. Judging from his unillustrated description especially of the apothecia, Mathes was probably dealing with *H. menthae*. Velenovský (1934: 191) placed Helotium vitellinum (as “H. vitellum”) in synonymy of Helotium repandum W. Phillips by giving a spore length of 6–12 μm for Bohemian samples on various herbs. From this he distinguished *H. repandum var. rumicis* Velen. with 15–30 μm long spores, which is a synonym of *H. menthae* according to the present study of the type. Also on page 407 Velenovský (loc. cit.) appears to have included *H. menthae* in his concept of *H. repandum* by giving a spore length of 10–18 μm. The name *H. consobrinus* was not mentioned, either by Velenovský or by Mathes.
Fig. 2–10. *Hymenoscyphus menthae*. – a. mature ascospores (7 and 10: overmature; N = nucleus); b. simple-septate ascus bases; c. apex of nearly mature ascus in IKI; d. rehydrated apothecia. – Living state (except for 8, 9a2, 10). In Fig. 9a1 a thin membrane slips off the spores; note that in the dead spores of Fig. 9a2 the lipid pattern was distorted in the fresh apothecium by fusion of the LBs (compare also *H. scutula*, Fig 59), but in those of Fig. 8a it remained undistorted when the apothecium was rehydrated. – Fig. 3a: based on a drawing by R. Thate; Fig. 7: del. T. Richter; Fig. 9: taken from BARAL (1992: fig. 22).
Based on a reexamination of type material, SVRČEK (1985) considered four of Velenovský’s taxa as conspecific with his concept of *Hymenoscyphus vitellinus*. The present study of these types revealed three of them to be synonyms of *H. menthae*, whereas one (*H. geophilum*) is undoubtedly not *H. menthae* because of its predominantly scutuloid spores, but probably conspecific with *H. vitellinus* in its original sense (see under *H. scutula*).

HENGSTMENGEL (1984 and pers. comm.) studied the species (as *H. consobrinus*) from 48 collections from the Netherlands and sometimes Belgium made mainly between 1943–2013, a few also in the 19th century (between 1865–1895). DECLERCQ (pers. comm.) lists *H. menthae* 236 times for Belgium for collections made between 1985–2014. I examined 8 of his herbarium specimens, and confirmed 7 of them (one was *H. cf. repandus*: B.D. 94/117, on a leaf gall of *Salix*). Declercq observed multiguttulate spore contents in most of his collections, while he found a “very wide range” of ascus and spore dimensions: the living spores exceptionally attained a size of 30 × 4.5 μm, and the living asci 135 × 12 μm. Ascus dimensions in the available literature, including Boudier’s protologue and drawing, all clearly refer to the dead state and are, therefore, considerably smaller than those given for the living state in this paper. In only a few collections (made mostly late in the year, e.g. B.D. 87/194.1) Declercq observed some overmature 1–3-septate spores with “pale brown to brown walls” (Fig. 10a).

SIEPE (1988) studied 13 collections from western Münsterland (Nordhein-Westfalen) and included a drawing of living multiguttulate spores and paraphyses for one of them, but he neglected the ascus base. VAN VOOREN (2009) described and illustrated a collection from a subalpine site in Fribourg (Switzerland) on stems of *Caltha* in the living state, with multiguttulate spores and paraphyses, and asci without croziers. The description given by LIZOŇ (1992: 15) under the name *H. consobrinus* lacks remarks on spore guttulation and croziers. I feel it is a mixture of *H. menthae* (or *H. macroguttatus*) (CUPG 1061, spores fusoid) and true *H. vitellinus* (PRM 614219, spores scutuloid), but the data are too insufficient. GRAUWINKEL (1987: 61) described a single collection of what was probably *H. menthae* (judging by the macroscopic description). Yet, he studied the specimen in dry state in “L4”, and thus illustrated in his drawings and photos.
only dead spores with confluent lipid content. Siepe (loc. cit.) drew attention to this inferior method of studying herborized samples, resulting in the loss of species-specific characters.

A report by Zhao & Hosoya (2014) under the name H. menthae refers to a collection on fruits of Hydrangea, with slightly scutuloid spores, judging by their photo (fig. 4 I) and description (“rounded at the proximal end, pointed towards the distal end”), whereas their drawing (fig. 5 E) shows homopolar spores. The asci are said to be “arising from croziers but obscure”, and the original oil drop pattern is unknown due to the study of dead herbarium material. The authors refer to the concept of H. menthae in Baral & Kriegsteiner (1985), i.e., in the sense of H. macroguttatus. A still unreleased sequence of the illustrated collection (TNS-F-40052, AB926063) is said to be 100% identical to another one in GenBank (AY348588, HMAS 75934, as H. cf. menthae), a sample described by Zhang & Zhang (2002: 36) on unidentified wood from Sichuan (as H. cf. consobrinus), with spores “very slightly narrower at one end”. This sequence, however, is very unrelated to both H. macroguttatus and H. menthae. Koukol (pers. comm.) found a fungus on Fraxinus petioles in Czechia with almost exactly the same sequence as HMAS 75934.

Figs 14–17. Types of Velenovský’s taxa reidentified as Hymenoscyphus menthae. – a. ascospores; b. simple-septate ascus bases; c. apices of immature or mature asci in IKI; d–e. dry and rehydrated apothecia. – All in dead state.
The brief original description of *Helotium menthae* by Phillips (in Phillips & Flowright, 1881: 69; Shropshire, on stems of Mentha) does not permit recognition of the species. It includes an egg-yellow disc, a white slender stipe, and fusiform, often curved spores (14–20 × 3–5 μm) being pointed at one or sometimes both ends, containing two to four “nuclei” (oil drops). Probably because no illustration was supplied, the name was rarely taken up by authors. Also no mention was made by Phillips about its relationship to other species. Later, Phillips (1887: 137) reduced his taxon to a variety of *H. scutula*, a view which was followed by other authors, and altered the description to “disc bright yellow”, spores with “2 to 3 guttulae”.

Authentic material of *H. menthae* was reexamined by Oudemans (1890: 315) and Dennis (1956: 78, fig. 71E). When Oudemans described his *Phialea appendiculata* Oud., an accepted later synonym of *H. scutula*, on Mentha in the Botanical Garden of Amsterdam, he examined also authentic *H. menthae* sent to him by W. Phillips for comparison. Oudemans concluded that *P. appendiculata* represents a clearly different species, based on its strongly heteropolar, much larger spores (20–26 × 4–5 μm) with distinct setulae at the ends, also in the quantity of oil drops (2–6 medium-sized guttules in a row). Detailed data on *H. menthae* were not given by him.

Dennis (loc. cit.) was apparently unaware of Oudemans’ study when he reexamined W. Phillips’ type “Elv. Brit. No. 188” in Herb. M.C. Cooke, for which he figured homopolar, non-septate spores with both ends pointed. Dennis placed it in synonymy of *Helotium scutula* var. *solani* (P. Karst.) P. Karst., based on an authentic specimen in Herb. Karsten which, however, deviates by consistently slightly scutuloid spores (Dennis, 1956: fig. 71B). In both specimens, Dennis did not see any setulae on the spores, and his drawings do not show the spore contents. Based on Dennis’ restudy, *Hymenoscyphus scutula* var. *solani* (P. Karst.) Ahmad is here assumed to be a synonym of *H. vitellinus* (see below).

In the present reexamination of the type of *H. menthae* (Phillips Elv. Brit. 188, two specimens studied from K, one from M), the spores were found to be very often multiguttulate when still inside the asci (Figs 12–13 [a1]). A size of (14–)16–20(–22.5) × (3.1–)3.3–4(–4.5) μm was evaluated in KOH or KOH+CR. The spores arise from simple septa and measure 173–100 × (7.5–)8–9.3 μm, and react medium to stron-

![Figs 26–29. Hymenoscyphus menthae. 26a. mature and immature ascus; 26b, 27a. paraphyses with refractive vacuoles (VBs) in upper part and minute yellow-orange LBs (carotenoid) in lower part; 26c, 27b, 28, 29. mature, freshly ejected ascospores (in Fig. 29 with delicate sheath). – All in living state. – 26. H.B. 8581 (Rehna), 27. H.B. 8493 (Obwalden), 28. H.B. 8854a (Schwerin), 29. H.B. 8866a (Chemnitz).](image)
since preparing the manuscript. A little doubt remains, however, practice of gathering microscopic data from herbarium mate-
spores derives from dead material. This would concur with the com-
state, I am forced to suppose that his statement of 2–4-guttulate
condition, two of which I have examined (Fig. 13).

"Phillips, Elv. Brit. No. 188, deposited in K(M) 52786" as the lectotype must be chosen as lectotype. I designate here the specimen
menclatural rules demand that one of the syntypes cited in the pro-
potologe read: "Elv. Brit., No. 188 (...) On dead stems of
Mentha. Shrewsbury". The above spore size falls in the scope given in the pro-
tologue. However, since the substrate is identified at the species level and particularly because of the granular spore contents as op-
posed to large guttules in the protologue, K(M) 31758 is probably a different collection, as was also suggested by HENGSTMENGEL (1996: 203). Dennis was obviously unaware of the fact that Phillips origi-
nally published H. menthae at the species level, since he cited as ba-
sionym "Hymenoscypha scutula var. menthae Phill., Brit. Discom.,
p. 137, 1887"; and I was likewise unaware in BARAL & KRIEGSTEINER (1985) of the true basionym Helotium menthae W. Phillips.

Although my studies indicate that Phillips’ material is homoge-
nenous, a lectotype must be chosen. It is most likely that further dup-
icates of “No. 188” exist, distributed by Phillips in other herbaria, and it seems to be impossible to be sure on which duplicate Phillips
based his diagnosis. Phillips issued his exsiccatea in the year when his publication appeared (HENGSTMENGEL, 1996: 201), therefore he possibly made the description before dividing the material. The no-
menclatural rules demand that one of the syntypes cited in the pro-
tologue must be chosen as lectotype. I designate here the specimen
“Phillips, Elv. Brit. No. 188, deposited in K(M) 52786” as the lectotype of Helotium menthae. This consists of ca. 30 apothecia in good
condition, two of which I have examined (Fig. 13).

Although Phillips probably described the apothecia in the fresh state, I am forced to suppose that his statement of 2–4-guttulate spores derives from dead material. This would concur with the com-
mon practice of gathering microscopic data from herbarium mate-
terial when preparing the manuscript. A little doubt remains, however, since H. menthae may rarely occur as a mixture with H. macrogutta-
tus (see below).

In contrast to H. menthae, BOUDIER (1909: pl. 488) detailed illus-
tration of the holotype of Helotium consobrinum was based on a fresh specimen and shows living multiguttulate paraphyses and spores, the latter with a homopolar, ellipsoid-fusoid shape (Fig. 30).

BOUDIER (1907: 114) considered his new taxon to differ from H. vir-
gulatum and H. scutula by multiguttulate spores being acute at both ends, and by a bulbous stipe base. As further characteristics he emphasized the yellow disc and the white, downy stipe. Boudier did not study the ascus base, nevertheless the correct interpretation of H. consobrinum by HENGSTMENGEL (1984), BARAL & KRIEGSTEINER
(1985) and SIEPE (1988) is beyond doubt, and does not necessitate reexamination of the type. A revision of authentic material of H. consobrinum seems not to have been done in the past, and type
material was not requested in the present study. GRELLET (1949: 53) merely copied Boudier’s description.

BOUDIER (1911: 284) stated to have repeatedly seen the species in autumn, always on Rumex acetosa ("Oseille"). His measurements of living spores as given in the text ("15–26 × 3–5 μm") concur very well with my present description of H. menthae ("15–26 × 3.5–4.5 μm"). Also when evaluating the spore size from his plate, the gained mea-
surements ("12.5–18.5–25 μm") well correspond to mine. This suggests that Boudier’s calibration was quite correct, contrary to the current assumption that he gave 10% too high values. In fact, discrepancies in measurements can be explained but the study of herbarium material or the use of lethal reagents (SIEPE, 1988; BARAL, 1992: 347).

The spores of Helotium julianum Velen. (from culms of a small
grass) are described by VELENOSKY (1940: 185) as containing minute granules, while they are said to be “eguttulate” in Svrček’s (1985: 153) revision (the guttules in the dead spores were invisible because Svrček mounted in water or media like MLZ). The apothecia are said to have originally been white. The synonymy with H. menthae was suggested by Svrček (as H. vitellinus) and is confirmed here from the reexamened holotype (Fig. 14): nearly all spores are multiguttulate (in KOH), homopolar, and measure 15–20 × 3.4–3.8 μm (Svrček: 17–
20.5 × 3–3.5 μm, Velenovský: 20–25 × 5–6 μm). The asci arise from simple septa and are relatively small (70–80 × 6.7–7.4 μm, Svrček: 70–
100 × 7–10 μm, Velenovský: 70–80 × 6–8 μm); the apical ring reacts only faintly blue in IKI (Svrček “amyloid”). The large spore
width in the protologue is in conflict with the given ascus size which does not permit biseriate spore arrangement, while the spore
length excludes a uniseriate arrangement.

Helotium repandum var. rumicis Velen., is, according to Svrček’s (1985: 172) revision, clearly a synonym of H. menthae: Svrček descri-
ted and figured the spores as “filled with minutely granular content”. Also VELENOSKY (1934: 191) reported them as densely filled with gut-
tes, and the hymenium as egg-yellow (“vitellina”). Velenovský assi-
gned to this variety records on various herbs and grasses. Actually,
totally of 30 specimens are deposited by him under this name at PRM (Svrček, 1985: 172). The present reexamination of the lectotype (Fig. 15) concurs with Svrček’s species concept: nearly all spores are multiguttulate (visible already in water), homopolar, and measure in water 14.5–21 × 2.6–3.1 μm (Svrček: 14–21.5 × 3–4 μm, Ve-
lenovský: 15–30 μm). The asci arise from simple septa and measure 110–120 × 7.5–9.2 μm (Svrček: 80–95 × 7–8 μm, Velenovský: 80–
125 μm). The apical ring is strongly blue in IKI, while Svrček stated “very slightly amyloid”. Svrček reported “numerous irregular lumps or crystals in the excipulum (in NH4OH)”. This I consider extracellular lipid which forms round drops upon squeezing. These drops did not stain in CRB, and did not disappear in KOH.

Helotium stramineum Velen., on culms of Triticum, was described by VELENOSKY (1940: 185) without indicating the spore content. The yellow apothecial colour and the large asci and spores suggest H. menthae. Svrček (1985: 178) found “eguttulate” spores (probably in water or MLZ). Reexamination of the holotype (Fig. 17) confirms Svrček’s opinion nearly all spores are multiguttulate (in KOH), ho-
ropolar, and measure 17.5–23 × 3–3.5 μm (Svrček: 16–17.5 × 3–
3.5 μm, Velenovský: 18–25 × 2–3 μm). The asci arise from simple
septa, and measure 80–105 × 6.7–7.5 μm (Svrček: 85–90 × 8–9.5 μm, Velenovský: 100–130 × 8–10 μm); the apical ring is strongly blue in IKI (Svrček “amyloid but often also inamylid”).

Helotium alismaceum Velen. was considered by Svrček (1985) as “close probably” to H. menthae (as H. vitellinus), but to represent a “very distinct species” which differs in the fresh state in a grey-lilac disc and lilac receptacle, also in an angular, almost dentate margin. According to Svrček, the lilaceous colour is due to a “vacuolar pig-
ment” in the slender hyphae of the marginal excipulum (pale viola-
ceous), also in the excipular cells at the base of the receptacle (violaceoviolaceous). Abundant crystals up to 12 μm across were seen by him in the excipulum (unclear whether outside the cells, not
drawn on his sketch). Svrček saw both pigment and crystals in the “lectotype” and a presumed toptype collected shortly afterwards on the same substrate (IX.1926, Mnichovice, PRM 148281). Since Ve-
lenovský did not mention this second collection in the protologue, the first collection (30.VIII.1926, PRM 147258, Svrček erroneously as 148258) should be taken as the holotype of H. alismaceum.

In the present reexamination of the holotype (Fig. 16, 24), no vio-
laceous pigment could be discerned at all, either macroscopically
or inside the cells. The disc was yellowish-cream when rehydrated (Fig. 24), or dirty reddish-brown in the large overmature apothecia. Apparently the ilacceous pigment fades with the age of the material. Whether this pigment represents a species-specific character remains unknown. Also no crystals could be discerned. In KOH the spores are consistently multiguilltigate and measure (15–)17–21(–23) × (3.2–)3.4–3.8(–4.2) μm (Svrček: 18–20.5 × 3.5–4.5 μm, Velenovsky: 15–20 × 3 μm). The ascii arise from simple septa and measure 80–120 × (8–)8.5–9.9–12 μm (Svrček: 85–100 × 10–12 μm, Velenovsky: 100–120 × 10–12 μm), and the apical ring is strongly amyloid when KOH-prepared. The overmature spores were 1(–3)-septate and up to 26.5 × 5.5 μm when 3-septate. 3-septate spores were otherwise only seen by Declercq (pers. comm.) in a collection on Agrimonia (Fig. 10) and by HengstMengel (pers. comm.) in one on Rubus (H.B. 400b).

Both the holotype and the authentic specimen represent a mixture with the type of Helotium septembrinum Velen., a taxon which was thought to be a synonym of Calycina cyathoides (Bull. ex Méré) Thuem. by Svrček (1985: 176, as Conchatium cyathoidenum), but in the present reexamination it is considered to be a synonym of Calycina discreta (P. Karst.) O. Kuntze. There is, however, a very sparse third species in association with the more senescent apothecia of H. alismaceum in the holotype, with short and stout apothecial stipes, separte hairs and larger spores (10–14 × 2.5 μm), which Svrček did not mention and which might be a Calycina too.

Two specimens in Velenovsky’s herbarium under the name Helotium microsorum Velen., a taxon which was identified by Svrček (1985: 160) as H. vitellinus (in his sense), while the lectotype of Helotium microsorum (on Lysimachia vulgaris) was found by him to represent Lachnum salicinae (Rehm) Velen. or H. menthae (KRIEGLSTEINER, 1999). Both are closely related to Helotium menthae (KRIEGLSTEINER, 2004) for the Rhön region (between Thüringen, Hessen and Bayern) as further hosts (genera not mentioned above) reidentified by J. HengstMengel and B. Declercq (Crepis paludosum, Geum urbanum, Glechoma hederacea, Heracleum spondylyum, Humulus lupulus, Juncus effusus, Melandrium rubrum, Scutellaria galericulata, Stachys palustris, Teucrium scordonaria, and Valeriana officinalis). The most frequent hosts of H. menthae in Declercq’s list are Epilobium hirsutum, Rubus fruticosus, and Urtica dioica.

Further herbaceous hosts (genera not mentioned above) recorded by J. HengstMengel include Centaurea sp., and by B. Declercq (Cirsium setosum var. echinatum, Cirsium setosum subsp. Sabaudium, Scutellaria galericulata, Stachys palustris, Teucrium scordonaria, and Valeriana officinalis). The most frequent hosts of H. menthae in Declercq’s list are Epilobium hirsutum, Rubus fruticosus, and Urtica dioica.

**Phylogeny:** Three European strains of Hymenoscyphus menthae (from Mecklenburg-Vorpommern, Baden-Württemberg and Liechtenstein) were sequenced from dry apothecia for the ITS rDNA region by QueLoo (pers. comm.). The three sequences were completely identical in the entire ITS region, and one of them is present in GenBank (KM114537). A sequence of H. repandus (H.B. 9057, ITS+LSU, KT876975) differs by 5.5% in the ITS from H. menthae, whereas other species of Hymenoscyphus show much higher distances to these two species. It seems probable that also H. peruni (Velen.) Svrček will be in contrast to most of those species which are closely related to H. scutula and H. fructigenus. This early fruiting of H. menthae was also stated by other authors: Siepe (1988) gave 1. June to August, rarely until 1. Oct., based on 13 collections from NW-Germany. Svrček (1985: 173) wrote “already in May” in HengstMengel (1984) noted (May)–July–Sept.–(Nov.) for 16 collections from the Netherlands. A further 23 collections from his country which he studied at a later date, are mainly from (May)–June–Aug. but also Sept.–Oct. Declercq’s 236 samples derive with rather equal frequency from May–Oct., but rarely also from Apr. and Nov. This suggests that the fungus shows a longer fruiting period in (sub)atlantic regions due to a milder climate (see Tab. 1). Engel (1987), however, gave 9.IX.–12.XI. for 8 collections on Sambucus ebulus, and Boudier’s holotype was even collected in December (Bouider, 1967).

During my visit to R.P. Korf’s laboratory in Ithaca (New York) in 1985, I was able to collect and examine H. menthae near his estate Eze Island in Canada in the fresh state. The species seems therefore to be widespread in the northern hemisphere.

**Specimens included** (all on dead herbaceous stems or culms if not otherwise indicated):

Fig. 31. Known distribution of Hymenoscyphus mentheae [including data from J. Hengstmengel, B. Declercq and T. Lassee (pers. comm.) which are not in the list of included specimens). Further records within Germany are found, e.g., in the database of the German Mycological Society (DGfM).
15 km NW of Chemnitz, 3 km NW of Burgstadt, Brausel; MTB 5042/4, 270 m, indet. dicot. herb, 19.VIII.1995, M. Eckel (M.E. 95/2606). – 2 km NNW of Ziegelhütte, Irrweiher, MTB 6838/1, 360 m, Indet. dicot. herb, 27.VI.2008, S. & P. Rönsch (P.R., d.v.). – 15 km NW of Amberg, 1.3 km SW of Horben, Ittingerwald, MTB 8419/1, 485 m, ± spored, spores (*) obliquely biseriate, (†) biseriate or ± uniseriate 8–10.3–11.8 μm {2}, †(70–)75–100(–107) × (7.7–)8–10(–11) μm {13}, 8- or sometimes orange with age.


**E:ymatography: **referring to the large lipid bodies in the mature living ascomycetes; *pteridicola* growing on ferns.


**Apothecia:** erumpent from minute cavities beneath the epidermis (5), also superficial if epidermis absent, scattered to ± gregarious in rather small groups, solitary, rarely two emerging from one spot; disc fresh 0.5–2.5 mm diam. (18), milky-white to pale cream (11), sometimes paling yellow (7), round, slightly concave to flat with somewhat raised margin (12), becoming medium convex with age (4), margin and exterior smooth or finely pubescent, whitish; stipe 0.2–0.4 mm (1–1.5) mm (5), 1.5–3 mm (6), 7–10(–11) mm (5) long. 0.1–0.15–0.3 (–0.35) mm (11), below receptive sometimes 0.4–0.5 mm wide (3), concolorous, smooth or finely pubescent-velvety, towards base partly pale to bright (reddish) brown (9), usually narrowed, rarely slightly bulbous; exterior of senescent apothecia turning cream-chromaceous to redbrown (9), hymenium becoming yellowish-cream or sometimes orange with age. Asc* = 90–115–120 × 9–10.8 (8) or 10.3–11.8 μm (2), (70–)75–100(–107) × (7.7–)8–10(–11) μm (13), B- spored, spores (‡) obliquely biseriate, (‡) biseriate or ± uniseriate
below, *pars sporoforma* 41–52 μm long (6), ±55–75 μm (2); apex of dead asci slightly to strongly conical-truncate, apical dome 1.2–2.6 → 1–2.2 μm thick, apical ring strongly (11) or faintly (1) blue (BB) in *KOH*, occupying the lower 1/2–2/3 (7) or 2/3–9/10 (13) of dome, *Hymenoscyphus* type (sometimes also *Calycina*-like, Fig. 49b); base with ± short stalk arising from croziers (28) (very rarely with an "arch" surrounding a small perforation, Fig. 41). **Ascospores** free *(14.5–)16–20(–21) × (2.5–)3.5–4.5(–5.5) μm (18), t(15)116.5–21(–22.3) × (3.2–)3.5–4.3(–5) μm (13), always non-septate within the living mature asci, cylindrical-fusoid-naviculate (cigar-shaped), rarely with slight median constriction, homopolar with both ends shortly tapering (27) (obtuse to subacute), sometimes very slightly scutellate (4), straight to very slightly curved or inequilateral, no sheath observed, without setulae (22) but sometimes with ca. 0.3–0.5(–1) μm long minute appendages (6) (difficult to see); living spores consistently with two large refractive LBs (1.8–2.5–3 μm wide; VBs multiguttulate, rather strongly refractive, 3–4(–4.3) μm, lower cells *9–25(–30) × (2.2–)3–3.5(–5) μm (3), 50(–58) μm × 2.5–4 μm or (3–)4–5.6 μm, †15–58 μm × 2–3 μm or flated (fusoid-submoniliform) (4), rounded, terminal cell *(23–)28–35 μm × 2–3 μm smooth. Paraphyses apically straight, cylindrical (11) or slightly in-

**Remarks:** *Hymenoscyphus macroguttatus* is easily recognized by its predominantly homopolar ascospores which contain large gut-
tules in the living state, and by the asci arising from croziers. Dimen-
sions of asci and spores are almost the same as in *H. menthae* (see Tab. 3). A further feature was found in the cells of the ectal excipu-
lum which are often distinctly shorter and more gelatinized com-
pared to *H. menthae*. Notable variation was observed in the length of the apothecial stipe which is considerably longer and also narrower in some collec-
tions, also in spore size, particularly in width. Samples on woody substrates showed partly slightly wider spores. One of them, which grew on a xeric *Crataegus* twig up to 1 m above ground, was not in-
cluded in the description because of extraordinarily short and wide spores (Fig. 42). This sample deviates from *H. subferruginus* (Nyl.) Dennis by the large LBs in its spores. Another not included speci-
men, on *Castanea* leaves from Tenerife, deviates by rather short, partly slightly scutelloid spores *(14.5–15.5–17) × (3.3–)3.6–
3.9(–4.2) μm.*

Distinct yellow colours were rarely observed in *H. macroguttatus*, but this feature is of minor value since also *H. menthae* may some-
times deviate by being almost white. Based on morphology and ascus croziers, *H. macroguttatus* might be confused with *Phaeo-
heliotium epiphyllum* (Pers.) H. Hagstrom or *P. monticola* (Berk.) Dennis, which differ in a much thicker, always rather short stipe and an ectal excipulum of *textura angularis*, at least at the lower flanks.

The name *H. menthae* was earlier misapplied by me (Baral & Krieglsteiner, 1985: 131) and subsequently by Hengstmengel (1996: 201) for the present taxon based on Dennis’ (1956: 78, fig. 71 E) brief re-
description and illustration of the type material and his remark that a specimen on *Teucrium* (fig. 71 E) represents "exactly the same form." While Dennis figured the type without spore guttules, the *Teu-
crium* sample shows two very large though mostly ellipsoid oil drops in the spores, reminiscent of *H. macroguttatus*, and also the cited protologue includes 2–3-guttulate spores. In the absence of in-
formation on the ascus base, the identity of the two collections remain-
ned obscure, however, and only when the type of *H. menthae* was reexamined by me (see above), this misinterpretation became ob-

### Habitat:
Predominantly in damp places (in open moist meadows, ditches, in bank communities of rivulets or small lakes, e.g. *Glycerie-
tum maxima* but also in shady woods or in gardens remote from water bodies, the substrate lying on very wet to rather dry ground, rarely up in 1.5 m above ground; on previous year’s, rather rotten

### Notable variation was observed in the length of the apothecial stipe which is considerably longer and also narrower in some collec-
tions, also in spore size, particularly in width. Samples on woody substrates showed partly slightly wider spores. One of them, which grew on a xeric *Crataegus* twig up to 1 m above ground, was not in-
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formation on the ascus base, the identity of the two collections remain-
ned obscure, however, and only when the type of *H. menthae* was reexamined by me (see above), this misinterpretation became ob-

## Hengstmengel (1996) distinguished *H. macroguttatus* (as *H. men-
thea*) from *H. menthae* (as *H. consobrinus*) and *H. scutula* mainly by the presence of croziers (from the latter also by spore shape). Spore
guttulation was neglected by him because his work was mainly based on herbarium material. In the present study which was conducted between 1997–2015, Hengstmengel’s observation on croziers was fully and independently confirmed based on material different from Hengstmengel’s. However, Hengstmengel followed my earlier interpretation of *H. menthae* without examining type ma-
terial of the two species.

Under the name *H. menthae*, Hengstmengel (1996) described *H. macroguttatus* from herbarium material of three collections from Netherlands, on stems of *Rubus* and *Fallopia japonica* (as *Polygongum cuspidatum*). The asci he reported to arise from croziers, and the dead spores as 14–21 × 3–4(–5) μm, sometimes slightly scutelloid though predominantly ellipsoid-fusoid. The depicted dead spores show 3–6 medium-sized, ± globose, partly regularly arranged gut-

### **Tab. 2 – Phenology of the here included collections of Hymenoscyphus macroguttatus.**

<table>
<thead>
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<th>Month</th>
<th>April</th>
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Figs 32–39. *Hymenoscyphus macroguttatus*. – a. mature ascospores, containing refractive guttules (LBs, the minute guttules are omitted in Figs 34–35 and partly 37; N = nucleus); b. ascus and paraphyses, the latter containing refractive vacuolar guttules (VBs), mature ascus bases with croziers; c. ascus apices of immature and nearly mature asci in IKI, with euamyloid apical ring; d. apothecia. – Living state, except for Figs 32c, 36b, c, 39a–d.
Tab. 3. Comparison of teleomorph features between Hymenoscyphus mentheae and H. macroguttatus (important characters in bold)

<table>
<thead>
<tr>
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<th>H. mentheae</th>
<th>H. macroguttatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hymenium (fresh)</td>
<td>pale to bright yellow-ochreous, rarely milky-white</td>
<td>milky-white to pale cream, sometimes light yellow</td>
</tr>
<tr>
<td>Base of stipe (fresh)</td>
<td>white, often 2 bulbous</td>
<td>white or light greyish–brown, not bulbous</td>
</tr>
<tr>
<td>Height of amyloid ring</td>
<td>1/2–2/3 (–5/6) of dome</td>
<td>(1/2–) 2/3–9/10 of dome</td>
</tr>
<tr>
<td>Asci</td>
<td>*(90–120 (–130) × 8.5–10.5 (–12) µm) simple septa</td>
<td>*(90–115 (–120) × 9–11 (–11.8) µm) croziers</td>
</tr>
<tr>
<td>Ascospore length (free)</td>
<td>*(13–(15–) 17–22 (–26) µm)</td>
<td>*(14.5–) 16–20 (–21) (–25) µm</td>
</tr>
<tr>
<td>Width (free)</td>
<td>*(12.8–) 3.5–4.2 (–4.5) µm</td>
<td>*(3.5–) 3.8–5 (–5.5) µm</td>
</tr>
<tr>
<td>Length/width ratio (free)</td>
<td>*(4.5–) 4.8–5.8 (–6.1)</td>
<td>*(3.7–) 3.9–5 (–5.5)</td>
</tr>
<tr>
<td>Slightly scutuloid spores</td>
<td>absent straight to medium curved</td>
<td>rarely present straight to slightly curved</td>
</tr>
<tr>
<td>Curvature</td>
<td>absent</td>
<td>sometimes present</td>
</tr>
<tr>
<td>Short polar appendages</td>
<td>sometimes present</td>
<td>absent</td>
</tr>
<tr>
<td>Membranous sheath</td>
<td>multiguttulate 0.7–1.3 (–1.5) µm diam., numerous</td>
<td>oligoguttulate (1.8–) 2.5–3 (–4) µm diam., 2–6 per spore</td>
</tr>
<tr>
<td>Content of living mature spores</td>
<td>1 (3–) septate</td>
<td>1-septate</td>
</tr>
<tr>
<td>Large LBs</td>
<td>Sublanceolate paraphyses</td>
<td>present or absent</td>
</tr>
<tr>
<td>Overmature spores</td>
<td>Yellow LBs (carotenoids)</td>
<td>absent</td>
</tr>
<tr>
<td>Phenology</td>
<td>pale to bright yellow-ochreous, rarely milky-white</td>
<td>milky-white to pale cream, sometimes light yellow</td>
</tr>
</tbody>
</table>

Table 3 shows a comparison of teleomorph features between Hymenoscyphus mentheae and H. macroguttatus. The table lists various features such as hymenium, base of stipe, height of amyloid ring, asci, ascospore length, width, length/width ratio, slightly scutuloid spores, curvature, short polar appendages, membranous sheath, content of living mature spores, large LBs, overmature spores, sublanceolate paraphyses, yellow LBs, and phenology. The comparison highlights differences in multiple features, with some being present in both species and others being unique to one or the other. For example, H. mentheae has a pale to bright yellow-ochreous hymenium, while H. macroguttatus has a milky-white to pale cream hymenium. Similarly, H. mentheae has a base of stipe that is white, often 2 bulbous, whereas H. macroguttatus has a base that is white or light greyish–brown, not bulbous.
quences of *H. scutuloides* no variance in the ITS region is observed. Two further strains under the name *H. scutula*, which are identical in the ITS, deviate from the former by only 1 nucleotide and are, therefore, to be considered as conspecific: AY789432 (WANG et al., 2005, strain MBH29259, without collection data) and an unpublished sequence (QUELOZ & BERNDT, pers. comm.; Switzerland, Zürich, rivulet at Wappenswil, indet. herbaceous stem, 3.X.1997, J. Schneller no. 87-284, Z Myc 337).

In the phylogenetic analysis of ZHENG & ZHUANG (2013), European and Chinese *H. macroguttatus* form with *H. scutuloides* a highly supported clade which clusters with medium support within the large genus *Hymenoscyphus*. Also in an unpublished analysis of the LSU region, in which only a few sequences are available, *H. scutuloides* (AY789431) clusters among *Hymenoscyphus* species with scutuloid spores. The distance of this clade to the *H. menthae*-*H. repandus* group is 17.5–18% in the ITS and 8.5% in the LSU (D1-D2).

**Ecology:** Unlike *Hymenoscyphus menthae*, *H. macroguttatus* was found fruiting only in late summer and autumn (Tab. 2). Except for this deviating though strongly overlapping phenology, ecological differences between *H. menthae* and *H. macroguttatus* could hardly be discovered. Both species inhabit a wide variety of hosts, mainly herbaceous stems (including monocots), rarely also woody substrates. Nevertheless, certain host preferences can be derived from the present data, for instance, *Hypericum* (mainly *H. perforatum*).
tum) was 13 times the host of *H. macroguttatus* (8× in Declercq’s list) but so far never of *H. menthae*. Extraordinary substrates of *H. macroguttatus* are seeds of *Prunus serotina* and twigs of *Alnus* and *Vitis*. Declercq’s records are almost always on herbaceous stems, often on *Lysimachia vulgaris*. Additional hosts in his list are stems of *Galeopsis tetrahit* and the two so far sole records on monocots, leaves of *Typha latifolia*.

The plant communities in which *H. menthae* and *H. macroguttatus* were found, appear to be more or less the same and are also very diverse. Obviously, both species prefer to colonize rotten herbaceous stems which lie on the ground under a condition of rather long-lasting humidity, often in close vicinity to standing or running water, but sometimes also far away from damp places. KRIEGLSTEINER (2005: 604) classified the vegetation at his only record in the Rhön region (H.B. 7034) as *Fraxino-Aceretum pseudoplatani*. The herbaceous stems on which the two species fruit appear to have died off in the previous year rather than two years ago. This would mean that colonization by ascospores during the period of fructification takes place on recently dead or perhaps still-living plant parts. The long fructification period of *H. menthae* suggests that several generations of apothecia occur in one year, perhaps on the same stem.

**Specimens included** (all on dead herbaceous stems or culms if not otherwise indicated):


Sachsen, 36 km N of Chemnitz, 4 km E of Leisnig, Klosterbuch, 160 m, Fallopia japonica, 15.IX.2013, S. Pohlers, vid. B. Mühler (unpres., d.v.). – 23 km ENE of Chemnitz, 4 km ENE of Gedeern, Kirchbach, 430 m, Fallopia japonica, 27.VIII.2012, B. Mühler (unpres., d.v.). – 6 km NNE of Chemnitz, 1 km E of Glosa, Indianerfeuchtwiesen, 325 m, Solidago canadensis, 28.VIII.2014, B. Mühler (unpres., d.v.). – 2.3 km SSW of Geyer, Waldschänke, 670 m, Rubus fruticosus, 9.IX.2011, B. Mühler (unpres., d.v.). – Nordrhein-Westfalen, 5.8 km NE of Monchengladbach, 1 km E of Neuwerk, S of Altzohf, MTB 4704/4, 40 m, Fallopia japonica, 28.VIII.2014, H. Bender (H.B. 9963).

Baden-Württemberg, 7 km E of Heidelberg, 1.5 km SE of Ziegelhausen, Kleingemünderstraße, Fallopia dumetorum, 16.X.2009, M. Bemmern (ø, d.v.); – 9 km E of Heidelberg, 1 km N of Neckargemünd, ESE of Kleingemünd, 130 m, twig of Vitis vinifera, on bark and leaf tendrils, 18.IX.2010, M. Bemmern (ø, d.v.). – 3.8 km NW of Stuttgart, 1.8 km SW of Feuerbach, Heimberg, MTB 7120/4, 370 m, Lysimachia vulgaris, 15.X.1975, H.O. Baral. – 5.5 km W of Stuttgart, 1.2 km E of Solitude, Nippenburgerle, MTB 7220/2, 440 m, Fallopia japonica, 15.X.1975, H.O. Baral (H.H. 10268). – ibid, Scrophularia nodosa, 22.IX.1975, H.O. Baral (ø). – 6 km WS of Stuttgart, 1.2 km NE of Busnau, Schattenseen, MTB 7220/2, 420 m, Lycopus europaeus, 18.IX.1975, H.O. Baral. – 5.3 km NE of Tübingen, ESE of Pfrondorf, Auchtert, MTB 7420/4, 395 m, Fallopia sachalinensis, 26.IX.1974, E. Weber (H.B. 7577, sq.: ined.). – 4 km WSW of Stuttgart, 1.2 km NE of Neckar, Küffinger, MTB 7420/4, 340 m, twig of Alnus glutinosa, on bark, 26.IX.1992, H.O. Baral (H.B. 4757b). – Emmendingen, Kloster Tennenbach, MTB 7813/3, 280 m, Persicaria hydropiper, 31.VIII.1975, H.O. Baral. – 8 km NE of Emmendingen, ~1.7 km SE of Freiamt, MTB 7813/1, 390 m, Rubus idaeus, 30.VIII.1975, H.O. Baral (H.B. 400a).

Hessen, 6 km SW of Frankfurt, Goldstein, Am Wiesenhof,

Not included: Luxembourg: Gutland, 10 km ESE of Esch-sur-Alzette, 2.3 km SE of Dudelange, Därebësch, 270 m, twig of Cotaegus, on wood, 9.XII.1997, G. Marson (H.B. 5999). – Macaronesia: Tenerife, 11 km ENE of Guía de Isora, Puerto de la Cruz, 1.7 km SE of La Matanza, N of Tabares, La Morra, 736 m, 15.IX.1992, W. Pohl (H.B. 4740, sq.: ined.).


Etymology: probably because of the brown apothecial colour.

This species was reported by THIND & SINGI (1972) only from the type collection (on herbaceous stems, Parbat [Parbat] valley, Kulu [Kullu] hills, Pulga, NW-Himalaya, Himachal Pradesh, India, 29.IX.1965, H. Singh (PAN 3115, as ”PUL”), holotype; isotypes in BPI, CUP, K). The protologue comes close to H. macroguttatus: the ascospores have a size of 116–20 × 3.2–4.2 μm and are figured with a homopolar shape (described as ”fusoid”). Although they are described as aguttulate and aseptate, the schematic drawing, which shows dead spores inside dead asci, seems to illustrate pseudosepta, i.e. plasma bridges, which indicate the presence of two large central and two smaller polar LBS. However, the dark brown exterior of receptacle and stipe, the latter almost black at the point of attachment” due to brown amorphous matter, and the long asci (1100–130 × 7.9–5 μm) deviate from H. macroguttatus. The entire fungus is said to be light to dark brown, but it remains unclear how the colour was in the fresh state. This species was not examined in the present study and awaits reexamination, especially for the ascus base, but also concerning the spore number and setulae, in order to exclude H. sharmae. Lambertella musooriensis K.S. Thind & H. Singh was described in the same paper. It shows very similar spores and might be a Hymenoscyphus too, being extraordinary in apothecia up to 12.5 mm diam. and truncate ascus apices (described as ”obtuse”).

Hymenoscyphus sharmae Baral, spec. nov. – MB 814405 – Figs 52–55

Diagnosis: Resembling Hymenoscyphus macroguttatus and H. trichosporus in ascospore size and shape, the former also in spore contents, the latter and also H. scutulaeoides, H. seminis-alni and H. trichosporus in the presence of conspicuous setulae at the spore ends, differing from all in 4-spored ascis.

Typification: India, Uttar Pradesh, Nainital, Kilbury, stems of Prunus cerasus, 11.VIII.1973, M.P. Sharma (TAAM 194665, holo-
type).

Etymology: referring to the collector, M.P. Sharma.


Apothecia rehydrated 0.3–0.7×(1–1) mm diam. (4), scattered to gregarious, small, pale yellowish-orange (Sharma: cream to light yellow when fresh), stipe 0.4–1.2×(1–1.7) mm high, 0.1–0.15 mm wide (4), somewhat glassy-translucent. Ascii 175–107 × (8.5–)9–11 (–12.5) μm (4), 4-spored (6), rarely a few ascis 3-, 5- or 6-spored (2), immature ascis 8-spored (~4 spores early aborting); apex of dead ascis medium to strongly conical, with pronounced apical dome 2.8–2 μm thick, with an amyloid ring reacting strongly blue (BB) in IKI, cupping only the lower half of the dome [3], Hymenoscyphus-type; base with ± long stalk arising from croziers (7). Asci [16.3–17–22×(4–)4.5–5.6–6.3] μm (7), non-septate, cylindrical-ellipsoid to fusoid, straight to slightly inequilateral, not or scarcely constricted at the centre, homopolar or very slightly scutuloid (he- teropolarity rarely recognizable in free spores), ends rounded to obus- tuse, sometimes subacute (especially at the base), each end with (1–2–4–6) very deltal, 1.5–3 μm long setulae (7); with 1 (4) or 2–4 (3) large LBS ~1.8–3.2–4 (μm diam. and many much smaller ones in each half (high lipid content). Paraphyses cylindrical, straight, or not or slightly widened above, apex rounded, ± equaling the ascis, terminal cell 121–48 × 1.7–4 μm (3), lower cells 118–30 × 1.5–2 μm (1), with anastomoses near base; remnants of VBs in terminal cells sometimes perceptible by a pale brownish, refractive content (in H2O). Ectal excipulum hyaline, of medium thick-walled (gelatinized) (5), horizontal textura prismatica in receptive cells, cells at (10)–26 × 4–7.5 μm (2), of t. porect in stipe; externally cove-
red by a loose network of narrow hyphae with a yellowish-orange (content of VBs). Medullary excipulum not studied.

The new species could only be studied in the dead state, based on several herbarium specimens collected in 1973 in India and pre-
served at TAAM. It seems to be closely related to H. macroguttatus. It has a very similar ascospore size, shape, and guttulation, and de-
viates only slightly in a tendency to a slightly higher number of large LBS and in slightly wider asci and distinctly wider ascospores. As in H. macroguttatus the ascis arise from croziers. The new species differs mainly in the two striking characters. (1) More than ca. 95% of the studied ascis were 4-spored, and the remaining ones 3-, 5- or 6-spored. Immature ascis are 8-spored, and it is not easy to detect the aborted spores in the dead state within the mature asci. Only 3–6 spores at-tain full size and are well visible, whereas 2–5 spores abort more or less half-sized and are collapsed, at least inside of dead asci. (2) Most of the free spores in a preparation possess very delicate, rather long setulae, 1–6 at each end. The setulae are usually invisible when the spores are still inside the asci, but can well be seen in free spores mounted in water, KOH, or KOH+CR. Despite their length and num-
ber they are not as apparent as in H. scutula. It is thus not surprising that they were overlooked by THIND & SHARMA (loc. cit.).

Four-spored ascis represent a rather unexpected character of Hy-
menoscyphus in its restricted sense. Perhaps therefore, not enough attention is paid to ascospore numbers in studies of this genus. THIND & SHARMA (1980) referred and illustrated 8-spored asci for the present material, apparently without carefully checking this feature. However, not all of the collections cited by these authors appear to exist at TAAM, and to clarify their conspecificity requires reexamina-

Only one potential Hymenoscyphus species with 4-spored ascis came to my notice: Heliotium tetra-ascosporum Rea (Scotland, on Phalaris). However, setulae were not reported there, and the spores are stated to be longer and narrower (21–27 × 3.5–4.5 μm) than in H. sharmae. There is also a Heliotium tetrascosporum (Feltgen) Boud., which is a synonym of Phialae winteri Rehm following Dennis (1964: 69), but was combined as Bisporella tetrascopora (Feltgen) S.E. Carp. by CARPENTER (1981). Some further discrepancies can be found between the present description of H. sharmae and that by THIND & SHARMA (1980). The ascis and ascospores are reported much smaller, 64–85 × 3.6–7.5 μm and 13–16.5(–18.2) × 3.7–4.5 μm, respectively, although the narrow ascus width hardly concurs with the given spore width, particularly since the authors describe the spores as bisericate. The remark ”gut-
tules disappearing at maturity” is possibly a misinterpretation that arose when the authors studied a water mount and saw not only li-
ving spores in their preparation but also dead, seemingly eguttulate ones. I have seen only a few free spores, and these always contained a high amount of lipid, with the LBs often fused, however (spores with fused LBs are intentionally omitted in my drawings). Overma-
ture spores showing reduced lipid contents could not be found.

All collections of H. sharmae examined by me were identified by Thind & Sharma (1980) as H. scutula var. solani. I have studied all seven specimens deposited in TAAM, but none of the duplicates which are said to be deposited at PAN, and none of the four further collections cited by Thind & Sharma. There is a discrepancy concerning the host genus. On the TAAM labels the host is indicated as "Pimpinella sp." or "Pimpinella acuminata" (Apiaceae), although Thind & Sharma say “Polygonum sp.” or “Polygonum amplexicaule” (Polygonaceae) for all eleven specimens listed. Based on the anatomy of the 1–4 mm thick stems in the holotype, this question could so far not be solved.

H. scutuloides Hengstm. and H. seminis-alni Baral, Grauw. & M. Eckel concur with H. sharmae in the presence of several setulae at each spore end, and in the presence of croziers. The two species differ in distinctly heteropolar (scutuloid), narrower spores, and 8-spored asci. I have studied H. scutuloides from a fresh collection from Liechtenstein, on stems of Filipendula ulmaria (H.B. 5845). H. trichos-
porus Dougoud differs in 8-spored asci and a lignicolous habitat.

Specimens examined (all on dead herbaceous stems, all issued as Helotium scutula var. solani):


Taxa with more or less heterophilop, scutuloid ascospores (H. scutula agg., H. vitigenus)


(?) = Hymenoscyphus vitellinus (Rehm) Kuntze, Revis. gen. pl., 3 (2): 486 (1898).

≡ Helotium vitellinum Rehm, Ber. naturhist. Augsburg, 26: 124 (1881).

≡ Helotium scutula f. vitellina (Rehm) Rehm, in Winter, Rabenh. Krypt.-Fl., Edn 2, 1.3(lief. 40): 794 (1893) [1896].

≡ Phiaeula vitellina (Rehm) Sacc., Syll. fung., 8: 262 (1889).


(?) = Hymenoscyphus scutula var. solani (P. Karst.) S. Ahmad, Asco-myctetes of Pakistan, 1: 207 (1978).


For further synonyms see in Species Fungorum.

**Typification:** scutula: location unknown, undated (type not located).


– scutula var. solani: South Finland, location unknown, stems of Solanum tuberosum, 27.X., P. Karsten (type not located).

**Etymology:** scutula: after the apothecial disc resembling a small shield; vitellinus: named after the apothecial colour, like egg yolk; geophilum, solani: after the host plant, Geum and Solanum.


**Hymenoscyphus scutula** is a very common and wide-spread species on herbaceous stems, known especially from Europe and North America (WHITE, 1942), characterized by strongly heteropolar (“scutuloid”) ascospores with one, rarely two conspicuous setulae at each end, which have a length of (0.5–)1–3 (–5) μm, and asci arising from simple septa. Spores of a typical collection are as illustrated on Fig. 59. The name has presently a long list of synonyms in Species Fungorum, although not all of them can safely be included in the scope of this rather variable species. Type material appears never to have been located or reexamined. WHITE (1942) studied various collections of H. scutula in which either some or almost all spores possessed setulae, but included also a sample entirely without setulae (type of H. scutula var. solani: named after the apothecial colour, like egg yolk; geophilum, solani: after the host plant, Geum and Solanum).

How widely the concept of H. scutula should be adopted is difficult to say. For instance, lichenicolous samples currently referred by me to H. virgultorum are not easy to separate. Perhaps only those populations with rather small LBs in the spores belong to the latter species. The caulicolous taxa H. vitellinum and H. geophilum as described here from their types (Figs 56–58) cannot safely be separated from H. scutula by morphology, therefore, I tentatively accept them as synonyms of H. scutula. Also a specimen on bark of Vitis might belong in the scope of H. scutula (Fig. 61).

According to SACCARDO (1889: 262), Rehm established Helotium vitellinum based on a specimen on rotten stems of Filippendula ("Spiraea") ulmaria from Augsburg, with yellow, 2 mm tall apothecia with discs eventually becoming orange-red, 1.5 mm diam., asci 75–80 × 9 μm, and heteropolar spores 18 × 4 μm containing 1–2 large “nuclei”. Later REHM (1893: 794) included collections from Berlin on Filippendula and Lysimachia vulgaris, and reduced it to a form of H. scutula. He distinguished it from typical H. scutula by smaller spores (18–20 × 3–5 μm vs. 18–25 × 4–5 μm), also by smaller apothecia (0.3–1.5 vs. 0.3–3 mm diam.), with shorter (rarely up to 1 vs. 0.5–5 mm) and much more delicate stipites.

CARPENTER (1981: 270) examined the “holotype specimen” from S ("Augsburg, auf Spiraea ulmaria, Oct 1878, Britzelmayr s.n. [ex Herb. Rehm]") and agreed with the opinion of WHITE (1942) and DENNIS (1956) that H. vitellinus is a synonym of H. scutula. Without personal study, LIZOŇ (1992: 43, 46) accepted this synonymy. The present type study confirms this opinion. However, since this species complex is in bad need of molecular work, conclusions about synonymies are to be considered as premature at the moment.

H. vitellinum was examined by me from holo- and isotype material in M (X.1991) and S (VIII.1999) (Figs 56–57). Although CARPENTER (1981) did not mention the existence of different convolutes at S, the online database of the herbarium in Stockholm lists two specimens, S-F10431 (“lectotype”) and S-F10432 (“isolateotype”). Since Rehm’s private herbarium is located in S, the specimens there frequently contain his original handwriting and sketches, which is also the case on the label of S-F10432, which bears a sketch of two spores (Fig. 57 a2b) and a diagnosis, including asc = 75–80 × 9 μm and spores ~18 × 3.5–4 μm. It seems, therefore, that convolute S-F10432 was the one that Rehm had used when preparing the protologue. However, this convolute contains only very few apothecia, and only a single ascospore was found by me in the examined apothecium (Fig. 57 a2a), but no asci. In contrast, S-F10431 contains abundant apothecia rich in asci and spores (two of them are documented on Fig. 57 a1). CARPENTER (1981) obviously meant with “holotype” the convolute S-F10432, and in naming it so he followed a current practice. Yet, this specimen seems to be rather useless for microscopic examination. I here follow the printed denomination on the two convolutes and designate specimen S-F10431 as lectotype of Helotium vitellinum.

Differences among the examined convolutes in M and S were noted in stipe length (1–1.3 mm in S-F10432, 0.2–0.6 mm in M, 0.1–0.5 mm in S-F10431), while stipe width was always in the range of 0.1–0.2 mm (rehydrated). The microscopic characters were found to conform, except that in S more spores with distinct setulae were observed. All spores were found to be strongly heterophylar (scutuloid), which is in accordance with Rehm’s illustration on the label of S-F10432 which shows strongly clavate spores with obtuse apex and acute base. They are frequently multiguttulate though often with some rather large globose LBs, and generally longer than stated by Rehm. What Rehm illustrated on the label (Fig. 57 a2a) and described as “with 1–2 large oil drops” in the protologue, refers to spores in which the oil drops fused to large elongate aggregations. Comparatively short setulae (0.5–2.5 μm) were seen in a few spores only, 1–2 at each end. The asci arise from simple septa and have strongly reactive apical rings (blue without KOH-preparation, type 8B).

Thus, H. vitellinum does not significantly differ from typical H. scutula, including the presence of setulae. An error occurred in the statement of LIZOŇ & KUCERA (2014) about the setulae which are in fact only predominantly absent in this collection, not entirely.

When SVRČEK (1985: 150, Tab. VII, fig. 1) revised the lectotype of Helotium geophilum Velen. (Vysoké Tatry, VII.1924, on rhizomes of Geum rivale, PRM 147239), he found the spores to be fusiform, rounded at both ends or very slightly attenuated towards the base, eguttulate, 15–19 × 3.5–4 μm, sometimes 1-septate and distinctly greysish in MLZ. The contents of the paraphyses stained reddish-brown in MLZ. VELOVSKÝ (1934: 193), however, described them as guttulate, but gave only a very brief description without illustration. With some hesitation, SVRČEK suggested H. geophilum to be conspecific with “Hymenoscyphus vitellinus” (s. SVRČEK = H. menthae), but he was unsure as the apothecia were described by Velovský as entirely flesh-coloured.
Rehm 513: Augsburg, stems of Filipendula ulmaria. Holotype (57-2) and isotypes of Hymenoscyphus vitellinus (56: M-0206430, 57-1: S-F10431, 57-2: S-F10432)

PRM 147239: Vysoké Tatry, on rhizomes of Geum rivale. Lectotype of Helotium geiphilum

H.B. 3290: Tübingen, on stems of Tanacetum vulgare

(c7: H.B. 1951, Ditzingen, on Chrysanthemum)

Figs 56–58. Types of Helotium vitellinum and H. geiphilum, here reidentified as Hymenoscyphus (?)scutula; Fig. 59. H. scutula (typical collection). a, mature ascospores (but overmature in Fig. 58a2; N = nucleus), b, ascus base with simple septa, c, immature and mature ascus apices (emptied in c7), d, apothecia. – Dead state, except for 59a1, c1, c3, c5. Fig. 59a is taken from BARAL (1992: fig. 21), Fig. 59c from BARAL (1987: fig. 10). – Note that the dead spores in Fig. 59a2 died in the fresh apothecium, therefore, the lipid bodies have undergone complete coalescence (compare also H. menthae on Fig 9). Fig. 57a2b: drawing by Rehm on label in S-F10432. Coalescence masks the striking difference between the two taxa.
Reexamination of the lectotype (Fig. 58) revealed the spores to be predominantly slightly scutuloid so that, for most of the free spores, it is possible to recognize their upper and lower ends. They are finally 1–(3)-septate, and setulae were not observed on any of them. Several spores were found to be multiguttulate (in KOH). The asci arise from simple septa and the apical ring reacts strongly blue in IKI. Despite the smaller and less scutuloid spores and the much narrower asci (Svrcěk: 95–110 × 6–7 μm), I consider this taxon as conspecific with the type of *H. vitellinus* (non s. Svrcěk).

The taxon *Hymenoscyphus scutula var. solani* was repeatedly applied to collections, even in the modern literature, and appears to be applied to collections, even in the modern literature, and appears to be conspecific with the type of *Vitis vinifera* of Tübingen, Pfrondorf, Blaihofstr., 430 m, stem of *Vitis vinifera* holotype (De Not.) Dennis, 1873: 78, fig. 71 B) examined an authentic specimen from Herb. H. menthae Karsten, yet without indicating any collection data. Dennis’ illustration shows five slightly but distinctly scutuloid, 1-septate, probably overmature spores. Based on their consistently heteropolar shape, it seems quite improbable that *H. scutula var. solani* is a synonym of *H. mentheae or H. macroguttatus* (see above).

Regrettably, the identity of *H. scutula var. solani* could not better be clarified in the present study. It was impossible to locate authentic material at H (Niemela & Huttinen, pers. comm.), although Dennis (1956: 78, fig. 71 B) examined an authentic specimen from Herb. Karsten, yet without indicating any collection data. Dennis’ illustration shows five slightly but distinctly scutuloid, 1-septate, probably overmature spores. Based on their consistently heteropolar shape, it seems quite improbable that *H. scutula var. solani* is a synonym of *H. mentheae or H. macroguttatus*. Rather, the taxon might be conspecific with *H. vitellinus* (= *H. scutula*).

**Phylogeny:** In GenBank the name *H. scutula* appears to be frequently misspelled. The only seemingly trustable ITS sequence concerns a sample from tropical Cuba collected by G. Verkley (CBS 480.97, KC481695), which is genetically close to an unpublished sequence from a Swiss collection identified as *H. scutula* (ZT 4292, Qieloz, pers. comm.), though probably not conspecific as it deviates by 10 nucleotides.

**Specimens of *H. scutula* s.l. examined and illustrated here:** Germany: Hessen, ~6 km NW of Mainz, around Budenheim, ~100 m, branch of *Vitis vinifera*, on bark, undated (autumn), L. Fuckel (Fuckel Fungi Rhen. Exs. 2685, M, H.B. 6010a ø [mixture in syntype of *H. vitigenus* (Fuckel) Fuckel, De Not., 1873: 78, fig. 71 B] identified a collection from Pakistan with relatively short, heteropolar spores as *H. scutula var. solani*. I can only speculate that this might be conspecific with *H. vitellinus*.

The brief, unillustrated original description of *Helotium vitigenum* by De Notaris (1864) concerns a fungus collected on a xeric (“secco”) branch of *Vitis vinifera* at Lago Maggiore (Italy) in autumn 1863. Its features include a pale straw-coloured disc, a stipe of moderate length, and 4-guttulate, ellipsoid-fusoid spores 20 μm long. Saccardo (1875: 137; 1883: tab. 1343; 1889: 229) referred to this species two samples from mountainous vineyards in Venetia (Padova and Treviso), on fallen twigs of *Vitis vinifera*, with homopolar, fusoid-guttulate spores 18–20 × 6 μm, and 8-spored asci 110 × 12 μm (Fig. 64).

**Etymology:** *vitigenus*: named after the host plant (*Vitis*); *hyalopes*: after the translucent stipe.

*Helotium vitigenum* and *H. hyalopes* are currently believed to represent a single species. Their original descriptions recall a possible relationship to *H. macroguttatus* because of their fusoid (homopolar), 2- or 4-guttulate ascospores, or to *H. mentheae*, given that the oil drops in the spores have fused during drying. However, when revising a syntype of *H. hyalopes* at M, it turned out to be a mixture of two different species growing in close proximity on the same branch, both possessing distinctly scutuloid spores and asci without setulae. Since the identity of the type of *H. vitigenus* was not clarified in the present study, its synonymy with *H. hyalopes* seems questionable. A published thorough redescription of De Notaris’ type material does not appear to exist.

The brief, unillustrated original description of *Helotium vitigenum* by De Notaris (1864) concerns a fungus collected on a xeric (“secco”) branch of *Vitis vinifera* at Lago Maggiore (Italy) in autumn 1863. Its features include a pale straw-coloured disc, a stipe of moderate length, and 4-guttulate, ellipsoid-fusoid spores 20 μm long.

**Specimens of *H. hyalopes* examined and illustrated here:** Germany: Hessen, ~6 km NW of Mainz, around Budenheim, ~100 m, branch of *Vitis vinifera* in autumn, with oblong-fusiform, biguttulate, subinequilateral spores 16 × 5 μm, “often aggregated in upper part ofascus”, and ascii 126 × 18 μm (obviously in living state). Rehm (1893: 789) restudied his portion of the exsiccatum and observed rather large (0.5–2 mm diam.) apothecia with long stalks (up to 2.5 × 0.2 mm), fusiform spores 15–20 × 5–6 μm with two large oil drops (possibly by fusion), and 4–8-spored asci 80–120 × 10–12 μm.

*Helotium hyalopes* in Fückel (1873), issued as Fungi Rhen. Exs. 2685, concerns a fungus collected near Budenheim (NW of Mainz), on xeric (“arid”) bark of *Vitis vinifera* in autumn, with oblong-fusiform, biguttulate, subinequilateral spores 16 × 5 μm, “often aggregated in upper part of asci”, and asci 126 × 18 μm (obviously in living state). Rehm (1893: 789) restudied his portion of the exsiccatum and observed rather large (0.5–2 mm diam.) apothecia with long stalks (up to 2.5 × 0.2 mm), fusiform spores 15–20 × 5–6 μm with two large oil drops (possibly by fusion), and 4–8-spored asci 80–120 × 10–12 μm.

**Specimens of *H. scutula* s.l. examined and illustrated here:** Germany: Hessen, ~6 km NW of Mainz, around Budenheim, ~100 m, branch of *Vitis vinifera* on bark, undated (autumn), L. Fuckel (Fuckel Fungi Rhen. Exs. 2685, M, H.B. 6010a ø [mixture in syntype of *H. hyalopes*, H.B. 6010b]). – Baden-Württemberg, 10 km NW of Stuttgart, Ditzingen, Mittlere Str., 300 m, stems of *Chrysanthemum*, 18.X.1975, H.O. & O. Baral (H.B. 1951). – 5.5 km NE of Tübingen, Pfondorf, Blaiforstr., 430 m, stem of *Tanacetum vulgare*, 24.X.1987, H.O. Baral (H.B. 3290). – Bayern, Schwaben, Augsburg, ~500 m, *Filipendula ulmaria*, X.1878, M. Britzelmayer (Rehm Ascomyc. Exs. 513, S-510431 lectotype of *H. vitellinus*, isolecotypes in S-510432 and M-0206430, H.B. 4497 ø). – Schweiz: Schaffhausen, 6.3 km NE of Schaffhausen, 1.2 km NW of Thayngen, Geiger (vineyard, 460 m, on leaves (petioles) and fruit stems of *Vitis vinifera*, 15.XI.1987, P. Blank (P.B. 689, H.B. 6015b ø [mixture with *H. vitigenus*, H.B. 6015a]). – Słowacja: Prešov, ~75 km W of Tatranská Lomnica, Vysoké Tatry, ~1800 m, on rhizoms of *Geum rivale*, VII.1924, A. Pilat (PRM 147239, lectotype of *H. geophilum*, H.B. 5819 ø).

**Hymenoscyphus vitigenus** (De Not.) Dennis, Persoonia, 3 (1): 74 (1964) – Fig. 64. – *H. vitigenum De Not.*, Comm. Soc. cirttig. Ital., 1 (5): 377 (1864) [1863].

*Calycina vitigena* (De Not.) Kuntze, Revis. gen. plant., 3 (2): 449 (1898).


**Typification:** *vitigenus*: Italy, Lombardia, Lago Maggiore, Valle Intrasca, branch of *Vitis vinifera*, autumn 1863 (not located according to Lazor & Kucerova, 2014); *hyalopes*: Germany, Hessen, Budenheim, branch of *Vitis vinifera*, autumn (L. Fuckel, Fungi Rhen. Exs. 2685, M, syntype, four apothecia with shorter and wider spores).

**Etymology:** *vitigenus*: named after the host plant (*Vitis*); *hyalopes*: after the translucent stipe.
separate population differ in having a more yellowish disc (rehydrated, 0.3–0.5 mm diam.) and a hyaline, glassy stipe 0.3–0.5 mm long. One of these apothecia was tested (Fig. 60); it differs in shorter and wider asci, and shorter and wider spores. The spores contain 1–2 large and many small LBs in each half, and no setulae were seen at their ends. As in the other species, the asci arise from simple septa and have a strongly euamyloid apical ring. Obviously, this is the taxon which Fuckel had under the microscope, judging from ascus and spore size and from the translucency of the stipe. Only these four apothecia should be considered as the type of *H. hyalopes*.

Because PIROTTA (1877) did not see marked differences between the descriptions of *Helotium vitigenum* and *H. hyalopes*, he considered them to be synonymous. The given differences in spore guttulation (4-guttulate in *H. vitigenum*, 2-guttulate in *H. hyalopes*) he considered to be incorrect because he observed variation in the number of drops in Mycoth. Veneta n. 959. Also REHM (1893) believed that the two taxa are synonymous, based on the descriptions of De Notaris, Fuckel and Saccardo, and on his reexamination of Saccardo Mycoth. Veneta n. 959 and Fuckel Fungi Rhen. Exs. 2685.

THÜMEN (1878: 87), however, doubted the synonymy of *Helotium vitigenum* and *H. hyalopes* because of differences in ascus length. However, this difference can easily be explained by the shrinking effect of the ascus which Fuckel measured in the living state (126 μm) and Saccardo probably in the dead state (90–110 μm).

Fresh collections and molecular data are needed to clarify the taxonomic relationship of *Hymenoscyphus vitigenus* and its asserted synonym *Helotium hyalopes*. DENNIS (1956: 92) described under *Helotium vitigenum* a British sample on unidentified twig, which he considered to agree well with Fungi Rhen. Exs. 2685. Contrary to his opinion, however, delimitation of *H. hyalopes* against *Hymenoscyphus subferrugineus* is easily accomplished by the presence of croziers in *H. subferrugineus* (a detailed restudy of the lectotype of that species will be presented in a separate paper). Possibly, the British sample belongs in the scope of that species. On the other hand, de-

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I never saw fresh material referable to Hymenoscyphus vitigenus or Helotium hyalopes. Like Dennis (loc. cit.), the following authors examined only a recent collection (under the name Hymenoscyphus vitigenus): Raitviir & Faizowa (1983, Salix twigs), Sacconi (1985, Vitis vinifera twigs), and Blank (1989, Vitis vinifera leaves). Blank’s specimen (P.B. 689) was reexamined by me and found to be a mixture of two different species growing on different petioles, both with a similar range in spore size: (1) a sparse population with slightly scutuloid spores which represents the fungus illustrated by P. Blank, and which hardly differs from H. caudatus (H.B. 6015a, Fig. 62); (2) a more abundant population with strongly scutuloid spores, perhaps belonging in the scope of H. caudatus in a wide sense (H.B. 6015b, Fig. 63). Both possess euamyloid asci without croziers. Blank’s drawing shows oblong ellipsoid-fusoid to clavate spores, but is obviously not accurate enough to recognize that the spores are predominantly heteropolar. His description includes also the macroscopy of the associated second taxon, and his remark “reddening with age” appears to refer to that species rather than H. vitigenus.

The present study indicates that Vitis hosts at least three different species of Hymenoscyphus: H. macroguttatus (Fig. 48), H. caudatus (Figs 60, 62–63), and H. scutula (Fig. 61). Whether H. vitigenus sensu Saccardo (Fig. 64) belongs to H. macroguttatus or to H. menthae remains to be clarified. Even more important would be to locate the type of limitation of H. hyalopes against H. caudatus (P. Karst.) Dennis seems more problematic: H. caudatus is a collective species on leaves of deciduous trees, being until now little understood. The name was applied to collections which obviously belong to different taxa, featuring scutuloid spores with a very different size and guttulation, differing also sometimes in the ascus base. However, collections on wood or bark so far appear never to have been referred to H. caudatus.

H. vitigenus and to find out whether it might represent an earlier synonym of H. macroguttatus or H. menthae.

Lizǒn & Kučera (2014) accepted a name change by Kuntze (1898) from hyalopes to hyalopus, but I feel there is no reason for doing so because the prefix hyalo- is both Greek and Latin and can, therefore, be used in compositions of pes as well as pus.

Specimens examined (H. cf. vitigenus): Germany: Hessen, ~6 km NW of Mainz, around Budenheim, ~100 m, branch of Vitis vinifera, on bark, undated (autumn), L. Fücker (Fucked Fungi Rhen. Exs. 2685, M, syntype of Helotium hyalopes, H.B. 6010b ø [mixture with H. scutula, H.B. 6010a]). – Switzerland: Schaffhausen, 6.3 km NE of Schaffhausen, 1.2 km NW of Thayngen, Geiger (vineyard), 460 m, leaves of Vitis vinifera, on petioles, 15.XI.1987, P. Blank (P.B. 689, H.B. 6015a ø [mixture with H. scutula/caudatus, H.B. 6015b]).

General comments

Shrinking effect. Ascus and spore size were found to be comparatively variable characters. Although there are tendencies for different mean values of length, width, and l:w-ratio between the species treated here, such data gained from a single collection helps little in species identification. Variation may even occur within single collections. F. ex., in the type material of those names here referred in synonymy with H. menthae, ascus length and width were often found to be very different from Srzcek’s data. Likewise, spore length in H. menthae may vary between 15–19 and 18–24 μm depending on the collection.

In addition to this variation, the shrinking effect of the asci (difference in size when comparing living with dead asci, Baral, 1992: 345), which is a general feature of ascomycetes, is very remarkable in the genus: asci shrink ca. 15–20% in length and ca. 8–20% in width, therefore, they lose up to about half of their volume (the same shrinkage takes place during active spore discharge). In comparison to this, shrinkage of ascospores is comparatively unimportant: ca. 1–2% in length and ca. 2–5% in width. Size differences in relation to the mounting medium (H2O, KOH, MLZ, CB etc.) are quite unimportant when dealing with dead elements.

Iodine reaction of ascus apex. The intensity of the iodine reaction as well as its colour (eu- or hemiamyloid) is usually a relatively constant feature within a species. Different observations gained
from the same material occurred, however, for instance in the types of *H. repandum var. rumicis* and *H. stramineum* between Světíček's and my data. Possibly this was a result of different reagents, although hemialloy reactions which cause such divergences were not observed in the species treated here. The shape of the apical apparatus is highly consistent within the genus *Hymenoscyphus*. It therefore does not aid species distinction, but it permits recognition of misplaced taxa: for instance, *Calycin a herbarum* (Pers.) Gray has for a long time been treated in *Hymenoscyphus*.

**Croziers.** The ascus base is currently neglected with regard to the presence or absence of croziers because in squash mounts the detection of this character needs a higher amount of patience and often the use of KOH in combination with Congo Red. However, in not too thin median sections of living material mounted in water, the character is usually very promptly seen.

White (1942, 1943, 1944) carefully examined and depicted the ascus base and ascogenous hyphae for simple septa and croziers as a species marker of *Hymenoscyphus* (as *Helotium*). In spite of these excellent and at that time new observations, White's knowledge was not taken up by most later workers. Only in recent times the character received due consideration, either at the species level (e.g., HengstMengel, 1996; Baral et al., 2013; Baral & Bemmann, 2013, 2014), or variety level (Huntinen, 1990). French workers, e.g. Berthet (1964), often use the equivalent terms 'pleurorhynque' (for croziers) and 'apo-rhynque' (for simple septa).

In my studies on the *Helotiales* after ca. 1987 I began to examine ascus bases for the presence or absence of croziers, stimulated by White's studies. This character turned out to provide a useful species marker in many genera, allowing clear separation between taxa which were previously considered to be difficult. It proved also very helpful in the genus *Hymenoscyphus* which is evident in the treatments by White (loc. cit.), HengstMengel (1996), Baral (1997), Baral et al. (2013) and Baral & Bemmann (2014). HengstMengel’s (1996) important paper deals with some species related to *H. scutula*, most of them with scutuloid sporodex but, in contrast to *H. scutula*, all being characterized by having croziers. The presence of croziers turned out to be a rather rare character state in species of *Hymenoscyphus* with scutuloid sporodex, while species having ascus arising from simple septa are in the majority (HengstMengel, 1996: 192; Baral, 1997: 255).

White and HengstMengel predominantly worked with dead herbarium material. Essential with such material is to swell the elements in KOH or NH₄OH. Staining with CR noticeably increases contrast of croziers and supports the taxonomic validity of a taxon. The relative abundance of croziers, based on results published in Bresinsky et al. (1987: 311). Six species of *Lachnaceae* were measured in this study: two of them had approximately a double relative DNA-content (77–90, ploidy level 4×) compared to the other four (37–45, ploidy level 2×). Although these samples have never been studied for the ascus base, I knew from different collections that the first two species have croziers, whereas the latter four had simple septa. However, further examination of similar pairs of species by Weber (1992) could not confirm such a correlation.

The relative DNA-content of *H. menthae* was 57 (ploidy level 3×; Weiss, 1992: 122, as *H. consobrinus*). The method of measuring the DNA content requires fresh collections, and such were not available during this study for *H. macroguttatus* and other species treated here.

**Ascospore shape.** The two main character states of spores, homo- and heteropolar, are not sharply delimited in the genus *Hymenoscyphus*. In *H. macroguttatus* most spores are homopolar, i.e., it is impossible to determine the upper end of an ejected spore. A few spores in some collections were found to be very slightly scutuloid: the upper end is then quite well recognizable (Fig. 32a, right spore). In species with scutuloid spores nearly all spores feature a well-recognizable upper (rounded) and lower (pointed) ends. Due to the bilateral symmetry of scutuloid spores, their oblique apex and unilaterial flattening is visible only in side view and, therefore, seemingly absent in ca. 50% of the spores.

Homopolar spores are more primitive compared to heteropolar spores, and scutuloid (bilateral-symmetrical) spores represent a higher specialized type compared to the more simple clavate spore shape. It seems, therefore, imaginable that *H. menthae*, *H. repandum* and *H. macroguttatus* represent an ancient group compared to the core of *Hymenoscyphus* around the type species *H. fructigenus*. However, unpublished phylogenetic results indicate that this is only true for the former two species, whereas *H. macroguttatus* belongs near species with scutuloid spores. *Cyathicula coronata* (Bull.) P. Karst., on the other hand, has almost the same type of ascospores as *H. menthae* in regard to shape, size, and guttulation, and even the apical apparatus of the ascus is indistinguishable. It differs in an ectal excipulum of strongly gelatinized texture obliqua and in long marginal teeth. However, collections of what seems to be *C. coronata* were seen in which the marginal teeth were completely absent (e.g., H.B. 9971, Cataluña, L. Sánchez). Genetically, *C. coronata* is rather distant to *H. menthae*, though both cluster in the family *Helotiales* as currently circumscribed.

**Ascospore guttulation.** Spore guttulation is frequently neglected by workers, for two reasons: the guttules (oil drops = lipid bodies = LBs) are more or less invisible (masked) when mounting living or dead spores in MLZ or CB, or often also when mounting dead spores in H₂O; they are well visible when mounting living spores in water, or likewise when mounting dead spores in KOH or NH₄OH. When studying dead spores, however, the guttules show a very high variation in size and shape among the spores, if the lipid content is medium or high. This variation is caused by coalescence in some or most of the spores. Further variation is due to the developmental stage of the spores: immature spores have few or smaller oil drops while they again decrease by consumption during the first stages of germination. For a correct interpretation of guttules in dead material, such secondary changes, as well as immature and overmature stages of spore development, must be disregarded. These effects have misled workers into disregarding cell contents, despite their high taxonomic value in living material. Lipid patterns in spores probably indicate differences in adaption concerning the first phase of colonization.

The taxonomic value of the guttule or lipid pattern (size and arrangement of the oil drops in spores) can hardly be overestimated. Nevertheless, it is frequently neglected in descriptions due to its seemingly high variation within a single preparation, as we have shown elsewhere (Baral, 1992), this variation is the result of (1) living vs. dead spores, and (2) different developmental stages of the spores. When mature living material is mounted in water, highly constant lipid patterns are observed if the attention is focused on living spores, either within the turgescent mature asci, or when recently forcibly ejected. Secondary changes of lipid bodies (oil drops = LBs) such as coalescence (fusion, e.g., Fig. 9a1 – a2 or Fig. 59a1 – a2), or degradation after septum formation and during germination (Figs 10a, 11a2, 40a2, 58a2) can easily be recognized in fresh living
apothecia. In fact, such altered spore contents do not occur inside living ascii, but only inside ascii which have lost their turgor some hours or days ago, or when they were ejected prior to that time.

Lipid patterns were found to be highly consistent in virtually all of the freshly ejected spores of a preparation. This character allows a clear and rapid distinction between H. mentheae and H. macroguttatus, but only if fresh specimens are at hand. Similarity or related species that differ markedly in this way occur in many groups of ascomycetes. The species of such species contain a comparable amount of lipid but differ in size and number of single drops. For example, Aleuria cornubiensis (Berk. & Broome) Moravec (= Melas
tiza chateri W.G. Sm.) differs from A. aurantia (Pers.) Fuckel in multi
guttulate vs. biguttulate spores, whilst dead spores are biguttulate in either species (BARAL, 1992: figs 15–16). Likewise, Ascoscyore cy
lichnium (Tul.) Korf differs from A. sarcoides (Gray) J.W. Groves & D.E. Wilson with a similar consistency in the same way. This species pair is remarkable because its spores resemble those of H. mentheae and H. macroguttatus in all respect.

Multiguttulate spores easily turn oligoguttulate by coalescence (fusion) of the small LBs when treated with chemicals such as lacto
phenol, or when heating a slide. Those large drops characteristic of Aleuria aurantia, Ascoscyore sarcoides, or H. macroguttatus are not formed by fusion but, like the small drops of multiguttulate spores, increase in size during spore ontogeny. This means that, under na
tural condition, fusion of oil drops does not take place. Large oil drops in living mature spores develop from one privileged minute drop out of a few LBs in the immature spore, the other LBs remain
ning more or less small. During growth the LBs always keep their perfectly globose shape. In dead spores, on the contrary, the lipid content often forms elongate drops or aggregations, and often shows a variable and asymmetrical pattern (see BARAL, 1992: 357).

Spores that show the original lipid pattern of the living state can often be detected in old herbarium material, even in species with a high lipid content which shows a stronger tendency to fuse. Howev
ver, sometimes all spores show more or less distorted lipid contents, e.g., when mounted in KOH. This was the case, e.g., in the specimen illustrated on Fig. 41, in which I noticed in the fresh state that the spores had 2–4–6 large globose LBs, and where I failed to make a drawing at that time. In specimens that were carefully collected and preserved, the regular original guttule pattern is conserved in most of the spores, especially those inside the asc. This was found to be the case in all of the type and some other herbarium specimens stud
died here, even in those being older than 100 years (compare Figs 11–17, 38–40, 52–58, 60–61).

On the other hand, a distorted lipid pattern is frequently seen in fresh material as well. Often a small number of dead, mainly free spores are found on the hymenium. Uncritical workers often desc
scribe spores with variable contents of oil drops in a single collection and consequently consider spore guttulation as being of little taxo
nomic value. As an example, HENSTENGEN (1984: 114) described the spores of H. consobrinus “with 1–4 relatively large guttules and/or a certain number of small guttules, later granulose to non-granulose". Apart from the fact that the observed variation is not a true one, the asserted development from a few large drops to many small dro
plets is actually impossible, and lacks any evidence.

Mountants such as MLZ or CB which contain lethal ingredients, but also water in the case of dead spores, often mask the lipid contents of cells. This further explains why authors who describe spores frequently disregard internal guttules. Contradictory reports in the literature about spore guttulation are frequently the result of this masking effect. Reviewers of preserved collections often won
dered why they could not see any guttules inside the spores, al
though the descriptor of the fresh sample reported conspicuous oil drops. Even if both used water as mounting medium, they will arrive at contradictory results. In order to be sure about the presence of intracellular lipid in herbarium specimens, mounting in KOH or NaOH (ca. 1–5%), or NH₄OH is obligatory. These alkaline mountants considerably diminish refractivity of the cytoplasm but do not affect lipid contents.

The frequent presence of undistorted lipid in dead spores can be explained by the fact that spores of recently dried herbarium mate
rial are often still alive. This is easily seen when rehydrating the spores in water. Such rehydrated living spores are usually indistin
guishable from those of the fresh state. Desiccation-tolerance of spores is indeed common in ascomycetes. Spores survive in a dor
mant state: the cytoplasm is completely dehydrated and the spores collapse due to water loss (in thick-walled spores of mainly non-he
lotialean fungi, de Bary bubbles are formed instead). When spores are rehydrated after a period of time which they do not survive, and they still show their original guttule pattern, it can be concluded that they lost viability in the dry state during storage in the herba
rium. Coalescence of lipid bodies appears to require a hydrated cy
toplasm, therefore, no coalescence took place in the dry spores.

Irreversible distortion happens when the spores die in the hydrated state, either in the field during senescence of an apothecium, during prolonged storage in a moist box, when treating a water mount by chemicals or heat, or during drying by means of hot air. Therefore, the lipid pattern which we see in KOH-mounted herbarium material strongly depends upon the circumstances during the desiccation process.

Coalescence of LBs in hydrated cytoplasm can sometimes be ob
served in a water mount and is the first visible sign of injury to a li
v-ing cell. I have demonstrated in a video film at the IMC 4 (1990, Regensburg) that the application of CB or MLZ to living hydrated multiguttulate spores of a Pezicula induces complete coalescence of the LBs within a few seconds. Interestingly, no or only slight co
alescence of the LBs occurs when KOH is added to the living spores. KOH-provoked coalescence I have repeatedly noticed in herbarium specimens: in water the dead spores still showed a rather undistor
ced guttule pattern, while a certain degree of fusion of the oil drops happened when KOH was added. Furthermore, the LBs may become elongated by forces of the contracting cytoplasm. This is especially apparent when applying KOH to living asci of taxa in which the asci are multiguttulate when immature.

Hypothesis on the biological sense of guttule patterns: Lipid bodies in spores undoubtedly serve as a reserve substance that sup
plies energy and carbon during the first phase of germination (for literature see BARAL, 1992: 357). Drought tolerance of spores is not linked to their lipid content, since dry dormant spores do not show metabolism at all. This is obvious from the fact that the LBs are still present in full size in herbarium specimens, hence they were not in the least consumed during storage.

Since constant differences in guttule patterns between closely related species occur rather frequently in ascomycetes, the biological function of LBs in spores should be of some importance in regard to the colonization of substrate. The differences in spore guttulation can be represented by two parameters: (1) the absolute lipid content of the spores, and (2) the size of the oil drops. These two pa
rameters are supposed to function in the following way:

(1) A high lipid content seems to indicate that the nutritive condi
tions in the field during spore germination are frequently poor. The high amount of reserve substances accomplish optimal growth in the first phase of colonization. Species which are adapted to better conditions for germination refrain from storing high amounts of lipid in their spores. Because of the rather high consistency within a species, the two parameters are obviously genetically fixed.

(2) Many small lipid bodies are more rapidly consumed by en
zymes during germination than a few large LBs, since the total sur
face area of all LBs is distinctly higher in a multiguttulate spore. A species with a multiguttulate spore is advantaged in substrate co
lonization over one with a few large drops, for instance, during short periods of humidity.

The disadvantage of the multiguttulate pattern lies in the fact that in a spore of a given volume more lipid can be stored when a few large LBs are formed. The tendency to store as much lipid as possible
living asci in any species of and overmature spores. I have never detected septate spores inside however, his observations were mainly derived from herbarium material asci regularly contain 1- or 3-septate spores). Overmature spores were also seen to be aseptate when studied immediately and are sometimes found (compare, e.g. the rareness of brown spores in the genus by BARAL, 1992: fig. 1c). In his unpublished identification criteria, which underlines the taxonomic value of VBs.

Ascospore septation: One of HENGSTMENGEL’s (1984: 116; also in ARNOLDS et al., 1984: 313) characters of H. consobrinus which distinguishes it from H. scutula was the frequently seen 1-septate spores. However, his observations were mainly derived from herbarium material, in which it is nearly impossible to distinguish between mature and overmature spores. I have never detected septate spores inside living asci in any species of Hymenoscyphus s.l., and forcibly discharged spores were also seen to be aseptate when studied immediately after discharge (except for two unpublished species in which the living asci regularly contain 1- or 3-septate spores).

Ascospore pigmentation: Overmature spores were found to be colorless in some collections of H. menthae. As pointed out in GALAN & BARAL (1997), this is quite a common feature in Hymenoscyphus, though occurring inconsistently within a species. Such a delayed spore pigmentation is not uncommon in various groups of Helotiales and is, therefore, not useful for separating the genus Phaeohelotium as was done by some authors (the recent acceptance of the genus by BARAL et al. (2013) is based on other features). The amount of brown spores in a preparation depends mainly on the senescence of the apothecia. For some other reason, perhaps unfavourable field conditions, some populations produce many such brown spores when senescent, while in others none or only a few can be found (compare, e.g. the rareness of brown spores in H. fraxineus, BARAL & BEMMANN, 2014). In any case, brown spores have never been seen inside living asci of the genus Hymenoscyphus, therefore, freshly ejected spores are always hyaline.

Paraphysis guttulation (refractive VBs). All species in this study examined from living material contain very similar, more or less refractive guttules (vacular bodies, VBs) in the terminal cells of paraphyses (see BARAL, 1992: 363f). VBs usually cannot be seen in herbarium material, also they disappear instantly when mounted in lethal media such as KOH or MLZ, therefore, they are frequently absent in descriptions. Since some Hymenoscyphus species possess vacuoles of very low or even absent refractivity, VBs are of taxonomic interest and should be examined whenever fresh collections are available. Usually also the cortical cells of the ectal excipulum contain them, at least near the margin. Under vital staining with CRB, VBs yield a homogenously, finally deep turquoise stain which confirms their vacuolar nature, while in non-refractive vacuoles CRB precipitates to form a few small, globose, dark blue MCs (metachromatic bodies, BARAL, 1992: fig. 1c). In his unpublished identification key to the species of Hymenoscyphus recorded in Belgium, B. Declercq used ascospore shape, excipular cell shape, and VBs as entry criteria, which underlines the taxonomic value of VBs.

Apothecial colour. The yellow colour of the receptacle of H. menthae originates from minute carotenoid-containing LBs close to the septa in the cells of the subhymenium and lower part of paraphyses. This pigment fades away with the years during storage in the herbarium.

In contrast to lipid-bound pigments, the yellowish-cream to red-brown colour change of the originally white apothecia of H. macroguttatus and H. subferruginus is due to the presence of VBs in the paraphyses and cortical cells. When VBs disappear in dead cells of senescent material, they are replaced by a slightly refractive cytoplasm of very irregular structure which shows a secondary pigmentation due to an oxidation process. The red-brown macroscopic colour of senescent apothecia was considered by some authors to be characteristic of H. subferruginus, perhaps without being aware of the fresh apothecial colour which was whitish or pale yellowish in the present collections. Recognition of living cells at 400–1000× is necessary to avoid misinterpretations of apothecial colours. Red-brown hydrated apothecia like those of senescent H. subferruginus may look sound in external view, but under the microscope hardly any living cells can be found.

BOUDER (1909: 284, pl. 488) figured the upper instead of lower part of the paraphyses of H. menthae with a homogeneous yellow colour (Fig. 30), but described them as “remplies de granulations jaunes”. However, he illustrated these droplets smaller and not as densely packed as in the present illustrations (Figs 1b, 26b, 27a), therefore, their yellow colour might originate from a colour change due to oxidation of the distorted VBs.

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