Ombrophila hemiamyloidea (Leotiales), a new aquatic discomycete

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Summary: The new species is marginal in the genus *Ombrophila* (= *Neobulgaria*, *Leotiaceae*) on account of its hemiamyloid spore sheaths and apical rings, and its spores being septate when ejected, characters which are so far unknown in the genus. It shares some characters with the families *Mollisiaceae* and *Vibrisseaceae*. No anamorphic state was obtained in pure culture. A similar collection of an undetermined *Ombrophila*, and isotype material of *Zugazaea agyrioides* is compared with the new species. The new combination *Vibrissea catarhyta* replaces the later synonym *V. strossmayerioides*. *Graddonia coracina* is reported as possibly new to the American continent.

Zusammenfassung: Die neue Art ist charakterisiert durch hemiamyloide Apikalringe und Sporenhüllen, sowie durch Asci, welche mehrzellige Sporen abschießen. Diese Merkmale waren in *Ombrophila* (= *Neobulgaria*, *Leotiaceae*) bislang unbekannt. Die neue Art stimmt in einigen Merkmalen mit den *Mollisiaceae* und *Vibrisseaceae* überein. In Reinkultur wurde keine Anamorphe gebildet. Eine ähnliche Kollektion einer unbestimmten *Ombrophila*, sowie ein Isotypus von *Zugazaea agyrioides* werden verglichen. Die Neukombination *Vibrissea catarhyta* ersetzt das spätere Synonym *V. strossmayerioides*. *Graddonia coracina* wird als möglicherweise neu für den Amerikanischen Kontinent berichtet.

Introduction

Within the past ten years I have had the opportunity to study a strange aquatic leotialean discomycete, discovered at three remote sites of Central Europe, and sent to me in the fresh state by three different collectors. Although hardly to be overlooked in the field on account of its quite large size, white colour, and occurrence in great numbers, no report of this species was found in the literature. Obviously, it is a rare one. The exceptional combination of characters seems to indicate that it forms a link between the *Mollisiaceae*, *Vibrisseaceae*, and *Leotiaceae*.

Abbreviations: CR = congo red (in NH_4OH), CRB = cresyl blue (c. 0.5 % aqueous), KOH = potassium hydroxide (5%), IKI = Lugol's solution (1% I2, 3% KI), MLZ = Melzer's reagent; MEA = malt agar; LB = lipid body (lipid content in the spores: categories 0-5 = no - maximum lipid), VB = refractive vacuolar body; * = living state of a cell, † = dead state. The numbers of examined samples in which the reported character was tested and observed, are indicated between { }.

Herbaria: CUP = Plant Pathology Herbarium, Cornell University, Ithaca; FH = Farlow Herbarium, Harvard University, Cambridge; M = Botanische Staatssammlung, München. Private herbaria: A.G. = Andreas Gminder, E.R. = Ernst Rasch, H.B. = author's herbarium.

Ombrophila hemiamyloidea Baral & A. Gminder, sp. nov.

Apothecia sub aqua fluente formantia, subgregaria, raro fasciculata, 1-8 mm diam., subconvexa ad hemisphaerica, sessilia, pure alba, valde gelatinosa. Excipulum ectale e textura globulosa non gelatinosa, excipulum medullare e textura intricata hyphis tenuis valde gelatinosis, crystallis numerosis impleta. Asci in statu vivo 125-218 x 12-17 µm, octospori, apice conico, cum annulo ope IKI intense rubrescente (hemiamyloideo), ex uncis orti. Ascosporae in statu vivo 21-39 x 4.5-6 µm, cylindraceae, polis obtusis vel attenuatis, hyalinae, plerumque triseptatae, guttulis minutis paucis impletae, extus cum gelatina tenue, ope IKI purpurascente. Paraphysae rectae, apice leniter vel valde capitatae, vacuolis refringentibus impletae.

Habitat: ad ramulos decorticatos putrescentes Carpini, Fagi, Fraxini, in aqua non polluta rivulorum immersos, late autumno.

Holotypus: Germany: Baden-Württemberg: Heidelberg, Ziegelhausen, „Bärenbachtal", *Carpinus betulus* L., 22.XI.1997, J. Haedeke, H.B. 5985 (holotypus in M, isotypi in H.B.).

Fig. A: *Ombrophila hemiamyloidea*, fresh apothecia on natural substrate; 3:1 phot.: Baral

Apothecia (1-) 1.5-4 (-8) mm diam. {4}, scattered to gregarious, sometimes 2-5-fasciculate, superficial or very slightly erumpent; **hymenium** slightly convex to completely hemisphaerical, somewhat irregular (subcerebriform) with age, chalky or milky white with a very pale (bluish-) grey to cream tint, non-translucent; **margin** indistinct, not protruding, often ± lobate, exterior watery-white; **flesh** very strongly gelatinous (very difficult to cut), watery-translucent; sessile on a short and broad stipe (mostly hidden by the margin); total height c. 0.4-1.8 (-2.5) mm {3}; dry apothecia deeply sunken, cupulate with thick margins, cream-ochraceous.

Asci *(115-) 140-208 (-218) x 11.3-14 or finally 15-17 µm (full turgescence) {4}, KOH (93-) 110-140 (-177) x (10-) 11-12 (-13.5) µm {2}, cylindrical with a ± flexuous short stipe, arising from croziers {4}; pars sporifera *43-50 µm {1} or *(60-) 70-78 (-87) µm {1} long, 8-spored, spores obliquely biseriate; living mature asci protruding 15-30 (-50) µm beyond paraphyses {2}, dead asci retracted to the level of the paraphyses (-10 µm to +10 µm); apex strongly conical, with an apical ring staining (medium to) strongly redbrown (type RR) in IKI {5} (without KOH, negative in MLZ, deep blue in IKI or MLZ after KOH-treatment), rings in dead asci immature 3.5-4.5 (-5.5) µm, mature 1.5-3.5 µm high, 2-3 µm wide {2}, resembling those of *Bulgaria* or *Pezicula.*

Ascospores free *(21-) 22.5-33 (-39) x (4.5-) 4.8-5.5 (-6) µm {3}, KOH (18-) 20-30 (-33) x 4.5-6 µm {3}, cylindrical with obtuse to sometimes strongly tapered ends, straight or distinctly curved, hyaline, thin-walled, smooth, 3-septate within living mature asci {5}, rarely some 1-2-septate {3} or 4-5 (-6)-septate {1}, septa usually already present in submature asci, slightly constricted at septa (not so within living asci); lipid content low (category 1-2), with several minute LBs, 1 nucleus and 1-3 glycogen bodies in each cell; with a mostly thin sheath staining pale to strong purplish-red or pink in IKI (spores dead or alive, mature or ± aged, some also IKI-, especially in herbarium material), MLZ-, CRB-; overmature spores 3(-6)-septate, not increasing in size, hyaline, finally without lipid bodies, constrictions stronger, sometimes disarticulating at middle septum, rarely or very abundantly germinating in senescent apothecia, normal germ tubes formed at each end (rarely from middle cells), no conidia produced {3}, anastomoses sometimes observed between germ tubes emerging from different cells of a single spore.

Paraphyses gradually to abruptly inflated at apex, ± moniliform {1} or mostly capitate {2}, terminal cell *(18-) 30-70 (-93) {2} x (4.5-) 6-8.5 (-10) µm {3}, lower cells *15-50 {1} x 2-4.8 (-6.3) µm {2}, towards base ± infrequently branching and anastomosing, total wall without {2} or with {1} a pale yellowish exudate; living paraphyses with 1-2 (-4) strongly refractive vacuolar bodies (VBs) in terminal cell, partly also in lower cells, total length of the VB-containing part (25-) 36-80 (-91) µm {2}, VBs containing groups of small transparent guttules, IKI-, KOH reaction (added to water mount) deep sulphur-yellow (sap exuded in the medium) {1}, or only slightly so (to nearly negative) {2}.

Ectal excipulum entirely hyaline or with a light brown tint near base, from base to margin of a thin-walled textura globulosa 40-100 µm thick {3}, very sharply delimited from medullary excipulum, cell rows oriented perpendicular to the surface, individual cells perfectly globose (or ovoid to pyriform), *(8-) 15-27 (-35) x (6-) 10-20 (-33) µm {3}, cortical cells *9-20 {1} x (6-) 8-15 µm {3}, those of uppermost margin (or including those of mid flanks) each ± completely filled by a refractive VB, here gradually passing over into the paraphyses.

Medullary excipulum c. 200-1500 µm thick, of a widely spaced hyaline textura intricata with c. 3-15 µm wide intercellular spaces filled by non-refractive gel (invisible in water), hyphae *1-3 (-4.5) µm wide {3}, near excipulum oriented ± perpendicular to surface, towards subhymenium and centre more dense and with parallel upward orientation; no perihymenial textura porrecta, no amyloid reactions; crystals (presumably of Calcium oxalate) very abundant {3}, especially in centre, mostly as small rhomboid crystals of c. (0.5-) 3-7 (-10-35) µm diam., densely covering some of the hyphae, also forming large druses (ectal excipulum, subhymenium, and hymenium with only very few crystals, possibly translocated during preparation {2}, but in one collection abundant between the paraphyses, fig. 14); gel of extremely mucilaginous consistency, extruding upon pressure on the cut apothecia as a slimy mass, filling the whole intercellular space, staining deep violet in CRB; also hymenium and ectal excipulum covered by a thin gel coat, invisible in water but staining pale to deep violet in CRB.

Subhymenium c. 40 µm thick, hyaline, of a more dense, less gelatinized textura intricata, composed of thin upwards oriented paraphysogenous hyphae and wider, irregularly oriented ascogenous hyphae, among these (in KOH) scattered bizarre cells with firm (0.3-0.6 µm thick) refractive walls (fig. 5) {1}.

Substratal hyphae sparse at base, hyaline {1} or pale brownish {1}, 2-2.5 µm wide, walls 0.2 µm thick, smooth, forming a loose, horizontal, c. 10-20 µm thick layer, without gel, CRB-, IKI-.

Ecology: acidophilous ?Alno-Fraxinetum with *Prunus avium* L., *Rubus fruticosus* agg. {Bärenbach}, acidophilous *Picea* forest {Walim}, narrow creek with acidophilous *Fagus-Picea* wood on steep slopes {Karkanosze}, calcareous *Quercus* forest {Osterholz}, in small fastly flowing rivulets {4} (width c. 0.5-1 m, water nearly unpolluted), completely submersed {6} or lying on very wet ground {1}, on decorticated {6} branches 5-10 mm {2}, 10-15 mm {1}, 15-28 mm {6}, or -150 mm thick {1}, of *Carpinus betulus* {3}, *Fagus sylvatica* L. {4}, *Fraxinus excelsior* L. {2}, surface layer (-0.5 mm thick) (little to) medium (to strongly) decayed (containing brown hyphae), mostly strongly eroded by the water (with many deep longitudinal furrows), inner parts still very hard, rarely moderately rotted, or perforated by old beetle galleries, Oct.-Dec. {6}, 145-700 m a.s.l. Associated fungi: *Ascocoryne sarcoides* (Jacq.) Groves & Wilson {1}, *A. solitaria* (Rehm) Dennis {1, anamorph}, *Hymenoscyphus ombrophilaeformis* Svrček {1}, but often without other fruiting fungi; on separate, adjacent branches (A. GMINDER, in litt.) *Mollisia ventosa* P. Karst., *M. uda* (Pers.) Gill., *Vibrissea decolorans* (Sautt.) Sánchez & Korf, *Scutellinia* sp., more remotely *Graddonia coracina* (Bres.) Dennis, *Pachyella babingtonii* (Berk.) Boud.

Drought tolerance: Asci died rapidly during drying (examined after c. 15 h exposure of branch fragments to air at room temperature); c. 50% of ascospores were still alive after 3 $^1/_2$ months in the herbarium, but all were dead after 10 months.

Fig. B: Collection site „Bärenbachtal" phot.: Haedeke

Cultural characters: (H.B. 5985a) Ascospores germinated very rapidly on MEA at 18°C. 18h after being shot on agar, germ tubes were already 80-130 µm long. 4 and 7 weeks after inoculation of new petri dishes the cultures had a diameter of 27-37 mm and 60 mm respectively. The central part of the culture was light greyish-brown and slightly zonate, the external region hyaline. Aerial mycelium was abundant, white, strongly pubescent, of hyaline, thin-walled, straight hyphae *2-3 (-4) µm wide (few cells inflated to *5-7 µm), frequently forming strands, lipid content low to high; towards the agar the hyphae formed a dense, irregular, intricate texture of mostly shorter, inflated cells *3-7 µm diam. with a high lipid content (LBs 1-5 µm diam.); the submersed mycelium was a loose intricate texture of *2-3.5 µm wide hyphae, with a low lipid content. Conidiophores and conidia were never observed. (A *Coryne*-like deuteromycete habitually resembling the apothecia of *O. hemiamyloidea* was observed at the Osterholz locality: 8.II.1989, E. Rasch, H.B. 3676a).

Specimens studied:

Germany: Baden-Württemberg: Schwäbische Alb, Bopfingen, „Osterholz", MTB 7128/1, 490 m, jura-malm, *Carpinus*, 23.X.1988, E. Rasch, H.B. 3601, E. R. 1907. - Heidelberg, Ziegelhausen, „Bärenbachtal", MTB 6518/4, 145 m, middle red sandstone, *Carpinus* and *Fraxinus*, 22.XI.1997, J. Haedeke, H.B. 5985a (*Carpinus*, holotype in M, isotypes in H.B.), H.B. 5985b (*Fraxinus*). - dto., 5.XII.1997, H.B. 5994 (*Fagus*), H.B. 5995a (*Fraxinus*), H.B. 5995b (*Carpinus*).

Poland: Silesia: Wałbrzych, c. 1-2 km E of Walim, 600 m, ?granite, *Fagus*, 10.X.1991, A. Gminder, H.B. 4523, A.G. 91/266, CUP 63529. - Karkanosze, Szklarska Poreba, 700 m, ?granite, *Fagus*, 21.X.1993, A. Gminder, A.G. 93/376, CUP 63528. - dto., 10.X.1996, *Fagus*, A.G. 96/359.

Remarks on morphology and chemistry

Cell size and shrinkage: Ascus and spore length varied among the collections. In H.B. 3601 the asci were *125-160 µm long, while they measured in the two other collections *155-208 (-218) µm. In H.B. 5985 the spores were *(21) 26-35 (-39) µm long, while they measured in the two others *22-27 (-33) µm. Linear shrinkage of asci is c. 19-20% in both length and width, i.e. an ascus of *218 x 15 µm measured only 177 x 12 µm when killed by MLZ (without spore release). Linear shrinkage of ascospores is approximately 4-8%.

Lipid bodies: Submature 1-3-septate spores have a somewhat higher lipid content with distinctly larger LBs (fig. 8, 1-septate spore). Spores in fully mature asci contained nearly always only minute LBs.

Vacuolar bodies: The VBs in the terminal cells of paraphyses and ectal excipulum stain bright turquoise in CRB. In aged but still alive excipular cells this substance may dehydrate to form even more refractive drops (fig. 12, lower cell) which likewise stain deep turquoise in CRB.

Iodine reaction: The iodine reagent was applied to the edge of the cover glass. Thereby, the pinkish-red IKI-reaction of the ascospore sheath is clearly evident already at less than c. 0.1% I_2. When more iodine has diffused inwards, the reaction of the sheath is masked while that of the apical ring becomes apparent. The MLZ reaction is negative for both structures if the fungal fragment is directly mounted in MLZ. When MLZ is applied to the edge of a water mount, however, a strong red reaction of the apical ring is obtained for some minutes since the iodine diffuses much faster than the chloral hydrate which finally completely suppresses the reaction (hemiamyloidity, cfr. BARAL 1987).

After 5% KOH-treatment (shortly boiled, or 3-5 min unheated) the rings stain deep blue in IKI or MLZ. The spore sheaths are then IKI-, or stain very pale greyish-lilac in overmature spores. When pretreated by 2% KOH for $^1/_2$ min unheated, the sheaths are still IKI pink (but the rings already IKI blue). The red IKI reactions of both structures were still fully present after storage for 9 years in the herbarium.

Although the red IKI reaction of the sheaths is not clearly changed to a clear blue by the influence of alcali, I tend to classify this reaction also as hemiamyloid. Similar reactions of ascospore sheaths are known from ***Vibrissea catarhyta* (Kirschst.) Baral comb. nov.** [Basionym: *Godronia catarhyta* Kirschstein, Hedwigia 80: 130 (1941)] (= *Vibrissea strossmayerioides* Korf & Iturriaga), *Loramyces macrospora* Ingold & Chapman („*Loramycetaceae*"), and *Obtectodiscus aquaticus* E. Müller, Petrini & Samuels („*Dermateaceae*"). In *L. macrospora*, a strong blue reaction could be induced after strong (heated) KOH-treatment (BARAL, 1987: 423).

Ecological remarks

Ecologically, *Ombrophila hemiamyloidea* closely resembles other submersed-growing lignicolous Leotiales like *Vibrissea* Fr., *Graddonia coracina*, or *Mollisia uda*. It may even be confused with them by habit at first glance. However, while the mentioned taxa produce their apothecia predominantly in spring and summer, *O. hemiamyloidea* so far is only found in late autumn (Tab. 1). *O. hemiamyloidea* seems to prefer colonizing hardwoods, mainly from trees which avoid a permanently high ground water level. It is therefore not surprizing that this fungus occurred at the Karkanosze site abundantly in a narrow creek where the steep slopes are covered by *Fagus*, *Picea*, and some *Betula*. Due to the narrowness of the valley, old branches of *Fagus* tumble down into the rivulet. About 100 m upwards the valley becomes more flattened. Here *Alnus* dominates and (more on the slopes) *Acer pseudoplatanus* L.; *Vibrissea decolorans*, *V. flavovirens* (Pers.) Korf & Dixon, *V. truncorum* (Alb. & Schwein.) Fr., and *Mollisia ventosa* were recorded, but no *O. hemiamyloidea* was found (A. GMINDER, in litt.). As the inhabited branches have the appearance of being submersed for several years (and have perhaps lost their bark already during several years decay in the crown of the trees), softwood might be unsuitable for the life style of this discomycete, perhaps on account of a too rapid decay by concurrant fungi.

Spore discharge was not observed in water mounts although hundreds of fully turgescent asci were present. The high amount of vacuolar water and the strongly amyloid apical rings indicate, however, that the asci are able to forcibly eject their spores. Indeed, spore discharge readily occurred when an apothecium was placed in a petri dish in order to obtain a pure culture. This seems to indicate that, in the field, the asci eject their spores mainly into the air as soon as the apothecia are, during a drier period, no longer submersed by the stream. The observed drought-tolerance of the spores supports this view. Air dispersal of the spores of aquatic fungi growing in running water seems reasonable since (1) active spore discharge below water level is ineffective, and (2) dispersal would exclusively be possible in the downward direction of the rivulet.

Therefore, I also doubt KORF's (1990: 23) belief that the *Vibrisseaceae* are adapted in „usually discharging their filiform ascospores under water". Likewise, INGOLD (1954: 17) appears to have only assumed the ability of spore release under water concerning discomycetes on substrata submerged in lakes. Actually, SÁNCHEZ & KORF (1966: 727) stated that „how spores are discharged under water seems to be unknown". As in *O. hemiamyloidea*, the ejection of the filiform spores of *Vibrissea* was not observed in water mounts by me, but is easily stimulated by the influence of dry air as soon as a box with the fungi is opened under the dissecting microscope.

Taxonomic relationship within the Leotiales

The textura globulosa, the yellow KOH-reaction (see BARAL 1992: 373) of the elongate VBs in the paraphyses, and the minute transparent guttules within the VBs are very typical of *Mollisia* (Fr.) P. Karst., a genus currently placed in the *Dermateaceae*. Within this genus, the species seems to have affinities with what NANNFELDT (1986: 196) segregated (unjustified in my opinion) as *Belonopsis* (Sacc.) Rehm on account of the abundant crystals of „Calcium-oxalate-hydrate" in the medullary excipulum. With the very close genus *Niptera* Fr. (including *Nimbomollisia* Nannf.; see BARAL 1994) it shares two further features: (1) the broad and septate spores, and (2) the IKI-red gel around the ascospores. However, no crystals occur in *Niptera*.

Very unexpected in the *Mollisia-Pyrenopeziza*-complex, however, *O. hemiamyloidea* has (1) a strong gelatinization of the medulla, and (2) a hyaline ectal excipulum. This character combination, together with the occurrence of crystals on the medullary hyphae, is well-known in many species of *Ombrophila* Fr. (including *Neobulgaria* Petrak, *Leotiaceae*). Here the excipular cells are also often large but mostly prismatic and oriented at a low angle to the surface, and the ascospores are non-septate within the living asci, with a thin sheath unstained in IKI but often violet in CRB. The close genera *Ascocoryne* Groves & Wilson and *Ascotremella* Seaver are also remarkably similar, especially in their vertical orientation of the excipular cells. However, in all these highly gelatinous fungi VBs are either lacking or without a yellow KOH-reaction, and both hemiamyloid apical rings and spore sheaths are unknown. Furthermore, many species produce phialoconidia immediately when the spores germinate in senescent apothecia.

Apart from the aquatic habitat, several morphological similarities with the *Vibrisseaceae* must be mentioned. Especially the paraphyses resemble those of *Vibrissea*, being very long, and containing in their terminal inflated cells long VBs which, in some species, show the yellow KOH-reaction. In one species (*Vibrissea catarhyta*) the spores have a sheath reacting bright violaceous in IKI, and the subhymenium contains similar bizarre cells with thick refractive walls (BARAL, ined.). *Vibrisseaceae* differ, however, (1) in the medullary excipulum being non-gelatinized, and (2) in a thin perihymenial textura porrecta reacting blue in iodine (euamyloid). No hemiamyloid rings are known, and several species have a distinct apical „nasse" (especially when the ascus apex is inamyloid). Most species of *Vibrissea* have a brown-walled excipulum, and crystals were never found.

However, in a recent European collection of *Leucovibrissea obconica* (Kanouse) Korf, the only known species of the genus *Leucovibrissea* (Sánchez) Korf, numerous rhomboid crystals and druses were found in the medullary excipulum and on the hyaline ectal excipulum (BARAL, ined.). This genus differs from *O. hemiamyloidea* in the amyloid perihymenial texture, and in the very long and narrow asci and spores of the *Vibrissea*-type. *Leucovibrissea* further differs from both *O. hemiamyloidea* and *Vibrissea* in the ectal excipulum on the middle flanks and margin being of rectangular cells with thick refractive walls, their longitudinal axis at a low angle to the outer surface of the receptacle (SÁNCHEZ & KORF 1966; KORF 1990; BARAL, ined.).

Vibrissea was traditionally placed in the *Geoglossaceae*, or even the Ostropales (KORF 1990). KORF (1990) erected the family *Vibrisseaceae* (Leotiales) because he saw no affinities with either group, nor with the *Dermateaceae*. I believe, however, that the affinities between the *Vibrisseaceae* and the „*Mollisia*/*Pyrenopeziza*-complex" (here referred to the *Mollisiaceae*) are much closer than between the latter and *Dermea* Fr., the type genus of *Dermateaceae*. *O. hemiamyloidea* complicates this view since it seems to form a transition between *Vibrisseaceae/Mollisiaceae* and *Leotiaceae*. For a long time I therefore believed *O. hemiamyloidea* to belong to a separate, undescribed genus with ambiguous affinities.

Comparison with an undetermined *Ombrophila* (figs. 19-26)

Recently, Christian Scheuer drew my attention to an undetermined collection of *Ombrophila* which shows striking similarities with *O. hemiamyloidea*. This collection convinced me that *O. hemiamyloidea* is a marginal species of *Ombrophila*.

The collection differs by smaller, euamyloid apical rings, somewhat smaller asci (KOH 90-100 x 7-9.5 µm) and ascospores (KOH 11-30 x 4-6 (-7) µm) with 0-3 septa. The rehydrated apothecia are mostly stipitate and have a flat or only very slightly convex hymenium. The variability in spore data is due to different stages of ascus development: as I studied this species only in the dead state, the question remains completely open whether or not the living asci eject septate spores as in *O. hemiamyloidea*, or aseptate spores as in typical species of *Ombrophila*. The spore wall does not react with IKI.

While the medullary excipulum is exactly that of *O. hemiamyloidea*, the ectal excipulum exhibits clear differences: (1) the inner ectal excipulum is of a short-celled textura prismatica oriented parallel to the surface; (2) an outer gel layer is present which becomes very thick at the base of the apothecium (figs. 20-22).

Specimen studied:

Austria: Tirol: Ötztaler Alpen, Untergurgl, „Sonnbergalm", lowest E-exposed slopes, W above „Dreihäusern", 1800 m, on decorticated branch of *Alnus viridis* (Chaix) DC. lying in a small rivulet (at least exposed to spray water), 24.VIII.1991, Ch. Scheuer, A. Nograsek & W. Pongratz (GZU [C.S. 2722], H.B. 6088).

Comparison with further similar species

In his thesis on some operculate aquatic discomycetes, PFISTER (1971: 14, 17) mentioned the existence of an aquatic „*Pezicula*". As this genus is characterized by hemiamyloid apical rings and large, finally septate ascospores, I requested material. Yet, in no case an identity with *O. hemiamyloidea* could be ascertained:

From FH I received two collections named „*Pezicula* sp.". These were found to belong to *Hymenoscyphus imberbis* (Bull.) Dennis (asci with croziers, FH, 112) and *H.* aff. *vernus* (Boud.) Dennis (asci without croziers, FH, 159):

USA: Minnesota: Lake Itasca, in swampy spot, twigs of *Alnus* (as „?*Acer*"), 3.VIII.1980, D.H. Pfister, C.K. Pfister & E.L. Pfister (112); dto., twigs of a ring-pored tree, D.H. Pfister (159). (In both the base of the apothecium reacts blue in IKI.)

From CUP and FH I received three samples labelled „*Pezicula aquatica* sp. nov. pro tem.". This represents *Graddonia coracina*, a species quite frequently collected in Central Europe (GMINDER 1993):

USA: Maine: Penolascot, La Grange, Birch stream, on decorticated log of deciduous tree submersed in a stream, 9.V.1971, R.L. Homola (CUP 52292, Homola 4161); Vermont: West Brattleboro, Ames Hill, on decorticated log of deciduous tree in a stream, 25.VIII.1983, H. Pofcher, D.H. Pfister & C. Pfister (FH) (spores KOH (13-) 17-20 (-22) x 7-10 µm.

France: Corsica: 32 km E of Ajaccio, Zipitoli, woods below Maison Forestière, 680 m, on wood of ?*Fagus* under water, 8.X.1972, R.P. Korf (CUP, R.P.K. 72-11): (spores KOH 11-15 x 6-7 (-8) µm) mm wide.

ITURRIAGA et al. (1998) described a new genus and species of uncertain affinities within the Helotiales, *Zugazaea agyrioides* Korf, Iturriaga & Lizoň. The habitus suggested a member of the *Peziculoideae*. It occurs on water-soaked rotted wood of ?*Eucalyptus* in Macaronesia. The detailed description shows some resemblance with *Ombrophila hemiamyloidea*. Reexamination of an isotype specimen (CUP-MM 2844), however, revealed that this species is clearly not an *Ombrophila*. The apothecia are described as deep dull yellowish-orange when fresh, and partly grow on the hymenium of post-mature apothecia of the same population. They are only slightly gelatinized, and therefore easy to section. A golden-yellow, KOH-soluble, resinous exudate occurs abundantly, especially in the ectal excipulum. The latter is formed of roundish, vertically oriented cells agglutinated by gel. Rhomboid crystals are absent. The asci are inamyloid (in IKI), the ascospores 0-3-septate, inamyloid, with large LBs (rather high lipid content).

On a recent separation of Leotiales ss. str. and Helotiales nom. cons. prop.

LIZOŇ et al. (1998) restricted the order Leotiales to four genera (*Geocoryne* Korf, *Leotia* Pers., *Neobulgaria* Petrak, and *Pezoloma* Clem.) which they consider to be „apparently far distinct phylogenetically" from the rest of the genera (including *Ombrophila*) now separated as „Helotiales nom. cons. prop.". The authors based the two orders mainly on a single distinctive character, the presence versus absence of an outer ectal excipulum of narrow, intricate hyphae immersed in gel. The gelatinized layer covers the complete exterior, or is only present near the apothecial base. The apical apparatus of *Leotia* is mentioned as a further character.

To select the external gel layer as single key character on ordinal level seems to me an arbitrary act. I cannot understand for what reason the authors did not instead select e.g. the gelatinization of the medullary excipulum (which in their concepts may be present or absent in both orders) as key character. Besides, the absence of a character, whether plesiomorphic or apomorphic, does not convincingly support a division into two natural groups.

Thick outer gel layers occur also in some typical species of *Ombrophila*, viz. *O. janthina* P. Karst. and „*Cudoniella*" *rubicunda* (Rehm) Dennis (BARAL, ined.), for which the new concept would necessitate transfer to *Neobulgaria*, and in some undetermined species of *Ombrophila* (including Scheuer´s collection), furthermore in *Discinella boudieri* (Quél.) Boud., the type species of *Discinella* Boud. (the latter has an apical apparatus reminiscent of *Leotia*). If I would follow LIZOŇ & al. (1998) I had to place Scheuer´s *Ombrophila* in the Leotiales, and *O. hemiamyloidea* in the Helotiales.

Pezoloma iodopedis Lizoň & al. was placed by LIZOŇ et al. (1998) in the Leotiales because of an (amyloid) gel layer restricted to the base of the stipe. The description fully recalls *Hymenoscyphus imberbis* which has typically such an amyloid base (BARAL, ined.), and which the authors did not mention when describing their new taxon.

It would be appreciated to do molecular work on this group in order to settle the contradictory opinions.

Tab. 1: Character matrix of studied genera showing affinities with *Ombrophila hemiamyloidea*

	Leotiaceae				*Mollisiaceae*			*Vibrisseacea*	
	Ascocoryne	*Ascotremella*	*Ombrophila*	*O. hemiamyl.*	*Mollisia*	*Niptera*	*Graddonia*	*Leucovibr.*	*Vibrissea*
Asci									
apex (IKI)	bb	bb	**bb**/–	rr	**bb**/rb/rr/–	bb/–	–	bb	bb/–
ring type	T	i	T/i	T	T	T	–	i	i
croziers	**+**/–	–	+	+	**+**/–	+	+	+	+
Ascospore									
sheath (IKI)	–	–	–	rr	–	rr/–	–	–	rr/–
septation	0→1-5	0	**0**→1-3	1-**3**-5	**0**-10	1-3	0→1	?15	3-23
l/w-ratio	3.2-6.2	1.8-2.5	1.8-9	4.2-6.5	2.4-16	2.7-6.5	1.8-2.6	≈120-180	14-210
Paraphyses									
VBs	–/mg	–	cy	cy	cy	cy/–	mg	?	cy
yellow KOH-react.	–	–	–	+/–	+/–	+/–	–	–	+/–
Ectal excip. (flanks)									
textura	glo	pri	**pri**/glo	glo	**glo**/pri	glo	glo	pri/glo	glo
orientation	vt	vt	**hz**/vt	vt	**vt**/hz	vt	vt	vt/hz	vt
brown pigment	–	–	**–**/+	–	**+**/–	+	+	–	**+**/–
external gel	–	–	+/–	–	–	–	–	–	–
Medullary excip.									
gel	+	+	**+**/–	+	–	–	–	–	–
crystals	–	–	**+**/–	+	**–**/+	–	–	+	–
IKI (perihym.)	–	–	–	–	–	–	–	bb	bb
Subhymenium									
refr. walls	–	–	–	+	–	–	–	–	**–**/+
Habitat	li	li	li/he	li	li/he	he	li	li/he	**li**/he
aquatic	**–**/+	–	+/–	+	**–**/+	+	+	+	**+**/–
Phenology*	VIII-I	VIII-XII	IV-I	X-XI	I-XII	V-IX	IV-VII	VI	IV-IX
Studied spp.	3	1	»15	1	»50	5	1	1	»7

*) in Central Europe

Explanation: **bb** = euamyloid (blue), **rr** = hemiamyloid (red), **rb** = intermediate (red at high, blue at low iodine concentration); **T** = T-shaped, **i** = only lower ring reactive; **cy** = large, long-cylindrical, refractive vacuoles, **mg** = multiguttulate (numerous globose vacuolar bodies) (requires living paraphyses!); **glo** = isodiametric cells (globose or angular), **pri** = elongated cells (prismatic); **vt** = orientation vertical to excipular surface (high angle), **hz** = horizontal (low angle); **li** = lignicolous (including corticolous), **he** = herbicolous (including monocotyledons and leaves). Prevailing states are given in bold-face.

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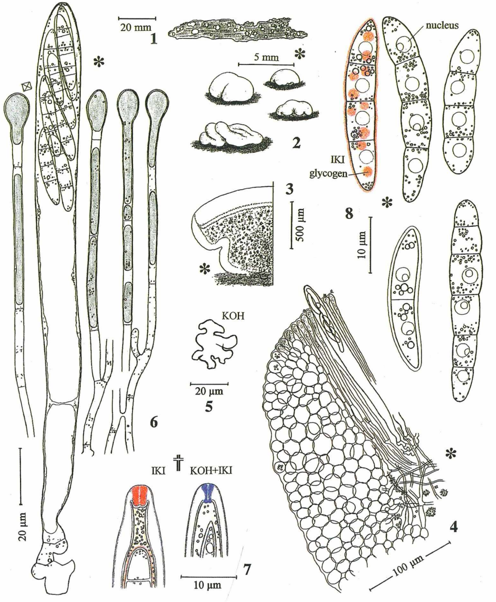
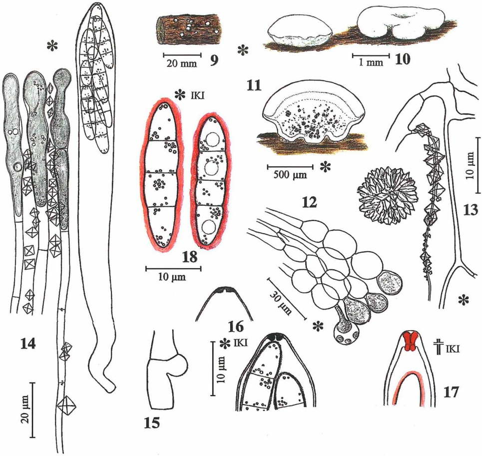
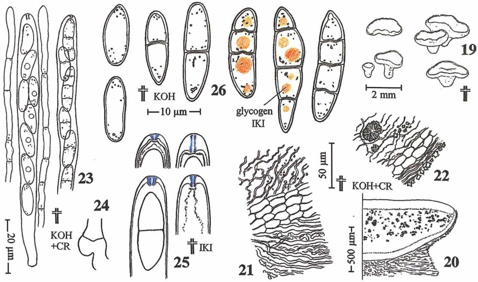


Figs. 1-18: *Ombrophila hemiamyloidea*, from living material. **Figs. 1-8:** H.B. 5985a (holotype). **Fig. 1:** apothecia on natural substrate; **fig. 2:** dto.; **fig. 3:** radial section of apothecium showing crystals in the medullary excipulum; **fig. 4:** ectal excipulum in radial section, upper most cortical cells containing refractive vacuoles (VBs), marginal region; **fig. 5:** bizarre cell in subhymenium; **fig. 6:** asci and paraphyses, the latter containing large refractive vacuoles (VBs); **fig. 7:** apices of mature asci showing hemiamyloid apical rings (red in IKI, blue in IKI after KOH-treatment); **fig. 8:** free ascospores, 3(-5)-septate, each cell containing one central nucleus, 2-3 glycogen bodies (redbrown in IKI), and small lipid bodies near the septa, exterior with a thin mucilaginous sheath staining pinkish-red in IKI; one immature spore (1-septate) with larger LBs.



Figs. 1-18: *Ombrophila hemiamyloidea*, from living material. **Figs. 9-18:** H.B. 3601. **Fig. 9:** apothecia on natural substrate; **fig. 10:** dto.; **fig. 11:** radial section of apothecium showing crystals in the medullary excipulum; **fig. 12:** ectal excipulum in radial section, cortical cells containing refractive vacuoles (VBs), middle flanks; **fig. 13:** medullary hyphae embedded in gel, with rhomboid crystals and druse; **fig. 14:** asci and paraphyses, the latter containing large refractive vacuoles (VBs); **figs. 16, 17:** apices of mature asci showing hemiamyloid apical rings (red in IKI, blue in IKI after KOH-treatment); note difference in height of the rings between living (fig. 16) and dead asci (figs. 7, 17); **fig. 18:** free ascospores.



Figs. 19-26: *Ombrophila* sp. from dead herbarium material (CS 2722 = HB 6088). **Fig. 19:** rehydrated apothecia (Ch. Scheuer: dirty white to pale yellowish when fresh); **fig. 20:** radial section of apothecium showing crystals in the gelatinized medullary excipulum; **fig. 21, 22:** radial section of ectal and medullary excipulum on lower (21) and middle flanks (22), outer ectal excipulum of narrow hyphae immersed in gel; **fig. 23:** asci and paraphyses; **fig. 24:** crozier at ascus base; **fig. 25:** apices of asci showing euamyloid apical rings; **fig 26:** ascospores with small LBs and 1-2 glycogen bodies in each cell.

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