

## Three new hypocrealean ascomycetes on bryophytes

Peter Döbbeler

Ludwig-Maximilians-Universität München, Fakultät für Biologie,  
Systematische Botanik und Mykologie, Menzinger Straße 67, D-80638 München,  
Germany

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The following bryophilous species of the order Hypocreales (Ascomycetes) are described as new: *Bryonectria tricellularis* grows biotrophically on *Frullania dilatata*. It is apparently rare and only recorded from the type collection in Romania. *Nectria hyperepiphylla* is a biotrophic parasite on an epiphyllous hepatic in New Caledonia. Apart from ascoma anatomy the species is characterized by elaborate infecting structures consisting of appressoria and intracellular haustoria. The necrotrophic *Nectria voratella* causes discolourations on different mosses in Italy and France. The species are illustrated in detail in order to facilitate their recognition and identification.

Keywords: bryophilous fungi, hepaticolous ascomycetes, Hypocreales, Nectriaceae, *Bryonectria*, *Nectria*.

During ongoing studies of bryophilous ascomycetes several undescribed hypocrealean species were detected. Three of them are presented here as new. They are quite heterogeneous regarding their anatomical and ecological features and geographical occurrence. Two of them are assigned to the genus *Nectria* Fr. Reasons for conceiving *Nectria* in the traditional broad sense are given by Döbbeler (2005). An alternative placement probably would require the creation of two new genera. At the moment, this would not be a step forward, as most of the nectriaceous ascomycetes on mosses and hepatics are rarely collected, poorly known and many additional species will be discovered. A more reasonable disposition must await further studies.

### Material and Methods

Apart from ascoma size and shape all measurements and illustrations were done in lactophenol cottonblue. *Bryonectria tricellularis* is found only on the ventral side of *Frullania dilatata*; ascomata are not easily observed unless potentially infected shoots are treated with a diluted solution of lactophenol cotton blue. Perithecia

and pycnidia are selectively coloured and easy to distinguish from host structures by microscopic magnification (100 fold). Reactions with KOH or iodine (Lugol's solution) have not been observed. Measurements are provided as observed spore size ranges, extreme values are given in brackets. Drawings were made with a camera lucida.

### Taxonomy

#### *Bryonectria tricellularis* Döbbeler sp. nov. Figs. 1–8

Species *Bryonectriae racomitrii*, crescentium ad folia *Racomitrii lanuginosi*, similis sed ab ea praecipue ascosporis longioribus (18.5–23 µm) et appressoriis minoribus (5–8 µm diametentibus) et hospite alio differt. Habitat parasitice in foliis hepaticae *Frullania dilatata*. Perithecia semper in latere ventrali plantarum evoluta.

Holotypus: ROMANIA, „Centralcarpathen, an den Stämmen der Nadelbäume“ (without further details on the voucher label), Kalchbrenner (Rabenhorst, Hep. Eur. 156, sub *Frullania dilatata*). (HBG; isotype M; no ascomata seen on a specimen from JE).

Perithecia (110) 130–170 × (80) 90–120 (135) µm, ovoidal to conical or mostly pyriform, colourless, without setae, surface sometimes uneven by shortly protruding groups of cells. Ostiole inconspicuous, ostiolar channel lined with periphyses. Excipulum in surface view with extremely thick-walled hyphae, only irregular, ramifying and anastomosing reduced lumina recognizable; lateral wall 13–25 µm thick. Paraphyses absent. Asci (55) 60–68 × 9–11 µm, cylindrical with slightly enlarged central part, straight or a little curved, the apex broadly rounded and without internal structures, foot short and thick, 4-spored, no degenerating ascospores seen; empty asci up to 85 µm long. Ascospores (17.5) 18.5–23 (25) × (4) 4.5–5 (6) µm, narrowly ellipsoid to cylindrical, straight or slightly curved, often longitudinally slightly asymmetrical, 3-celled, not constricted at the septa, colourless, epispore slightly rough and cyanophilic; several times 4 discharged ascospores lying side by side on the leaf surface observed; ascospores germinating with appressoria. Hyphae (2) 3–5 (6) µm wide, colourless, of varying diameter and wall-thickness, thick-walled, branched and anastomosing, with appressoria, growing superficially over the host cells, preferring the anticlinal cell walls. Appressoria 5–8 (10) µm diam, laterally arising from the hyphae, sessile, elliptical or slightly sinuate in outline, preferably formed over the junctions of the anticlinal cell walls. Anamorph comprising hemispherical aggregations of hyphal cells leaving a small hollow space between themselves and the leaf surface, seen from above 35–55 µm diam, roundish or slightly irregular, with roundish or angular cells, 2.5–6 µm diam, laterally seen about 17–35 µm high, outer hyphal

cells thick-walled, inner side of the dome-cells covered with up to 10  $\mu\text{m}$  long and 2–3.5  $\mu\text{m}$  wide, bottle-shaped phialides; conidia up to 4  $\times$  0.5–1  $\mu\text{m}$ , wedge- or rod-like.

Etymology: *tricellularis* (lat.) = with three cells; refers to the ascospores.

Host: *Frullania dilatata* (L.) Dumort.

Distribution: known only from the type collection from Romania.

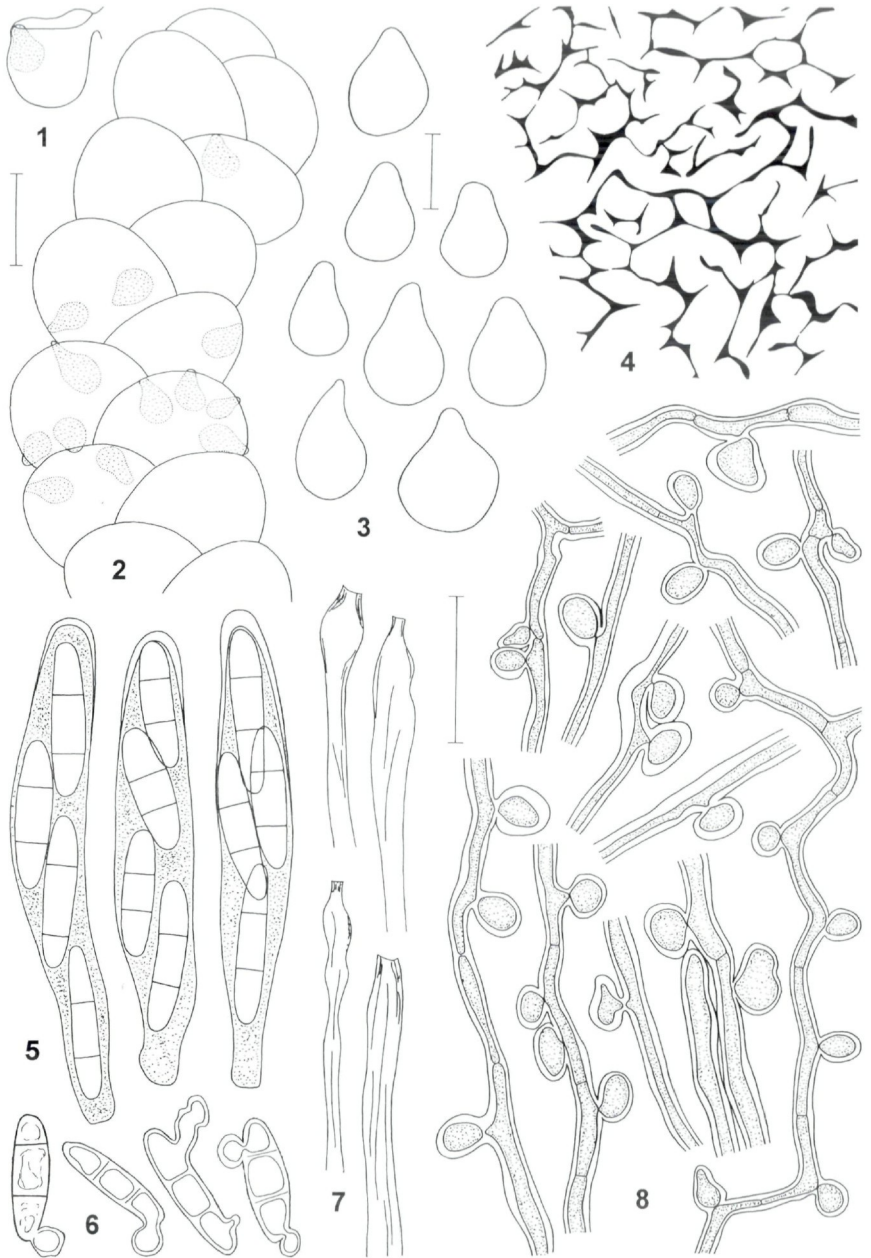
Ascomata are formed exclusively on the protected ventral side of the host plants. They are most numerous on the leaf border of the dorsal lobes where they are laterally attached. The ostiolar region is level with the leaf margin or protrudes slightly (see Döbbeler 2002, section 2.4.2.). There are also variously oriented ascomata on the dorsal lobes, the underleaves, the water sacs (ventral lobes) and even within individual water sacs. Normally only one ascoma is to be found per leaf, but occasionally up to four ascomata have been observed on a single leaf.

Conidiomata have been most often found at the ventral leaf border of the dorsal lobes. No opening of the cushion-shaped structures with phialides showing to the leaf-surface was discernible. Therefore liberation of conidia is unclear. Texture-types of ascomata and conidiomata are quite different.

*Bryonectria tricellularis* is related to *B. racomitrii* (Döbbeler & Hertel) Döbbeler on *Racomitrium lanuginosum* (Hedw.) Brid. from Marion Island with the same ascospore-type, but shorter ascospores (16–19  $\mu\text{m}$  long) and larger appressoria (8–11  $\mu\text{m}$  diam) (Döbbeler & Hertel, 1984).

Though several hundred specimens of *Frullania dilatata* have been checked for the presence of fungi during the last two decades, only one record of *B. tricellularis* exists. It is likely that this extremely inconspicuous species, has been overlooked, particularly when ascoma density is low.

*Bryonectria tricellularis* is one of several hypocrealean species recorded on *Frullania dilatata* and apart from *B. callicarpa* Döbbeler the second species of *Bryonectria* on that host. *Bryonectria callicarpa* is also confined to the ventral leaf side, has four-spored asci (of the eight ascospore initials only four reach maturity) and its appressoria and anamorph are very similar, including the above mentioned orientation of the phialidic cells towards the substrate (Döbbeler 1999). Ascospore characters readily distinguish the two species. In *B. callicarpa* the ascospores are one-septate and up to 14  $\mu\text{m}$  long, whereas *B. tricellularis* has two-septate ascospores which are longer than 18.5  $\mu\text{m}$ .



**Figs. 1–8.** *Bryonectria tricellularis* (holotype): 1. Perithecium within watersack of *Frullania dilatata*. 2. Perithecia on the ventral side of *F. dilatata*. 3. Perithecia in outline. 4. Excipulum seen from above, hyphal lumina black. 5. Asci with ascospores. 6. Germinating ascospores. 7. Empty, collapsed asci. 8. Hyphae with appressoria. Figs. 1, 2 bar = 0.5 mm, fig. 3 bar = 100  $\mu$ m, figs. 4–8 bar = 20  $\mu$ m.

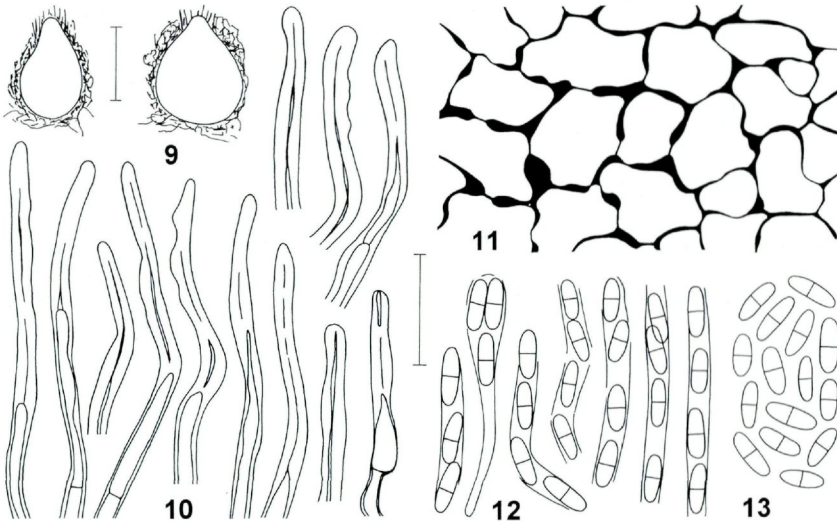
*Nectria hyperepiphylla* Döbbeler sp. nov. Figs. 9–13, 14.

Perithecia 130–180 × 100–140 µm, pyriformia ad ovoidea, incolorata, apicaliter nonnullis hyphis setiformis luminibus valde reductis praedita. Superficies perithecorum hyphis intertextis obtecta. Ostiolum punctiforme. Excipulum e stratis paucis cellularum tangentialiter extensarum compositum; extus cellulis subepidermicis, 10–18 µm magnis, parietibus irregulariter incrassatis. Asci 22–40 × 4 µm, cylindrici, tenuitunicati, 4-sporei, maturitate parietes ascorum evanescentes. Ascosporae 6–7 × 2–2.5 µm, subcylindricae ad anguste ellipsoideae, bicellulares, incoloratae. Hyphae 1.5–2.5 µm crassae, incoloratae, ramosae anastomosantesque, irregulariter supra cellulas hospitis repentes. Appressoria 9–12 µm diametentia, circumferentia plerumque elliptica, uni- vel bicellularia. Haustoria intracellularia, ex appressoriis orientia.

Habitat parasitica inter folia viva hepaticae epiphyllae indeterminatae.

Holotypus: NEW CALEDONIA, Grande-Terre: Prov. Sud: Monts Koghis-Dumbéa, ca. 15 km NNE from Nouméa, tropical rain forest, 22°14' S, 166°30' E, 550 m alt., on an epiphyllous hepatic, 23 Aug 1994, K. & A. Kalb (M).

Perithecia 130–180 × 100–140 µm, pyriform to ovoid, colourless, bearing around ostiolar plate blunt, somewhat irregular, bristle-like hyphae with very thick walls, up to 40 (60) µm long and 2–3.5 µm wide; perithecium surface covered by interwoven hyphae sometimes forming a thick and dense, apically up to 30 µm thick layer. Ostiolar plate about 20 µm diam; ostiole inconspicuous, punctiform. Excipulum with epidermis-like cells, about 10–18 µm diam, cell walls with irregularly thickened and unthickened pit-like parts; cells often hidden by adjacent hyphae; excipulum about



Figs. 9–13. *Nectria hyperepiphylla* (holotype): 9. Perithecia in outline. Bar = 100 µm. 10. Bristle-like setae. 11. Excipular cells seen from the outside. 12. Asci with dissolving walls. 13. Ascospores. Figs. 10–13 bar = 15 µm.



**Fig. 14.** *Nectria hyperepiphylla* (holotype): Hyphae with appressoria, haustoria (partly drawn in, stippled) arising from a circular penetration point within the (distal) appressorial cell. Above the outlines of two infected leaf cells in surface view. Bar = 15  $\mu$ m.

10  $\mu$ m thick, composed of few layers of strongly flattened cells. Paraphyses absent. Asci very delicate and early disappearing, 22–40  $\times$  4  $\mu$ m, normally cylindrical, without apical structures, with (3) 4 ascospores. Ascospores (5.5) 6–7 (8)  $\times$  2–2.5 (3)  $\mu$ m, sub-cylindrical to narrowly ellipsoid, 2-celled, colourless, episporium finely rough; mostly uniseriate. Hyphae 1.5–2.5 (3)  $\mu$ m wide, colourless, thick-walled, with ramifications and anastomoses, finely rough, irregularly growing over the host cells. Appressoria (6) 9–12

(14)  $\mu\text{m}$  diam, varying, mostly elliptical in outline, lateral and sessile or terminal, rarely intercalary, 1- or 2-celled, thick-walled, finely rough. Haustoria intracellular, arising from the appressorial cell (from the distal cell of 2-celled appressoria), thin-walled, rich in cytoplasm, first often gradually enlarged and funnel-shaped, then with thinner irregularly intercoiled ramifications, septa rarely recognizable, normally only one haustorium formed per host cell; perforation peg seen from above as a small point within the appressorium. Anamorph not observed.

Etymology: *hyper-* (gr.) = above, over, *epiphyllus* (gr./lat.) = growing on the upper surface of leaves; refers to the occurrence on an epiphyllous hepatic.

Host: unidentified epiphyllous hepatic.

Distribution: known only from the type collection from New Caledonia.

Ascomata develop especially at the ventral border of the leaves. Heavily infected leaves bearing several ascomata may stick together by hyphae. Apart from being a biotrophic hyperepiphyllous parasite the new species is characterized by ascomata covered by a layer of interwoven hyphae, mostly four-spored dissolving asci, small ascospores and the formation of appressoria and intracellular haustoria. Comparable infection structures exist in *Nectria contraria* Döbbeler growing on the epiphyllous liverwort *Radula flaccida* Lindenb. & Gottsche in Costa Rica (Döbbeler 1998, Figs. 13, 14)

### ***Nectria voratella* Döbbeler sp. nov.** Figs. 15–19

Perithecia 120–190  $\times$  100–150  $\mu\text{m}$ , pyriformia, dilute colorata, sine setis sed superficie hyphis adhaerentibus ornata. Ostiolum punctiforme. Excipulum cellulis 8–12  $\mu\text{m}$  diametentibus, angularibus, parietibus leptodermicis et cyanophilis praeditum; paries excipuli 8–14  $\mu\text{m}$  crassus, e stratis paucis cellularum appanatarum compositus. Asci 30–50  $\times$  5–6  $\mu\text{m}$ , cylindrici, tenuitunicati, 8-spori, maturitate parietes ascorum evanescentes. Ascospores 6–8.5  $\times$  3.5–4.5  $\mu\text{m}$ , late ellipsoideae, bicellulares, incoloratae. Hyphae 1.5–2  $\mu\text{m}$  crassae, incoloratae, supra cellulas foliorum hospitis repentes.

Habitat parasitice in foliis muscorum diversorum. Perithecia singulariter ad folia hospitis laxae affixa. Plantae infectae omnino vel in parte se flavide tingentes et emorientes.

Holotypus: ITALY, Tuscany, Prov. Arezzo: just north of Mercatale Valdarno at the road to Montevarchi (near the junction to Réndola), ca. 250 m alt., on *Homalothecium sericeum* and *Leucodon sciuroides* (growing together), 1 Sep 2000, P. Döbbeler 7462 (M).

Perithecia 120–190  $\times$  (70) 100–150 (160)  $\mu\text{m}$ , pyriform, yellowish to colourless, without setae but surface felted by adjacent hyphae,

apically rarely with few bristle-like hyphae up to 15 µm long; ascospores sometimes recognizable from the outside through the excipular wall. Ostiole inconspicuous, punctiform, ostiolar canal lined with periphyses. Excipulum loosely or densely covered by 2–3.5 µm wide colourless hyphae; excipular cells (5) 8–12 (14) µm wide, apically smaller, angular, sometimes slightly irregular, cells with thin, cyanophilic walls; excipulum (5) 8–14 µm thick, composed of few layers of appanated cells. Paraphyses absent. Asci 30–50 (60) × 5–6 (7) µm, cylindrical, apical structures missing, walls very delicate, at ascospore maturity disappearing, 8-spored. Ascospores (5.5) 6–8.5 (10) × (3) 3.5–4.5 (5) µm, broadly ellipsoid, 2-celled, longitudinally slightly asymmetrical, septum very fine, colourless, epispore finely rough, slightly cyanophilic, mostly uniseriate. Hyphae difficult to trace, colourless, in the immediate surroundings of a perithecium 1.5–2 (3) µm wide, growing over the leaf cells; wide hyphae with thin walls within individual dead host cells; aerial mycelium sometimes present, consisting of felty or arachnoid white hyphae stereomicroscopically visible between dead leaves and at the border of the advancing necrotic zone. Anamorph not observed.

**Etymology:** artificial form of *voratorellus*; *vorator* (lat.) = feeder, *-ellus* (lat.) = adjectival suffix indicating diminutive; refers to the parasitic behaviour of the species.

**Hosts:** *Ctenidium molluscum* (Hedw.) Mitt., *Homalothecium sericeum* (Hedw.) Bruch, Schimp. & W. Gumbel, *Leucodon sciuroides* (Hedw.) Schwägr.

**Distribution:** known from France and Italy.

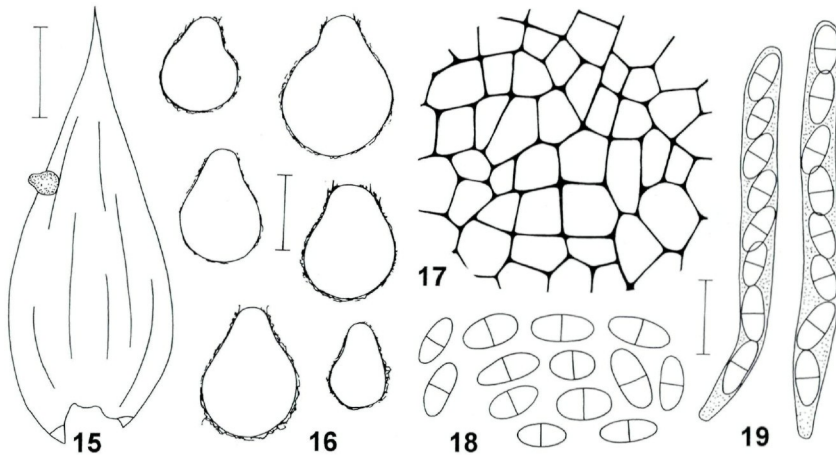
**Additional specimens examined:** FRANCE, Dép. Pyrénées-Atlantiques: South of Pau, at the road D 35 about 1 km east of Louvie-Juzon near Arudy, on *Ctenidium molluscum*, 27 Sep 1980, G. & P. Döbbeler 3924 (M); ITALY, Tuscany, Prov. Siena: West of Monteverchi, at the road 408 north of Gaiole (in Chianti), about 1 km south of the chapel Badia Coltibuona, ca. 600 m alt., on *Homalothecium sericeum*, 7 Sep 2000, P. Döbbeler 7517 (M).

Perithecia of *Nectria voratella* are formed superficially and mostly individually and sporadically on the ad- or abaxial side of dead leaves. They are often laterally and always very loosely attached. Two years after collecting some perithecia still retained a yellowish colour. In the living state they are presumably brightly coloured and therefore relatively easy to detect. Infected plants or parts of them are straw-coloured forming a sharp contrast to healthy green plants. Well delimited necrotic zones may even be present within individual shoots. Often their yellow basal parts have died off whereas the green apical region still remains living. Other colour sequences like yellow, green, yellow along the stems of infected



shoots have also been observed. Later on the dead plants show signs of decomposition. Leaves become fragile and algae begin to colonize them. Within the cell walls of such leaves a mycelium of unknown affiliation apparently degrading wall material can be demonstrated.

As in *Nectria hyperepiphylla*, the delicate ascus walls tend to disappear during spore maturation releasing the ascospores into the centrum. The spores seem to be passively dispersed as in other soft-textured hypocrealean ascomata, which periodically dry out and swell up again when rehydrated extruding the ascospores through the ostiole (Rossman et al. 1999).



**Figs. 15–19.** *Nectria voratella*: **15.** Leaf of *Leucodon sciuroides* with perithecium. Bar = 0.5 mm. **16.** Perithecia in outline. Bar = 100  $\mu$ m. **17.** Excipular cells seen from above. **18.** Ascospores. **19.** Asci (Döbbeler 3924). (Figs. 15–18 holotype). Figs. 17–19 bar = 10  $\mu$ m.

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