

Two new leaf-perforating ascomycetes on corticolous liverworts from Southeastern North America

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Abstract: Two leaf-perforating ascomycetes occurring on bark-inhabiting liverworts in Southeastern North America are described as new species and documented by numerous records. Their life style is regarded as biotrophic parasitism. *Epibryon ventrale* infects three species of *Cheilelejeunea* sect. *Leucolejeunea*, *Hypobryon bacillare* grows on *Frullania inflata*. Common characters are globose ascumata, lacking setae, asci surrounded by hymenial jelly, and brown, bacilliform, transversely septate ascospores. Both species form anamorphic structures along portions of their hyphae: *Torula*-like, disarticulating cell-chains in *E. ventrale* and phialidic conidiogenous cells in *H. bacillare*.

Keywords: Ascomycota, anamorphs, bryophilous, hepaticolous, frullaniicolous, microniches, *Cheilelejeunea*, *Frullania*.

Introduction

Perforation of host leaves in hepaticolous ascomycetes by fruit-bodies borne beneath the protective covering of the host leaf is a successful strategy that may reduce rapid water loss and allow unhindered ascospore discharge into the open air. The ascumata develop on the ventral side of the leaf and, by means of their apex, perforate the host leaf lamina that is only one cell-layer thick. The ostiolar region of the ascoma apex becomes level with the dorsal leaf surface following perforation. Perforation of incubously oriented leaves occurs almost exclusively in the exposed laminal region not overtopped by an older, proximal leaf.

More than 20 obligate leaf-perforator ascomycetes are currently known (perianthicolous species not included). They are always pyrenocarpous, infect almost exclusively foliose liverworts, and belong to different clades, e.g., *Chaetothyriales* and *Hypocreales* (DÖBBELER, 2002; DÖBBELER & DAVISON, 2017). In the following two further leaf-perforating ascomycetes are proposed as new species. They were detected while studying corticolous liverworts from Southeastern North America. Both species share similar features, such as the formation of transversely septate ascospores, but represent different genera.

Material and methods

The species were investigated by standard mycological methods. Initial screening of host specimens for the presence of fungal fruit bodies was performed on fresh specimens collected 2013–2019 from limited regions within the southeastern United States of America. Critical study of ascumata and hyphae was made after the specimens were dried. Lugol's solution was used to test the iodine reaction. Measurements and illustrations apart from fruit-body size were done in lactophenol-cotton blue (CB). The micrographs were taken from freshly collected material. Especially in *Epibryon ventrale* the number of septa in ascospores is variable. It is recommendable to analyze more than one well developed ascoma.

Taxonomy

Epibryon ventrale Döbbeler & P.G. Davison, *sp. nov.* (*Chaetothyriales*) – MycoBank MB 832830 – Figs 1, 2

Diagnosis: *Epibryon ventrale* differs from other *Epibryon* species by ascumata less than 100 µm diam, lacking setae, small ascumatal wall cells covered by dark papillae, bacilliform, light brown, fasciculate ascospores measuring 15–21 × 2.5–3 µm with 5 transverse

septa, variable hyphae on living and within dead host cells, presence of a *Torula*-like anamorph, and leaf-perforation and parasitism of *Cheilelejeunea* sect. *Leucolejeunea* species.

Type: U.S.A., North Carolina: Graham County, Nantahala National Forest, dry oak-hickory-pine forest above shore of Fontana Lake, 35.43783° N 83.74808° W, 549 m alt., host *Cheilelejeunea uncioloba* on trunk of *Carya tomentosa*, 18 March 2016, P.G. Davison 9336A (*holotypus* NY, *isotypus* M).

Etymology: From Latin *ventralis* = ventral; refers to the position of the ascumata on the ventral side of the leaf lobes.

Ascumata leaf-perforating, globose, black-brown to black, without setae, (65–)70–90(–100) µm diam. **Ostiole** inconspicuous. **Ascumatal wall** seen from the outside with angular to slightly meandering, 2.5–5 µm wide cells, cellular structure usually hidden by dark, papillate, irregular wall protuberances giving the ascumatal surface an uneven texture. **Interascal filaments** absent. **Hymenial jelly** dirty reddish to reddish-brown in iodine, dirty blue after pretreatment with KOH. **Asci** claviform to ellipsoidal with a short basal foot, thin-walled, 25–38(–45) × 9–14(–16) µm, 8-spored; ascus wall not or hardly recognizable when ascospores are mature; number of mature asci per ascoma between 5 and 25 depending on fruit-body size. **Ascospores** bacilliform to very narrowly ellipsoidal, rarely subclaviform or fusiform, straight or less often slightly bent, (3, 4) 5 (6)-septate, usually not constricted at the septa, light brown, (13–)15–21(–23) × (2–)2.5–3(–3.5) µm; epispore smooth, no reaction with CB; ascospores fasciculate, forming 19–32 × 9–14(–16) µm large packets. **Hyphae** light to dark brown, superficial on both leaf sides (but basally often slightly immersed into the outermost part of the host cell wall) and within the leaf cells, highly diverse, of three merging types: **1.** Superficial hyphae delicate, ca 1–1.5(–2) µm thick, light brown, with ramifications and anastomoses, on living and dead host cells, following the anticlinal cell walls mirroring the cell net of the leaves, usually only one hyphal strand; hyphae without ramifications forming a zig-zag line. **2.** Intracellular hyphae within dead host cells, (1.5–)2–3(–4) µm thick, light brown, often strongly constricted at the septa (sausage-shaped cells), adjacent to the anticlinal walls, not filling the host cells, crossing the walls of neighbouring cells by fine perforations. **3.** Superficial about 2 µm thick, dark brown hyphae on the anticlinal walls of dead host cells producing a *Torula*-like **anamorph** (only observed in the type collection), anamorph from a larger almost cubiform cell that divides and forms a chain of similar cells that disarticulate, fragments 1-, 2- or more-celled (up to 11 cells observed), chains up to 20(–28) µm long and 2.5–3.5(–4) µm wide, single cells up to 4 × 4 µm, terminal cell in the chain with a rounded apex; cells walls at the septa sometimes strongly constricted causing the impression of a row of globular cells.

Hosts: *Cheilolejeunea clypeata* (Schwein.) W. Ye & R.L. Zhu, *C. conchifolia* (A. Evans) W. Ye & R.L. Zhu, *C. uncioloba* (Lindenb.) Mal-
 ombe. All species belong to *Cheilolejeunea* (Spruce) Steph. sect. *Leu-
 colejeunea* (A. Evans) W. Ye, Gradst. & R.L. Zhu (*Porellales*).

Geographical distribution: U.S.A., Alabama, Mississippi, North
 Carolina, Virginia.

Additional specimens examined: U.S.A. Alabama. Clay County:
 Talladega National Forest, Horn Mountain, upland oak-pine forest,
 33.204° N 86.066° W, 525 m alt., *C. uncioloba* on *Quercus stellata*, 31
 Dec 2013, P.G. Davison 8505 (M, NY); same locality, *C. conchifolia* on
Pinus virginiana, 31 Dec 2013, P.G. Davison 8510 (M, NY). Lawrence
 County: Bankhead National Forest, Tennessee Valley Divide, upland
 oak-pine forest, 34.398° N 87.459° W, 305 m alt., *C. uncioloba* on *Nyssa
 sylvatica*, 17 Apr 2016, P.G. Davison 9382C (NY). Marion County:
 Rideout Branch below Rideout Falls, hardwood forest, 34.235° N
 87.991° W, 170 m alt., *C. clypeata* on *Symplocos tinctoria*, 5 May 2016,
 P.G. Davison 9420C (NY); North Fork Creek near Highway 43, edge
 of hardwood forest and sandstone glade, 34.234° N 87.894° W,

183 m alt., *C. cf. clypeata* on *Hamamelis virginiana*, 26 Dec 2013, P.G.
 Davison 8438 (NY). Monroe County: Haines Island Park along the Al-
 abama River, *Fagus-Magnolia* forest, 31.722° N 87.470° W, 52 m alt.,
C. uncioloba on *Magnolia macrophylla*, 13 May 2015, P.G. Davison
 8802 (NY). Winston County: Bankhead National Forest, above Sipsey
 Fork, loblolly pine stand with mixed hardwoods, 34.291° N 87.382°
 W, 250 m alt., *C. clypeata* on *Acer rubrum*, 27 Feb. 2016, P.G. Davison
 9275B (NY); same locality, *C. uncioloba* on *Acer rubrum*, 30 Jul 2019,
 P.G. Davison 10397 (NY); same locality, *C. uncioloba* and *C. clypeata*
 on *Cornus florida*, 30 Jul 2019, P.G. Davison 10398A (M, NY), 10398B
 (NY); same locality, *C. uncioloba* and *C. clypeata* on *Nyssa sylvatica*, 20
 Jul 2019, P.G. Davison, 10401 (NY); Bankhead National Forest, be-
 tween Hwy 33 and Davis Creek, oak-pine forest, 34.293° N 87.346°
 W, 244 m alt., *C. clypeata* on *Quercus rubra*, 5 Mar 2016, P.G. Davison
 9298A (NY), same data but 34.287° N 87.338° W, 274 m alt., on
C. conchifolia and *C. uncioloba* on *Quercus coccinea*, P.G. Davison
 9292E (NY); Bankhead National Forest, near Sipsey Fork, pine-oak
 forest, 34.295° N 87.384° W, 256 m alt., *C. uncioloba* on *Acer rubrum*,
 27 Feb 2016, P.G. Davison 9288B (NY), same data but 34.267° N
 87.392° W, 244 m alt., *C. uncioloba* on *Magnolia macrophylla*, 29 Mar



Fig. 1 – *Epibryon ventrale* (a, c, e PGD 10401, b PGD 10398B, d PGD 10398A, f PGD 10397). a–b. Black ascomata as seen through semitransparent leaves of living host (a: *Cheilolejeunea uncioloba*, b: *C. clypeata*), dorsal surface. c. Dorsum of perforated host leaf, damaged cell at arrow, surrounding host cells with chloroplasts (green) and large oil bodies (colorless). d. Ruptured ascoma with mature asci on ventral side of host leaf. e. Black ascomata on ventral surface of host leaves, photographed dry. f. Habitat on bark of *Acer rubrum*. Scale bars: a, b: 1 mm, c–d: 50 µm, e: 0.5 mm.

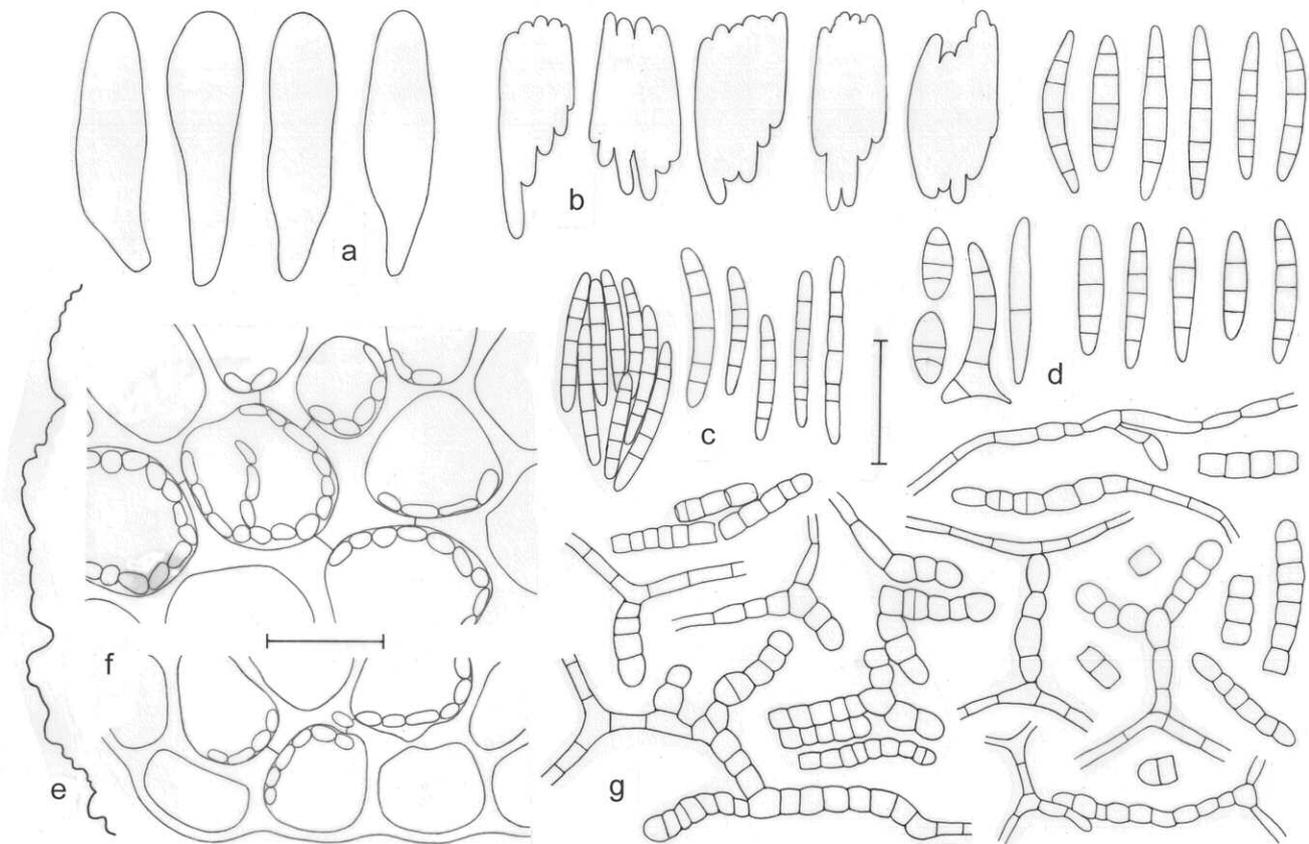


Fig. 2 – *Epibryon ventrale*. a. Immature asci in outline. b. Ascospore fascicles in outline. c. Ascospores, to the left a squashed fascicle. Ascospores, to the left deformed spores. e. Outline of an ascoma in the middle part. f. Hyphae within cells of a host leaf. g. *Torula*-like disarticulating hyphae in different developmental stages. a, d, f, g PGD 9336A, (holotype); b, e PGD 9354D; c PGD 9420C. Scale bars: a–e, g: 15 µm, f: 20 µm.

2016, P.G. Davison 9354D (M, NY). Mississippi. Alcorn County: Tributary to Coke Creek, 16 km SE of Corinth, bottomland hardwood forest, 34.856° N 88.382° W, 140 m alt., *C. clypeata* on *Liquidambar styraciflua*, 20 Oct 2016, P.G. Davison 9623B (NY). North Carolina. Graham County: Nantahala National Forest, cove hardwood-white pine forest along streamlet near Fontana Lake, 35.435° N 83.750° W, 549 m alt., *C. clypeata* on *Acer rubrum*, 18 Mar 2016, P.G. Davison 9334C (NY). Macon County: Nantahala National Forest, Blue Valley near Henson Branch, hardwood forest, 35.011° N 83.228° W, 792 m alt., *C. uncioloba* on *Liriodendron tulipifera*, 2 Aug 2015, P.G. Davison 8930 (NY). Virginia. Giles County: Little Stony Creek below the Cascades, cove hardwood forest, 37.368° N 80.575° W, 860 m alt., *C. clypeata* on *Betula alleghaniensis*, 17 May 2016, P.G. Davison 9486 (M, NY).

Remarks: Ascomata mostly occur on the ventral side of the leaf lobes and typically occur near the leaf margin. Occasionally ascomata occur within perianths and perforate the perianth wall. Superficially formed ascomata have rarely been found. Perforation, at least initially, seems to be through a single host cell. The life style is difficult to judge because ascomata develop on healthy, withering or dead leaves of the host. Intracellular hyphae typical for necrotrophs are present, but may be the result of colonization of already dead or dying cells. Furthermore, necrotrophic behaviour expressed by distinctly discoloured patches does not occur and is so far not known in *Epibryon* species. *Epibryon ventrale* is therefore regarded as a less harmful parasite that seems to be specific to *Cheilolejeunea* species. Though hundreds of specimens have been visually screened with great care, *E. ventrale* has never been observed on sympatrically occurring *Frullania* species. Notably, *E. ventrale* appar-

ently fails to infect *Frullania* plants even when occurring in intermingled mats with infected *Cheilolejeunea*.

Fruit-bodies begin to develop on the corners of the host cells. The wart-like coverings of the cells on the surface of the ascomata are already present in primordia. Anamorphs in the species-rich genus *Epibryon* have only rarely been recorded. Phialidic conidiogenous cells produced by germinating ascospores and by hyphae occur, e.g., in the pantropical *E. filiforme* Döbbeler & Menjivar on epiphyllous *Lejeuneaceae* and *Radula flaccida* Lindenb. & Gottsche (DÖBBELER & MENJIVAR, 1992, Figs 1: 4, 2: 5; DÖBBELER, 1998, Fig. 7: 2).

The host genus *Cheilolejeunea* contains approximately 150 species (STOTLER & CRANDALL-STOTLER, 2017) and is predominantly pantropical in distribution (YE *et al.*, 2015). Except for the hepaticolous lichen *Catinaria brodoana* R.C. Harris & W.R. Buck (LENDEMER *et al.*, 2016), *Epibryon ventrale* is the first-known bryophilous fungus associated with *Cheilolejeunea*. The three host species, *C. clypeata*, *C. conchifolia*, and *C. uncioloba*, have similar habitat requirements and may occur sympatrically. They are common members of the hepatic flora of the southeastern United States with *C. clypeata* extending northward to New England and westward to Oklahoma, and *C. uncioloba* extending northward to Rhode Island. *Cheilolejeunea clypeata* and *C. conchifolia* are endemic to the eastern United States; in North America *C. uncioloba* is restricted to the eastern United States and extends into South America and South Africa (SCHUSTER, 1980; STOTLER & CRANDALL-STOTLER, 2017). Specimens of *E. ventrale* were collected from upland, dry oak-pine forests (western North Carolina, northern Alabama), mesic cove hardwood forests (western North Carolina, western Virginia), a bottomland hardwood forest (northern Mississippi), and a beech-magnolia forest (southern Alabama). Given its lack of restriction to any one forest type, it is possible that *E. ventrale* occurs more widely than the current distribution reflects. As with

most bryophilous ascomycetes, the species is easily overlooked and many regions within the geographic ranges of the host species probably remain uninvestigated. A fourth species in *Cheilolejeunea* section *Leucolejeunea* occurring in the United States, *C. xanthocarpa* (Lehm. & Lindenb.) Malombe with a pantropical distribution, was not investigated due to its restricted regional occurrence (Florida).

The new species is assigned to *Epibryon* Döbbeler with only slight reservation. Though the type species of the genus, *E. plagiochilae* (Gonz. Frag.) Döbbeler, has superficial, setose ascomata and one-septate ascospores, *E. ventrale* shares similar features with other species in the genus. *Epibryon ventrale* seems to be closely related to *E. hypophyllum* Döbbeler growing on *Radula complanata* (L.) Dumort., and to a slightly deviating form of *E. hypophyllum* on the tropical *R. flaccida*. These differ from *E. ventrale* by distinctly smaller, 3-septate, ascospores that are not fasciculate (DÖBBELER, 1979, 1985, 1998). *Epibryon perrumpens* Döbbeler is also similar, but has somewhat larger, 5–7-septate ascospores, intercellular leaf-perforation,

and parasitizes *Frullania dilatata* (L.) Dumort. and *R. complanata* (DÖBBELER, 1982, 2006) [Tab. 1].

Hypobryon bacillare Döbbeler & P.G. Davison, *sp. nov.* (*Chaetothyriales*) – MycoBank MB 832834 – Figs 3, 4.

Diagnosis: *Hypobryon bacillare* differs from other species of the genus *Hypobryon* by polysporous asci, bacilliform, 3-septate, uniformly brown ascospores measuring 14–20 × 2.5–3 µm, and parasitism of *Frullania inflata*.

Type: U.S.A., Alabama: Lauderdale County, Shoal Creek Preserve north of St. Florian, 34.90778° N 87.61933° W, 195 m alt., host *Frullania inflata* on trunk of *Acer saccharum*, together with *Bryonectria callicarpa*, 26 May 2015, P.G. Davison 8848E (*holotypus* NY).

Etymology: From Latin *bacillaris* = rod-like, bacilliform; refers to the shape of the ascospores.

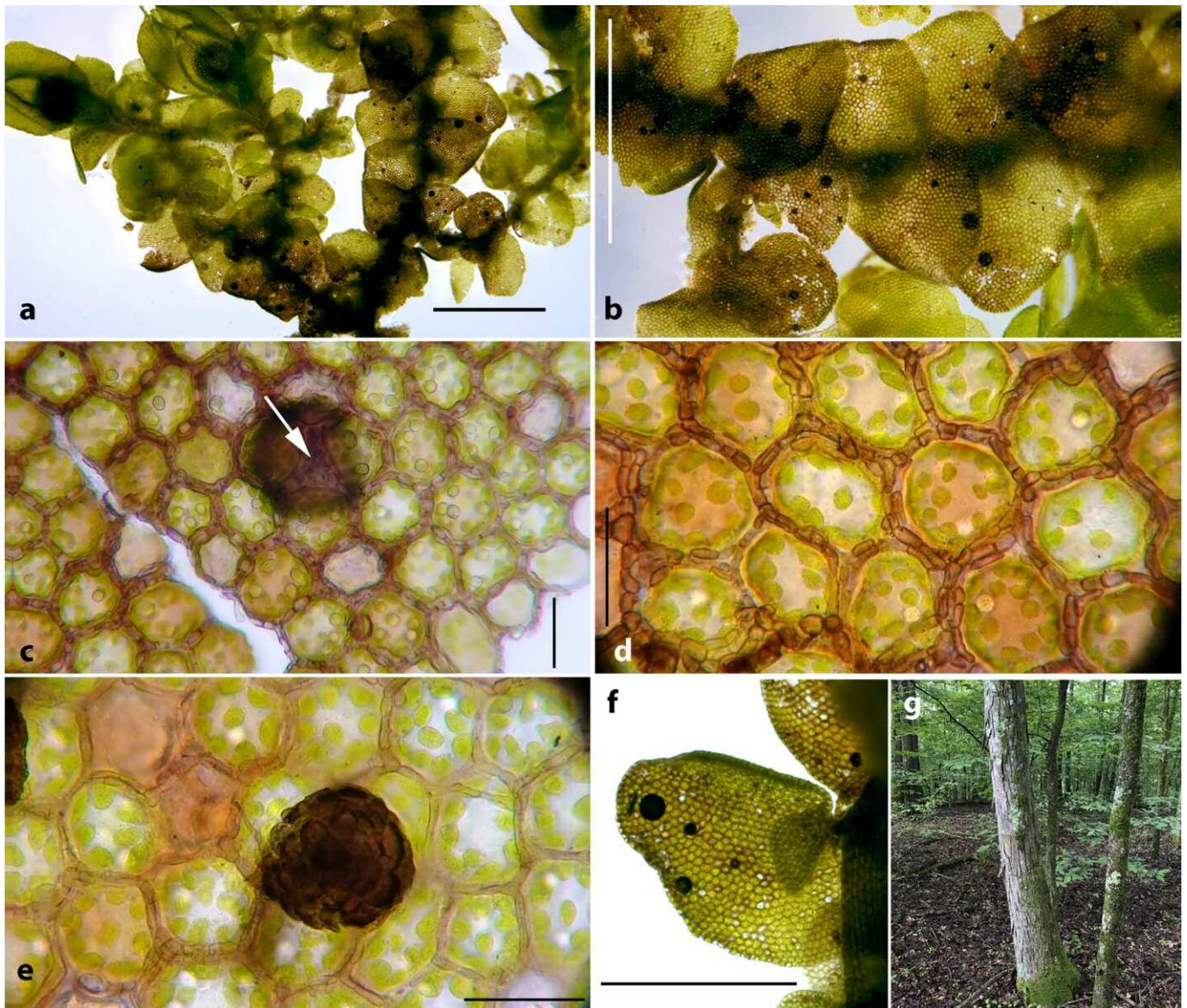


Fig. 3 – *Hypobryon bacillare* (a–f PGD 10389, g PGD 10386A). a, b. Black ascomata as seen through semitransparent leaves of host dorsal surface. c. Dorsum of perforated host leaf, intercellular perforation at arrow, optical section below host leaf surface with swollen hyphal cells between anticlinal walls of host cells. d. Dorsum of host leaf surface with hyphae over the anticlinal cell walls. e. Immature ascoma and anticlinal host cell walls with surface hyphae over ventral surface of leaf. f. Ascomata in formation on ventral side of host leaf. g. Habitat on bark of *Carya ovata*. Living host cells with chloroplasts (green) and oil bodies (colorless) visible in c, d, and e. Scale bars: a, b: 1 mm, c–e: 25 µm, f: 0.5 mm.

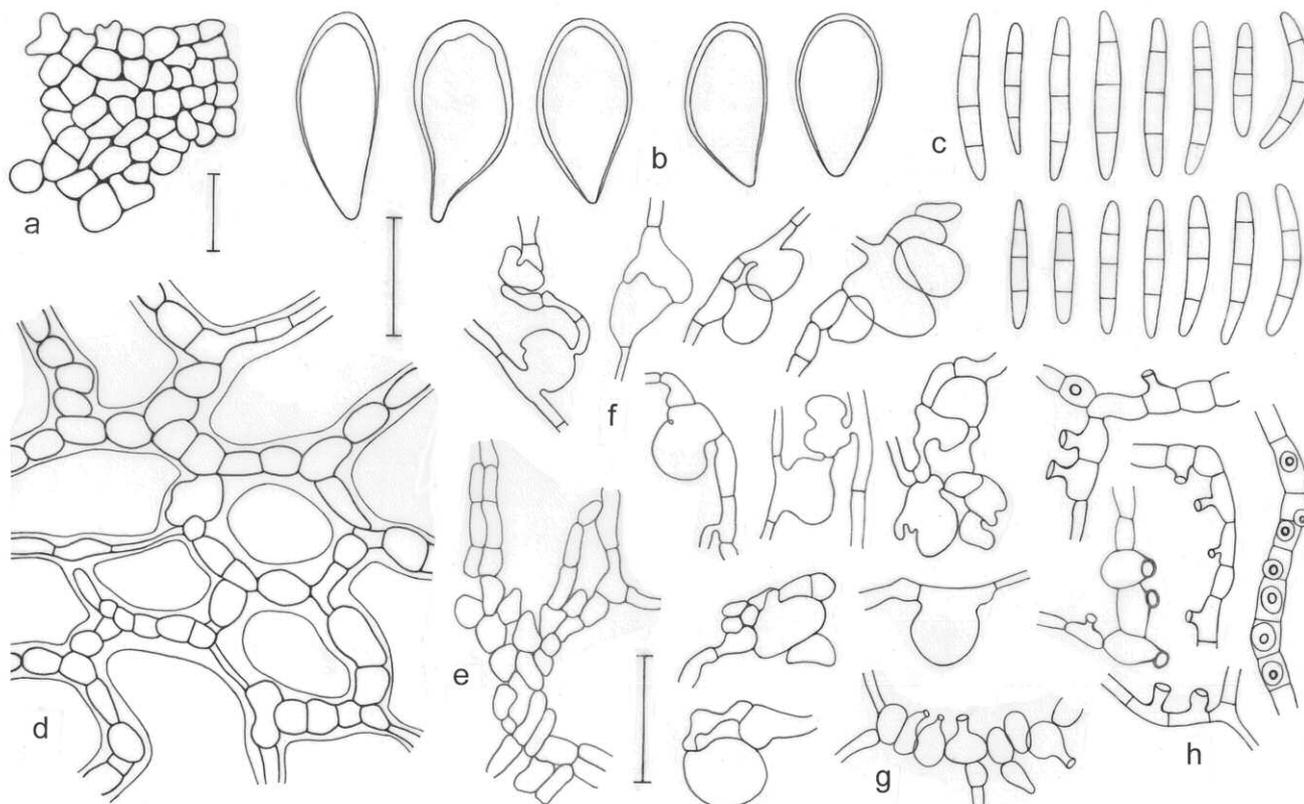


Fig. 4 – *Hypobryon bacillare*. a. Ascomatal wall cells seen from the outside. b. Asci in outline. c. Ascospores. d. Enlarged hyphae within anticlinal cell walls of a host leaf, optical section. e. Hyphal plate. f. Enlarged hyphal cells laterally seen. g, h. Phialids, laterally seen and from above (at the right). a PGD 8778; b different collections. c–f PGD 8848E (holotype), g PGD 8328, h PGD 8850D. Scale bars: a: 10 μ m, b, d: 20 μ m, c, e–h: 15 μ m.

Ascomata leaf-perforating, globose, dark brown to black, without setae, (55–)65–100(–115) μ m diam. **Ostiole** not visible as a light point. **Ascomatal wall** in surface view with 3–6(–7) μ m wide angular or slightly sinuose cells, outermost walls of individual cells covered by up to 3 μ m wide dark brown thickenings, surface uneven, cellular structure sometimes hard to recognize. **Interascal filaments** absent. **Hymenial jelly** in iodine reddish immediately following a light blue front, blue after pretreatment with KOH. **Asci** claviform to ovoid, with 16 or more, often 24 or 32 spores, (25–)30–45(–50) \times (12–)15–20(–22) μ m, spores filling the whole ascus lumen, few mature asci per ascoma (up to 12 in a large ascoma observed). **Ascospores** bacilliform, slightly claviform or subfusiform, straight or less often somewhat curved, somewhat asymmetrical, brown, end cells not lighter colored, 3 (4)-septate, not constricted at the septa, (12–)14–20(–25) \times (2–)2.5–3 μ m, episore smooth; spore orientation more or less parallel to the longitudinal axis of the asci. **Hyphae** abundant, variable, brown, relatively short-celled, occurring within the host cell walls, ca. (1.5–)2–3.5 μ m thick, forming pellicles especially within the anticlinal walls; pellicles consisting of hyphae and irregular hyphal cells with often sinuose walls, conspicuously enlarged cells usually present in the anticlinal cell walls, bulging the adjacent walls sideward; the enlarged cells elliptical, sometimes with a transverse wall, without dark thickenings at both smaller sides, 4–10(–13) \times 4–7(–8) μ m (seen from above in optical section); these cells seen laterally more or less circular in outline. **Conidiogenous cells** phialidic, 1-celled, originating from a hyphal segment, often forming a short row with one phialide behind the other, variously shaped, either bottle-shaped and longer than wide, or a hyphal cell with a neck-like protrusion, whole length of bottle-shaped cells (including the neck) 4–8 μ m, widest lower part 3–9 μ m, neck 1–3 μ m diam; phialides often oriented perpendicularly to the substrate. **Phialoconidia** not observed.

Host: *Frullania inflata* Gottsche (*Porellales*).

Geographical distribution: U.S.A., Alabama, Mississippi, Tennessee.

Additional specimens examined: All specimens on host *Frullania inflata*; the name of the tree or vine from which the specimen was collected is included in the citations below.

U.S.A. Alabama. Jefferson County: Turkey Creek Nature Preserve, 33.704° N 86.693° W, 205 m alt., *Carpinus caroliniana* along creek, 29 Sep 2013, P.G. Davison 8479 (M); same data, *Liriodendron tulipifera*, P.G. Davison 8483 (NY). Lauderdale County: Florence, King Spring, 34.857° N 87.655° W, 168 m alt., *Ligustrum sinense*, 18 Jul 2013, P.G. Davison 8263 (NY), 8264 (NY), 8268A (M); same location, *Ligustrum sinense*, 25 Jul 2013, P.G. Davison 8304 (NY), 8291 (M); Florence, Wildwood Park, atop bluff above Cypress Creek, 34.803° N 87.692° W, 152 m alt., at ground level on *Quercus montana*, 23 Jul 2013, P.G. Davison 8290 (NY); Wildwood Park along Cypress Creek, 34.817° N 87.693° W, 152 m alt., *Betula nigra*, 11 Apr 2015, P.G. Davison 8778 (NY); same data, *Ostrya virginiana*, P.G. Davison 8777A (NY); same data, *Ulmus* sp., P.G. Davison 8776A (NY); Wildwood Park, upland above Cypress Creek, 34.807° N 87.699° W, 152 m alt., *Celtis* sp., 22 Nov 2015, P.G. Davison 9018B (NY); same location, *Carya ovata*, 23 Jul 2019, P.G. Davison 10386A (NY); Wildwood Park, atop bluff above Cypress Creek, 34.801° N 87.697° W, 158 m alt., *Carya ovata*, 3 Jan 2016, P.G. Davison 9103A (NY); Petersville, UNA wetland, 34.850° N 87.689° W, 177 m alt., *Campsis radicans*, 28 Jul 2013, P.G. Davison 8318 (NY), 8321 (NY); same location, *Campsis radicans*, 24 Jul 2019, P.G. Davison 10389 (NY); Saint Florian, Brotherick Branch stream ravine and adjacent slope, 34.911° N 87.565° W, 180 m alt., *Fagus grandifolia*, 18 Feb 2016, P.G. Davison 9255A (NY); same data, *Ligustrum sinense*, P.G. Davison 9236A (NY); same data, *Liriodendron tulipifera*, P.G. Davison 9234 (NY); same data, bark of log, P.G. Davison 9246D (NY); same data, *Quercus montana*, P.G. Davison 9258B (NY); same data, *Vitis* sp., P.G. Davison 9253A (M); Shoal Creek Preserve,

34.908° N 87.619° W, 195 m alt., *Liquidambar styraciflua*, 24 May 2015, P.G. Davison 8839E (M); same location, 26 May 2015, *Acer saccharum*, P.G. Davison 8848A (M); same data, *Liquidambar styraciflua*, P.G. Davison 8850D (NY). Mississippi. Oktibbeha County: Starkville, MSU Research Park, 34.475° N 88.789° W, 91 m alt., *Cercis canadensis*, 28 Jan 2016, P.G. Davison 9211 (NY); Starkville, W of Sessums Road, 33.394° N 88.712° W, 79 m alt., *Cercis canadensis*, 28 Jan 2016, P.G. Davison 9219C (NY). Tennessee. Hardin County: floodplain border of Tennessee River, 35.176° N, 88.265° W, 113 m alt., *Ilex decidua*, 27 Oct 2016, P.G. Davison 9648B (NY); Horse Creek, 35.109° N, 88.120° W, 152 m alt., *Betula nigra*, 27 Oct 2016, P.G. Davison 9658B (M). Marion County: Little Cedar Mountain at base of slope along small creek, 35.030° N, 85.581° W, 207 m alt., *Celtis* sp., 4 Aug 2013, P.G. Davison 8329 (NY); same data, *Ligustrum sinense*, P.G. Davison 8327 (NY), 8328B (M, NY), 8332 (NY), 8333 (NY), 8335 (NY); same data, *Ulmus* sp., P.G. Davison 8330 (M).

Remarks: While it has been widely misunderstood, *Frullania inflata* as treated by ATWOOD (2017) is endemic to the United States ranging from Pennsylvania south to Florida and west to Kansas. The species appears to be absent from the higher elevations of the Southern Appalachians and occurs most abundantly in areas of high humidity often near water (SCHUSTER, 1992). *Hypobryon bacillare* was most often collected from lowland sites in wetlands or near streams. The upland locations in Lauderdale County, Alabama were humid sites atop a bluff 35 meters higher altitude than the nearby Cypress Creek.

As "one of the most common *Frullania* species in the southeastern United States" (ATWOOD, 2017), *F. inflata* is host to a rich assemblage of bryophilous ascomycetes. Within the genus *Frullania*, *F. inflata* is tied with the European *F. dilatata* (L.) Dumort. for hosting the highest number of hypocrealean ascomycetes (six species each). Three of the six hypocrealean species on *F. inflata* appear to be host specific: *Bryocentria biannulata* Döbbeler & P.G. Davison, *B. chrysothrix* Döbbeler & P.G. Davison, and *Periantria bellacaptiva* Döbbeler & P.G. Davison (DÖBBELER & DAVISON, 2017). Including *Hypobryon bacillare*,

F. inflata is host to four host-specific ascomycete species as currently known.

An ecological character of *Hypobryon bacillare* (and other *Hypobryon* species) is the preferred colonization of lower, old and moribund host leaves which may bear up to six ascomata of different developmental stages. However, mature fruit-bodies also develop on healthy leaves. Studying fresh material reveals that host cells immediately surrounding the perforation possess living chloroplasts and oil-bodies (Figs 3 c, d, e). The species is therefore regarded as a biotrophic parasite. Colonization of hepatics apart from *Frullania inflata* even in collections containing two or more liverwort species has never been observed.

The new species is a typical member of the genus *Hypobryon* Döbbeler which is characterized by common features like globose ascomata without setae, cellular structure of the ascomatal wall, 8- or more-spored asci, positive iodine reaction, brown ascospores with one or more transverse septa, presence of conidiogenous cells formed on the hyphae, biotrophic parasitism and microniche selection. A unique feature of *Hypobryon* species are the abundant mycelia within the host cell walls, especially the anticlines. The mycelia consist of irregularly growing hyphae that form more or less pronounced hyphal pellicles. Vesicular cells may strongly expand the infested cell walls which gives even sterile *Hypobryon* infections an unmistakable aspect.

Due to the intercellular mycelium, perforation of leaves or perianths is effected between host cells. Examination of developing fruit-bodies clearly shows that the enlarging fruit-body apex separates the middle lamellar region between two or several adjacent host cells, which are pushed further apart by the expanding fruit-body apex. The leaf surface is not elevated by the perforating ascomata as in *Bryocentria* species. In contrast this point-like perforation is depressed as in other members of *Hypobryon*. The copiously developed mycelium surely contributes to leaf decomposition. All anticlinal cell walls are colonized by hyphae in portions of heavily infected leaves. Several times *Bryocentria callicarpa* has been recorded as an associated species. Fruit-bodies of both species may

Table 1 – Comparison of leaf-perforating *Epibryon* species and *Hypobryon bacillare*

Characters	<i>Epibryon ventrale</i>	<i>Epibryon perrumpens</i>	<i>Epibryon hypophyllum</i>	<i>Hypobryon bacillare</i>
Literature	this paper	DÖBBELER (1982, 2006)	DÖBBELER (1979, 1985, 1998)	this paper
Ascomata	70–90 µm diam	60–110 µm diam	60–100 µm diam	65–100 µm diam
Ascomatal wall	cells angular, 2.5–5 µm wide with papillate wall protuberance	cells angular, 3–6 µm wide with wart-like wall thickenings	cells angular, in the lower part 4–8 µm wide; surface with few short hyphae or setae	cells angular, 3–6 µm wide with up to 3 µm wide wall thickenings
Asci	25–38 × 9–14 µm, 8-spored	25–40 × 10–13.5 µm, 8-spored	30–35 × 7–9 µm, 8-spored	30–45 × 15–20 µm, polysporous
Ascospores	15–21 × 2.5–3 µm, (3, 4)5(6)-septate, fasciculate	17–25 × 2.5–3 µm, (1–4) 5–7 (8–11)-septate, fasciculate	10–12.5 × 3–3.5 µm, (1, 2) 3 (4, 5)-septate, not fasciculate	14–20 × 2.5–3 µm, 3 (4)-septate, not fasciculate
Hyphae	superficial, intracellular	mostly superficial	superficial, intracellular	within cell walls
Anamorph	Torula-like hyphae	unknown	unknown	phialidic conidiogenous cells
Leaf-perforation	presumably intracellular	intercellular	intracellular through 1 host cell	intercellular
Hosts	<i>Cheilolejeunea</i> sp. div.	<i>Frullania dilatata</i> , <i>Radula complanta</i>	<i>Radula complanata</i> , <i>R. flaccida</i> , <i>Radula</i> sp.	<i>Frullania inflata</i>
Geographical distribution	Southeastern U.S.A.	France, Italy	Europe, U.S.A., Java, Afro-american tropics	Southeastern U.S.A.
Remarks	many records	few records	many records; specimens on <i>R. flaccida</i> with slightly smaller ascomata, asci and ascospores	many records

be on the same leaf. Phialidic conidiogenous cells were found in about a third of the specimens examined. They are likely more common than observed given that a prolonged, concerted effort is required to detect them when they are scattered and scarce. As in other *Hypobryon* species abnormal spores regarding size, shape and septation are not rare.

Hypobryon bacillare is easy to identify by the unique combination of polyspored asci and three-septate, narrow ascospores. In addition to *H. bacillare* there are seven species of *Hypobryon* named. *Hypobryon heterotropum* infects the moss *Leucodon sciuroides* and *H. insigne* infects epiphyllous neotropical *Lejeuneaceae*. The other five previously named species infect *Frullania* and *Porella* species in Europe (*H. bicolor* on *P. arboris-vitae*, *H. florentinum* on *F. dilatata*, *H. perforans* on *F. dilatata*, *H. poeltii* on *P. cordaeana* and *P. platyphylla*, and *H. validum* on *F. teneriffae*). Five species (*H. bacillare*, *H. bicolor*, *H. heterotropum*, *H. insigne*, and *H. poeltii*) have three-septate ascospores. Two species (*H. bacillare* and *H. florentinum*) have polysporous asci. Phialoconidia have been demonstrated in about half of the known species (DÖBBELER, 1983, 2006; DÖBBELER & MENJIVAR, 1992).

Discussion

Epibryon ventrale and *Hypobryon bacillare* share many morphological and ecological characters (Tab. 1). Both species described here have small, globose ascomata of similar size and colour, no setae, an ascomatal wall of cellular texture, lacking interascal filaments, asci surrounded by hymenial jelly that gives a hemiamyloid iodine reaction (BARAL, 1987), and brown, bacilliform, transversely septate ascospores of similar size. The number of septa in the ascospores distinguishes *E. ventrale* (five septa) from *H. bacillare* (three septa).

Common ecological features include the infection of living, corticolous liverworts where each fungus occupies the same microniche although the process of leaf-perforation may be different (intra- versus intercellular). Both species seem to be restricted to their indicated hosts. Hyphal features and the anamorphic structures are, however, quite different. The *Torula*-like, disarticulating cell-chains in *E. ventrale* may directly contribute to propagation of the species, whereas the function of the phialidic conidiogenous cells in *H. bacillare* is unknown. Due to the supposed very small size of the phialoconidia they may play a role in spermatization.

Owing to the similarities shown between *Epibryon* and *Hypobryon*, *Hypobryon* is tentatively included in the *Epibryaceae*. The family was established by GUEIDAN *et al.* (2014) and placed within the *Chaetothyriales* based on molecular data of various, mainly *Epibryon* species.

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