

Bryocentria octosporelloides (Hypocreales) – a new species on *Cololejeunea minutissima* from Asturias (Spain)

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Abstract: *Bryocentria octosporelloides* Döbbeler, J. Linde & E. Rubio (*Bionectriaceae*, *Hypocreales*) is proposed as a new species. It infects the foliose, bark-inhabiting liverwort *Cololejeunea minutissima* (*Lejeuneaceae*, *Porellales*). The species is morphologically characterized by its superficial perithecia, one-celled ascospores and intracellular haustoria, and ecologically characterized by exhibiting biotrophic parasitism. It is recorded from one locality in Asturias in Northern Spain.

Keywords: Ascomycota, biotrophic parasitism, bryophily, haustoria, hepaticolous ascomycetes, *Lejeuneaceae* as hosts.

Resumen: Se propone *Bryocentria octosporelloides* Döbbeler, J. Linde & E. Rubio (*Bionectriaceae*, *Hypocreales*) como nueva especie. El hongo infecta *Cololejeunea minutissima* (*Lejeuneaceae*, *Porellales*), una hepática foliosa cortícola. Esta especie morfológicamente se caracteriza por sus peritecios superficiales, sus ascosporas unicelulares y por sus haustorios intracelulares, y ecológicamente por su parasitismo biotrófico. El hongo fue recolectado en una única localidad de Asturias, en el norte de España.

Palabras clave: Ascomycota, ascomycetes hepáticas, briofilia, haustorios, *Lejeuneaceae* como huéspedes, parasitismo biotrófico.

Introduction

During field work in Asturias, in Northern Spain, many hundreds of small *Octosporella*-like ascomata were detected on a tiny, bark-inhabiting, healthy-looking liverwort. Closer examination revealed that the parasite belongs to the obligately bryophilous genus *Bryocentria* Döbbeler (*Hypocreales*). The species is described in this paper as new.

Material and methods

Living fruit-bodies were investigated using standard methods. Perithecia and ascospores were analyzed and documented in tap water (H₂O); all other structures including ascospores were measured and drawn in lactophenol cotton-blue (CB). Hymenial features were also illustrated in Waterman's blue ink and Lugol's solution (IKI).

Taxonomy

Bryocentria octosporelloides Döbbeler, J. Linde & E. Rubio, sp. nov. – *Bionectriaceae*, *Hypocreales* – MB 824603 **Figs 1, 2**

Diagnosis: *Bryocentria octosporelloides* differs from other *Bryocentria* species by having superficial, ovoid, up to 160 µm long, orange perithecia with short setae, angular to rounded, thick-walled cells in the perithecial wall, relatively few asci, 1-celled, 10–14 × 3.5–4.5 µm large ascospores with a rough, cyanophilous episporium, intracellular haustoria, and biotrophic parasitism on *Cololejeunea minutissima*.

Type: SPAIN, Asturias: Pimiango, alrededores del faro de San Emeterio, not far from the sea shore, 43°23'50"N, 04°31'59"W, 43 m alt., on branches of *Quercus ilex* L. lying on the ground, but still attached to the tree, 24 Dec. 2017, J. Linde s. n. (*holotypus* AH 47960, *isotypi* ERD-7413, GZU, M, NY, TUR). Type material consists of at least 1300 perithecia.

Etymology: *octosporelloides* is a composite name consisting of *Octosporella* and *-oides* (gr. suffix), indicating resemblance and refers to the ascomatal *Octosporella*-like habit of the novel species.

Perithecia superficial, subglobose, broadly ellipsoidal or ovoid, setose, yellowish or more usually orange coloured, in the dry state somewhat darker, after some months probably beginning to bleach, 100–160 × 100–120 µm, laterally collapsing during drying, apical opening inconspicuous, firmly attached to the substratum. **Setae**

densely arranged especially in the upper third of the perithecia, straight or bent, thick-walled, apically usually blunt, sometimes with rough surface, 10–40(–50) × 3–5 µm at the base; intermediate forms between setae and hyphae present in the lower part of the perithecia. **Periphyses** lining the ostiolar canal, individual filaments uniformly tapering, about 30 µm long, basally up to 5 µm thick and often with 1 or 2 septa, rich in cytoplasmic content, united at the base by rectangular cells forming a toothed ring, in squash mounts forming a comb-like structure. **Perithecial wall** in optical section 10–12 µm thick laterally and below, up to 20 µm thick above, seen from the outside with more or less isodiametric, angular or rounded cells (5–)7–12(–15) µm wide, 2(–4) µm thick-walled, especially cell corners thickened; pit-like, un-thickened wall perforations present. **Paraphyses** disappear early. **Asci** unitunicate, cylindrical or slightly claviform, in CB (34–)36–42(–45) × (8–)9–10(–11) µm, 8-spored, easily separating and freely floating; asci very thin-walled, often only spore packets visible. **Ascospores** narrowly ellipsoidal to subbacilliform, sometimes slightly asymmetrical, straight or rarely somewhat bent, both ends rounded, 1-celled, colourless to very pale yellow (when alive), in H₂O (9–)10–14(–15) × (3–)3.5–4.5(–5) µm, in CB 9–14(–15) × 3–4(–4.5) µm, episporium finely warty, irregularly biseriate lying in the asci. **Hyphae** irregularly growing over the surface of both leaf sides, with ramifications and anastomoses, colourless, thick-walled, (2–)2.5–3(–4) µm diam., thicker hyphae often finely rough; appressoria missing; aerial hyphae near perithecia connecting leaves sometimes observable by stereomicroscopic magnification (40×). **Haustroria** intracellular, irregularly shaped, 15–25 µm diam., consisting of lobed and coiled, sometimes delicately ramified hyphae, restricted to the perforated host cell; haustorial hyphae thin-walled, 1–1.5(–2) µm wide, rich in cytoplasmic content, septa hard to see, usually not filling the infected cell; insertion point at mother hypha rarely visible. **Asexual state** not observed.

Fruit-body colour caused by numerous yellow droplets concentrated within the inner perithecial wall cells, outermost perithecial wall often hardly coloured, resulting in an almost colourless external and a coloured internal layer; living periphyses, paraphyses and ascospores also with many yellow droplets.

Chemical reactions: Perithecial wall in KOH and lactic acid negative; hymenial structures in Lugol's solution negative; cell walls of perithecial wall, setae, periphyses, episporium and at least thicker hyphae light to stronger bluish in CB; ascospores without a cyanophilous band.

Host: *Cololejeunea minutissima* (Sm.) Schiffn. (*Lejeuneaceae*, *Porellales*). On the same pieces of bark plants of *Frullania dilatata*, *Radula*

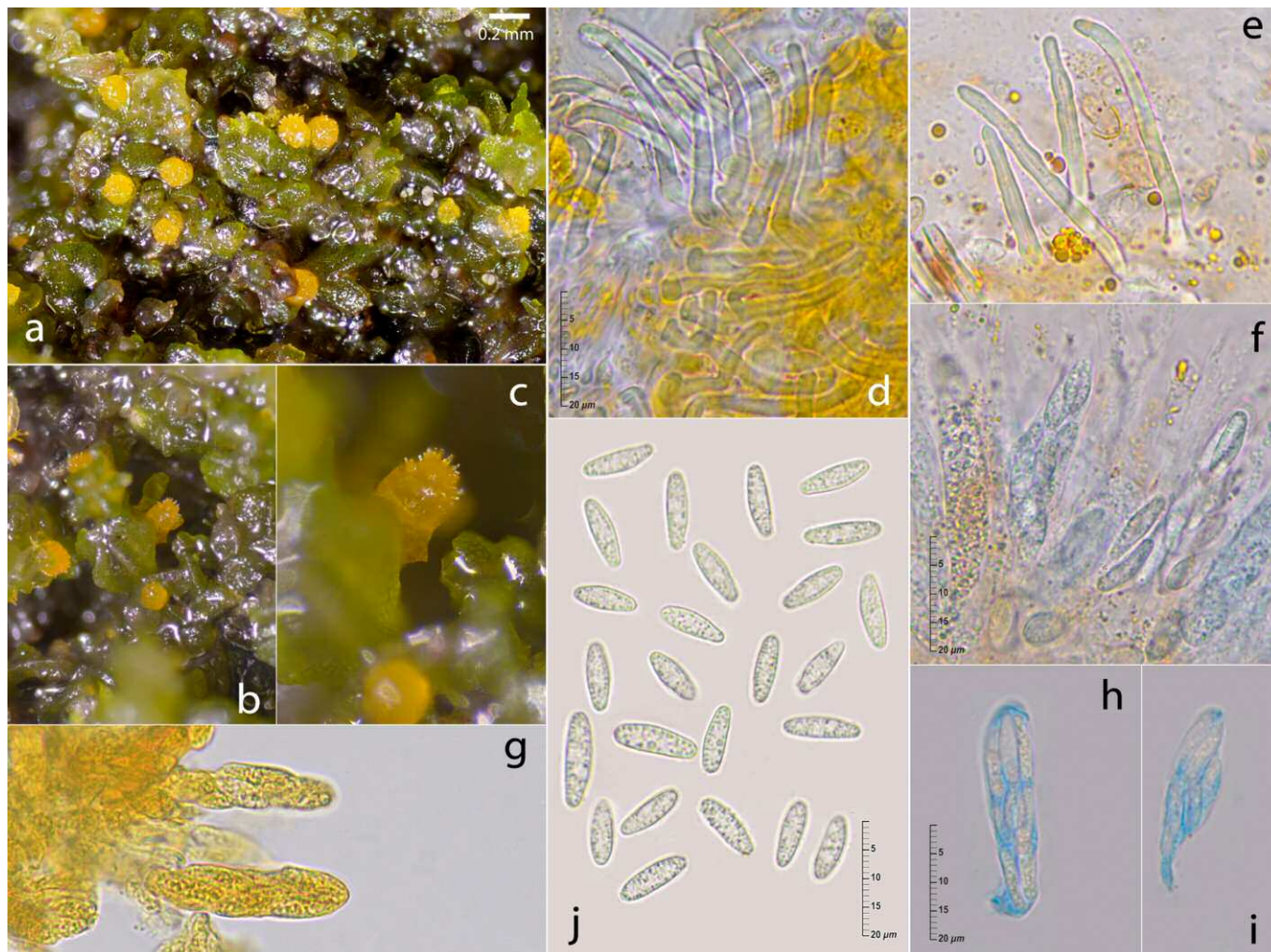


Fig. 1 – *Bryocentria octosporelloides* (holotype AH 47960). – a–c. Perithecia on *Cololejeunea minutissima* in situ. d, e. Setae in water, in d with perithecial wall. f. Part of hymenium with mature and immature asci in Waterman's blue ink. g. Asci in Lugol's solution (IKI). h, i. Asci in Waterman's blue ink. j. Ascospores in water.

complanata, *Orthotrichum* sp. and frequently conspicuous bunches of orange coloured *Trentepohlia* sp. (filamentous green algae) were present.

Known distribution: Known only from the type locality.

Additional specimen examined: With the same information as the holotype label, but: 16 Dec. 2017, E. Rubio s. n. (ERD-7392, M).

Remarks

The perithecia bear a striking resemblance to the perithecioid ascomata of species of *Octosporella* Döbber (Pezizales), but are considerably smaller. They are formed on and between the leaves, on the outer surface of perianths and on the bracts of male inflorescences. Although there is pleasant colour contrast between the light green host and the orange parasite, the tiny plants are dominated by the disproportionately large perithecia. The largest ascomata can be almost as long as the smallest host leaves.

Bryocentria octosporelloides can be regarded as a biotrophic parasite. Its close relation to the host plant is expressed by intracellular haustoria that are surrounded by healthy cells containing oil-bodies. Plants with and without perithecia may show no difference in habit and colour. However, when heavily infected they do seem to suffer. Such plants lose their vivid colour and are colonized by algae. Perithecia occur scattered or concentrated in patches on the liverwort. No less than 150 fruit-bodies were counted on an approximately 8 × 2 cm large piece of bark inhabited by *Cololejeunea*

minutissima. Interestingly, there were no mature sporophytes although numerous perianths, often with a globose capsule inside, were present. The sporophytes undergo a form of arrested development and do not release of spores. Microscopic analysis revealed mummified capsules infested by sterile fungal hyphae, which apparently prevent maturation of the capsules and spores. However, fruit-bodies formed by these hyphae have not been observed. An unnamed hypocrealean ascomycete with perithecia attacking developing sporophytes of *C. minutissima* is known to occur in south-eastern North America (DAVISON & DÖBBELER, unpubl. observation).

Cololejeunea minutissima is a tiny pioneer species with filiform shoots and 180–250 µm long, bilobed leaves. It bears antheridia and archegonia on the same (autoicous) plant. Perianths are usually present. The liverwort tolerates a wide range of moisture conditions and occurs on trunks, branches and twigs of different living, deciduous trees and shrubs. It is widespread in western Europe in (sub)oceanic regions, the Azores, Madeira, North Africa, and South-eastern North America (SCHUSTER, 1980; PATON, 1999).

The genus *Cololejeunea* has hitherto not been recorded as a substrate for fruit-body forming fungi (RACOVITZA, 1959; FELIX, 1988), but several *Lejeuneaceae* of the phyllosphere have been repeatedly found to be a suitable substrate for ascomycetes (DÖBBELER, 2018; DÖBBELER & MENJÍVAR, 1992).

Relationship of *Bryocentria octosporelloides*

Bryocentria is a rapidly growing genus with regard to the number of species. Currently, 17 species are described, including *B. oc-*

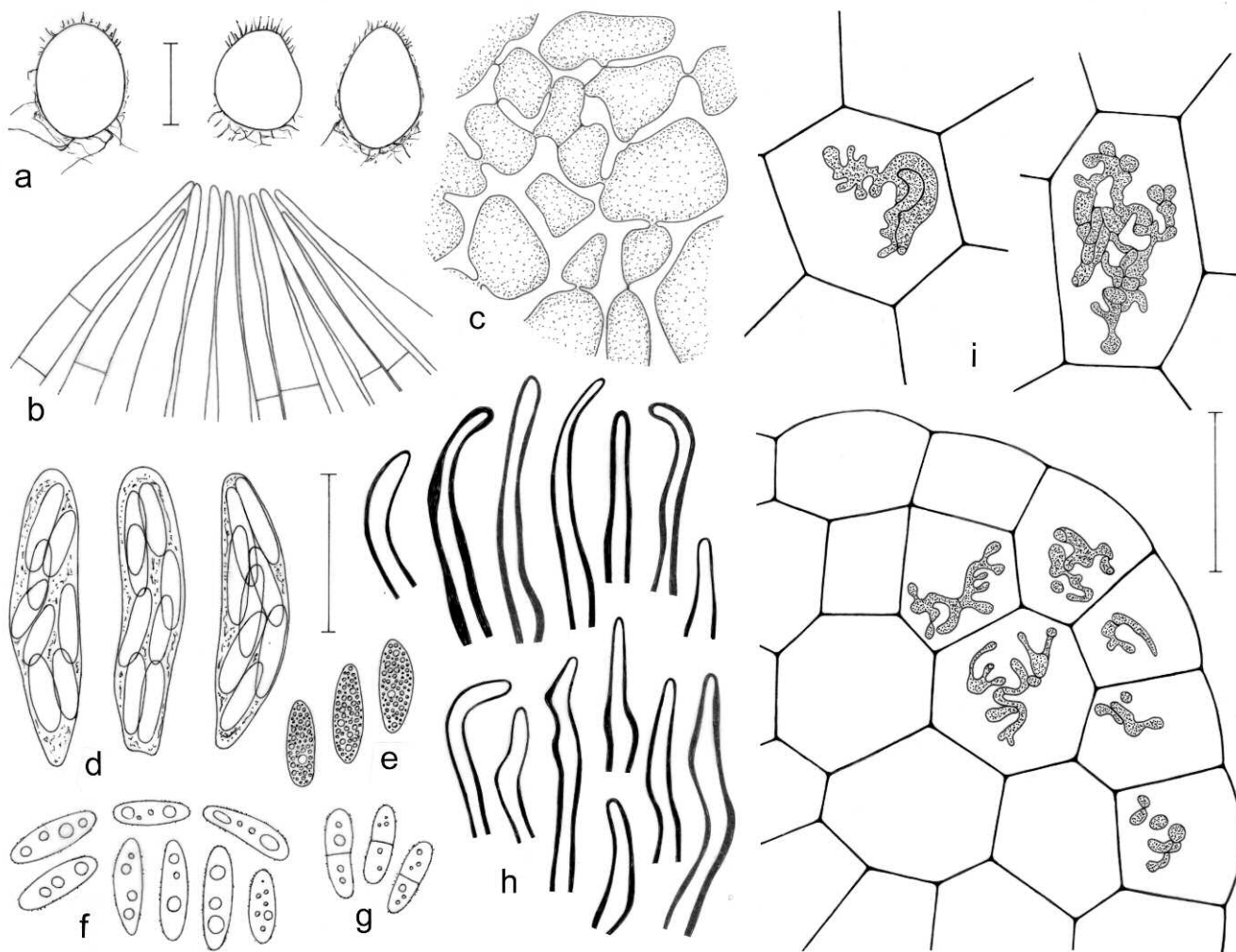


Fig. 2 – *Bryocentria octosporelloides* (holotype AH 47960). – a. Perithecia in outline. Scale bar = 100 µm. b.Periphyses. c. Perithecial wall cells seen from the outside. d. Asci. e. Ascospores in tap water. f. Ascospores in CB. g. Atypical, one-septate ascospores. h. Setae. i. Haustoria in leaf cells, superficial hyphae not indicated. Figs b–i, scale bars = 20 µm.

tosporelloides (DÖBBELER, 2004, 2010, 2018; NORDÉN *et al.*, 2015; DÖBBELER & DAVISON, 2017). The genus is well-defined by its (sub)globose, ovoid to pyriform, orange perithecia up to 280 µm diam. The setae — when present — are apical, short and blunt, the periphyses are tapering filaments, and the perithecial wall is of *textura angularis*, with a negative colour reaction in KOH. The paraphyses are *Opuntia*-like, and the asci are cylindrical to claviform, thin-walled, 8-spored, usually numerous. The ascospores are variable in shape, although in some species can be difficult to interpret by using light microscopy. Typically colourless and 2-celled, the ascospores are usually shorter than 10 µm, with cyanophilous structures often in the form of a central transverse band. The hyphae form intracellular haustoria without appressoria, and the cell walls of different hyphal structures (e.g. perithecial walls) are typically cyanophilous. Most species perforate the leaves forming fruit-bodies on the protected ventral leaf sides. Necrotrophic parasitism and infection of foliose liverworts prevails. The hosts grow under varying ecological conditions, including the phyllosphere of tropical vascular plants.

The new species fits the generic characters of *Bryocentria* very well, except that the ascospores have no central transverse septum. So far, one-celled ascospores have not been identified. In all cases where the ascospores appear non-septate, the central septum seems to be hidden by a cyanophilous band encircling the spore, e.g. in *B. phaeocarpa* Döbbeler (DÖBBELER, 2018). However, *B. octosporelloides* does have the capability to form a transverse septum. One-septate ascospores still in the hymenium have been observed several times. It is unclear whether these spores are over-mature or

not. The new species is defined by having aseptate, relatively large ascospores in combination with superficial perithecia, biotrophic parasitism and its host preference.

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