Plant and Fungal Systematics 70(1): 28–37, 2025
DOI: https://doi.org/10.35535/pfsyst-2025-0004

ISSN 2544-7459 (print)
ISSN 2657-5000 (online)

Bryocentria insolens (*Hypocreales*) – a new bryophilous ascomycete on *Lewinskya* and a preliminary phylogenetic analysis of European *Bryocentria*

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Article info
Received: 9 Mar. 2024
Revision received: 21 May 2025
Accepted: 26 May 2025
Published: 3 Jul. 2025

Associate Editor
Julia Pawłowska

Abstract. The bryophilous genus *Bryocentria* comprises nineteen species of mostly hepaticolous parasites exhibiting a range of distinctive ascospore features and host-parasite interactions. While *Bryocentria* has been relatively well studied in Europe, with several common and well-known species, new taxa continue to be encountered and most species lack molecular data. Here, we describe a new, muscicolous species of *Bryocentria* and provide molecular barcoding data for most European species. *Bryocentria insolens* sp. nov. parasitizes the mosses *Lewinskya affinis* and *L. speciosa*, with records from Switzerland and Belgium. The new species deviates from most congenerics by parasitising mosses rather than liverworts, and its ascospores lack the medial, cyanophilous band-like structures typical of most species in the genus. New molecular data from the LSU locus from *Bryocentria insolens*, *B. hypothallina*, *B. metzgeriae* and *B. cyanodesma* are combined with recent data from *B. brongniartii* and *B. confusa* in a preliminary phylogenetic analysis of the genus. These data support the hypothesis that the sequenced species form a natural group. Furthermore, they suggest that the distinctive transverse band-like structures in the medial regions of the ascospores of most *Bryocentria* species is a trait that arose after the acquisition of a bryophilous lifestyle by a likely necrotrophic hypocrealean ancestor. Finally, a key to the European members of the genus is provided to aid with identification.

Key words: bryoparasitic, hepaticolous, muscicolous parasitism, necrotrophic, *Orthotrichales*

Introduction

Hypocreales (*Ascomycota*, *Sordariomycetes*) include many species that grow on bryophytes, presenting a range of morphologies, lifestyles, and specific host-parasite interactions. This diversity spans broad-spectrum necrotrophic pathogens as well as biotrophic parasites that are often restricted to one or a few closely related host species (Döbbeler 1997). Most previous studies on bryophilous *Hypocreales* have concentrated on Europe, North America and the tropics, often focussing on individual host species or genera (e.g., Döbbeler 2006, 2018; Döbbeler & Davison 2017). The large number of species resulting from such studies show that the bryophilous *Hypocreales* are remarkably successful and numerous, suggesting that many new species remain undetected and undescribed.

The genus *Bryocentria* Döbbeler (*Hypocreales*, *Bionectriaceae*) currently comprises nineteen species of obligate parasites of bryophytes (Döbbeler 2004, 2010, 2018; Döbbeler & Davison 2017; Döbbeler et al. 2018; Greiff et al. 2024). While the type species of the genus, *B. brongniartii* (P. Crouan & H. Crouan) Döbbeler, is a biotrophic parasite specific to *Frullania dilatata* (L.) Dumort., other species, such as *B. hypothallina* B. Nordén, Gardiennet, Priou & Döbbeler, *B. metzgeriae* (Ade & Höhn.) Döbbeler and *B. cyanodesma* Döbbeler, are virulent necrotrophic pathogens, often with less host specificity or, in the case of *B. hypothallina* on *Metzgeria furcata* (L.) Corda, infecting a host that is abundant in many areas.

The plant-fungal interface is important for the assessment of the nature of parasitism in bryophilous ascomycetes, including *Bryocentria. B. brongniartii* produces superficial mycelium and occasional haustorium-like structures that penetrate host cell walls, leaving the cell cytoplasm healthy and intact (Döbbeler 2004). *B. octosporelloides* Döbbeler, J. Linde & E. Rubio on *Myriocoleopsis minutissima* (Sm.) R.L. Zhu & Y.Yu & Pócs produces a superficial mycelium that gives rise to specialized branched intracellular haustoria that seem not to kill the infiltrated cells (Döbbeler et al. 2018). The North

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American species, *B. navicula* Döbbeler & P.G. Davison and *B. pentamera* Döbbeler & P.G. Davison, have similar strategies (Döbbeler & Davison 2017). Necrotrophic parasites like *B. metzgeriae*, *B. septinensis* Döbbeler and *B. lusor* Döbbeler & P.G. Davison kill host tissues with superficial and intracellular hyphae, the latter not developing into branched haustoria (Döbbeler 2004; 2010; Döbbeler & Davison 2017). Infection by these necrotrophs is often marked by the absence of oil bodies (in liverworts) and green chloroplasts from host cells, resulting in conspicuously bleached tissues or entire plants. In *B. hypothallina*, infections can often be observed from several meters away as trees with infected populations have radiating white patches of dead tissue killed by the fungus.

Many *Bryocentria* species, especially the better-known European ones, produce bright orange perithecia covered by hair-like hyphae (tomentum) or by abundant web-like superficial mycelium, though tropical species may have colorless, yellow or brown perithecia (Döbbeler 2018). Setae may be present near the apices and the outer coverings of the ascomata are distinctively cellular with cyanophilous walls perforated by pit-like channels. The hymenia of the species often have inconspicuous, *Opuntia*-like apical paraphyses (Döbbeler 2004) and produce abundant asci and tiny 1-septate ascospores. Most species, including *B. brongniartii*, possess cyanophilous (staining strongly in lactophenol cotton blue) band-like structures that obscure the septum between 2-cells of the ascospores (see Döbbeler 2004), though the ultrastructure and possible functions of these bands are unknown. All hitherto described species except for *B. cyanodesma* and *B. septinensis* are parasitic on liverworts within the *Jungermanniopsida* (*Marchantiophyta*) and the majority have leaf-perforating (or thallus-perforating) ascomata – that is, ascomata which develop in the sheltered regions beneath bryophyte leaves (or thalli) and pierce through the host tissue with their apices when mature to liberate ascospores.

Here, we add to the diversity of *Bryocentria* by describing a new species and providing a phylogenetic analysis of the genus based on most European species known so far. *Bryocentria insolens* is found on mosses in the *Orthotrichaceae* family, recorded in Switzerland and Belgium. It is unusual based on its muscicolous parasitism and ascospores lacking the medial band-like structures found in most other species in the genus. We sequenced the LSU locus of the new species and compared it with newly generated and recently published sequences of European *Bryocentria* species (Stenroos et al. 2010; Greiff et al. 2024). We used these data to garner the first phylogenetic insights into the evolution of *Bryocentria*.

Methods

Morphological analysis

Specimens of *Bryocentria insolens* were examined fresh or a couple of weeks after collection. Squash mounts were prepared and measurements made in tap water and lactophenol cotton blue (LCB); hand sections of perithecia were produced from specimens stained in LCB.

European material of *B. cyanodesma*, *B. hypothallina*, *B. metzgeriae* and *B. octosporelloides* for sequencing was carefully collected and checked as above. Vouchers were stored in the dry state for up to three years ahead of sequencing. All sequenced voucher specimens have been deposited in the following public herbaria: Royal Botanic Garden Edinburgh (E), Botanische Staatssammlung München (M), the National Museum of Wales (NMW), and Herbarium der Eidgenössischen Technischen Hochschule Zürich (ZT).

Molecular biology

We followed a modified version of the Phire Plant Direct protocol (Fisher; F160S) as described in Greiff et al. (2024) and Greiff & Döbbeler (2024). ITS1, 5.8S and ITS2 rDNA fragments were amplified using ITS1F and ITS4 primers (White et al. 1990; Gardes & Bruns 1993), while LSU rDNA fragments were amplified using NL-1 with NL-4 (O'Donnell 1993) with annealing temperatures of 56°C and 62°C for ITS and LSU, respectively. PCR products were checked using agarose gels and purified using the Wizard SV Gel and PCR Clean-up System (Promega; A9282). Nucleic acids were quantified on a nanophotometer before being sequenced externally (by Eurofins Genomics) in accordance with the supplier's instructions.

Sequence analysis

Sequence traces were manually checked and the sequences were trimmed accordingly. Alignments were produced on the UGENE software (Okonechnikov et al. 2012) using the MAFFT method and trimmed to 478 characters. Following this, phylogenetic trees were constructed on UGENE using the PhyML Maximum Likelihood approach with a GTR substitution model and the number of substitution rate categories was changed to 6. The bootstrap value was set to 100. The type of tree improvement was set to 'SRT & NNI'. The Genbank accession numbers of the ITS and LSU sequences generated in this study are indicated in Table 1, along with additional sequences used in the phylogenetic analysis.

Results

Taxonomy

Bryocentria insolens Greiff, Stöckli, Bartholomeeusen & Döbbeler, sp. nov. Figs 1–3

MycoBank MB 859671

Diagnosis: *Bryocentria insolens* differs from other members of the genus based on muscicolous parasitism, superficial perithecia, and smooth to rough, ellipsoid ascospores lacking cyanophilous medial band-like structure. *B. insolens* grows on mosses in the genus *Lewinskya* F. Lara, Garilleti & Goffinet, namely *L. affinis* (Schrad. ex Brid.) F. Lara, Garilleti & Goffinet and *L. speciosa* (Nees) F. Lara, Garilleti & Goffinet, which have thus far not been noted as hosts for other species of *Bryocentria*.

Table 1. Genbank accessions and provenance of material used in the phylogenetic analysis.

Taxon	Country, collector, voucher (herbarium)	Host bryophyte	ITS	LSU	References
Bryocentria brongniartii	Austria, G. Greiff, L. Janošík & M. Vega, Greiff501 (ZT)	*Frullania dilatata*	–	OR872251	Greiff et al. (2024)
Bryocentria brongniartii	England, G. Greiff, Greiff456 (NMW)	*Frullania dilatata*	–	OR872250	Greiff et al. (2024)
Bryocentria brongniartii	England, G. Greiff, Greiff299 (NMW)	*Frullania dilatata*	–	OR872253	Greiff et al. (2024)
Bryocentria brongniartii	England, G. Greiff, Greiff264 (NMW)	*Frullania dilatata*	–	OR872252	Greiff et al. (2024)
Bryocentria brongniartii	Scotland, G. Greiff, L. Janošík & M. Vega, Greiff511 (NMW)	*Frullania dilatata*	–	OR872254	Greiff et al. (2024)
Bryocentria brongniartii	Germany, P. Döbbeler, M139 (TUR)	*Frullania dilatata*	EU940181	EU940105	Stenroos et al. (2010)
Bryocentria brongniartii	United Kingdom, S. Huhtinen, M190 (TUR)	*Frullania dilatata*	–	EU940125	Stenroos et al. (2010)
Bryocentria confusa	Wales, C. Preston, Greiff260 (NMW)	*Frullania dilatata*	–	OR872257	Greiff et al. (2024)
Bryocentria confusa	England, G. Greiff, Greiff238 (NMW)	*Frullania dilatata*	–	OR872258	Greiff et al. (2024)
Bryocentria confusa	England, G. Greiff, Greiff416 (NMW)	*Frullania dilatata*	–	OR872256	Greiff et al. (2024)
Bryocentria confusa	England, G. Greiff, holotype (NMW)	*Frullania dilatata*	–	OR872255	Greiff et al. (2024)
Bryocentria brongniartii (anamorph)	England, G. Greiff, Greiff709 (ZT)	*Frullania dilatata*	–	PP736106	Greiff et al. (2024)
Bryocentria brongniartii (anamorph)	Switzerland, A. Büschlen, AB20231226_4 (ZT)	*Frullania dilatata*	–	PP736107	Greiff et al. (2024)
Bryocentria brongniartii (anamorph)	Switzerland, A. Büschlen, AB20231226_5 (ZT)	*Frullania dilatata*	–	PP736108	Greiff et al. (2024)
Bryocentria cyanodesma	**Italy, E. Stöckli, s.n. (E)**	***Tortella tortuosa*; *Amblystegium serpens***	**OQ911494**	**OQ918670**	**This study**
Bryocentria hypothallina	**England, G. Greiff, Greiff283 (E)**	***Metzgeria furcata***	**–**	**OQ918671**	**This study**
Bryocentria hypothallina	**England, G. Greiff, Greiff414 (NMW)**	***Metzgeria furcata***	**–**	**OR872244**	**This study**
Bryocentria hypothallina	**England, G. Greiff, Greiff274 (NMW)**	***Metzgeria furcata***	**–**	**OR872246**	**This study**
Bryocentria hypothallina	**Scotland, G. Greiff, Greiff306 (NMW)**	***Metzgeria furcata***	**–**	**OR872247**	**This study**
Bryocentria hypothallina	**Wales, G. Greiff & S. BenJeddi, Greiff439 (NMW)**	***Metzgeria furcata***	**–**	**OR872245**	**This study**
Bryocentria insolens	**Belgium, F. Bartholomeeusen, s.n. (E)**	***Lewinskya affinis***	**OQ921084**	**OQ921086**	**This study**
Bryocentria insolens	**Switzerland, E. Stöckli, s.n. Holotype (ZT, M)**	***Lewinskya affinis***	**OQ921085**	**OQ921087**	**This study**
Bryocentria metzgeriae	Germany, P. Döbbeler, M140 (TUR 178068)	*Radula complanata*	EU940182	EU940106	Stenroos et al. (2010)
Bryocentria metzgeriae	**Austria, G. Greiff, L. Janosík, M. Vega, Greiff491 (M)**	***Radula complanata***	**–**	**OR872248**	**This study**
Bryocentria metzgeriae	**Scotland, L. Janošik, G. Greiff & M. Vega, Greiff525 (NMW)**	***Metzgeria furcata***	**–**	**OR872249**	**This study**
Geosmithia pallida	United Kingdom, BCIRA [*Bionectriaceae* outgroup for phylogeny]	NA	–	OQ055509	Hou et al. (2023)
Hypocrea citrina	Not provided [outgroup for phylogeny]	NA	–	EU481408	Miller & Huhndorf, direct submission
Nectria cinnabarina	Not provided [outgroup for phylogeny]	NA	–	MH876869	Vu et al. (2019)
Stephanonectria keithii	Netherlands, J. W. Veenbaas-Rikjs [*Bionectriaceae* outgroup for phylogeny]	NA	–	MH871546	Vu et al. (2019)
Stilbocrea macrostoma	France, C. Lechat [*Bionectriaceae* outgroup for phylogeny]	NA	–	OQ430123	Zhao et al. (2023)

Type: Switzerland, Jura, Les Breuleux, La Haisou Neuve, 47.2297°N, 7.0329°E; alt. 1,023 m, on *Lewinskya affinis*, 19 Feb. 2023, E. Stöckli s.n. (Holotype: ZT Myc 0066943; Isotype: M).

Etymology. *Insolens* (Latin): insolent, unfaithful – relating to the unusual muscicolous ecology, necrotrophic parasitism, superficial perithecia and ascospores lacking cyanophilous bands; features absent in the type species of the genus, *B. brongniartii*.

Description. Ascomata perithecia, orange to orange-red, fading to white during storage in the herbarium, superficial, solitary or gregarious, not merging, developing from a superficial mycelial web on the abaxial or adaxial host leaf surface, sometimes between leaves, ovoid, conical or pyriform, 110–150 µm tall, 90–130 µm wide, with a well-developed hyaline, irregular tomentum arising from the outer excipular cells in the lower and medial regions, lower regions with anchoring hyphae. Apical regions of the peridium with hyaline setae, especially around the ostiole in a corona-like form, 1–2(–3)-celled, mostly 1-celled around ostiole, up to 25 × 3 µm (in H_2O). Ostiole visible as an orange-red spot under low power magnification in dry material, ostiolar canal lined with delicate periphyses reaching 15 µm long and 1.5 µm wide at the base that emerge in a comb-like structure in squash preparations. Excipulum comprises ± isodiametric cells that may be angular or rounded, (4.5–)6–8(–9) µm wide, no reaction in KOH; cell walls distinctly cyanophilous, cells occasionally with merging lumina via narrow pit-like channels. Apical paraphyses not observed, likely ephemeral, delicate and dissolving early. Asci unitunicate, thin-walled, cylindrical, clavate or inflated medially, (35–)40–50 × 5–7 µm in H_2O, 32–35 × 3–4.5 µm in LCB, (7)8-spored, abundant, with uni- to biseriately arranged ascospores. Ascospores hyaline to subhyaline, narrowly ellipsoidal, 1-septate, one half usually wider than the other, not or hardly constricted at the septum, epispore appearing smooth or finely rough to distinctly rough when overmature, spore halves occasionally separating in squash mounts, cyanophilous band absent, (6.2–)6.5–8.5(–9.5) × 2.2–2.8(–3.3) µm in H_2O, (6–)6.5–7.5(–8) × (2–)2.5–3(–3.5) µm in LCB; living ascospores contain small numbers of yellowish droplets, epispore cyanophilous in LCB. Hyphae hyaline to greyish close to the perithecia, arachnoid, becoming hyaline and more inconspicuous further away, growing superficially over both surfaces of infected leaves and occasionally penetrating the basal host leaf cells and growing intracellularly within them, 1.5–3 µm diam., intracellular hyphae not obviously differentiated, but traversing anticlinal host cell walls by fine perforation hyphae. Appressoria and haustoria not observed. Asexual morph not observed.

Hosts. *Lewinskya affinis*, *L. speciosa*.

Habitat. Specimens were found on trees and shrubs bordering predominantly open areas of managed grassland. The type population was found in hedgerows at the border between two grazed pastures, while the Belgian samples were found on bushes of *Sambucus nigra* beside a wooded path along the banks of a river in a wider area of open grassland.

Known distribution. Belgium, Switzerland.

Additional specimens examined. BELGIUM. Frans Segersreservaat, 2300 Turnhout, 51.2932°N, 4.9163°E, alt. ~12 m, on *Lewinskya affinis* on *Sambucus nigra*, 22 Feb. 2023, F. Bartholomeeusen s.n. (E); additional collection with the same metadata, but on 16 Mar. 2023, F. Bartholomeeusen s.n. (E). SWITZERLAND. Berne, Tavannes, Pâturage d'Orange, 47.23182°N, 7.15348°E, alt. 851 m, on *Lewinskya affinis* [and possibly sterile *L. speciosa*] on trees of *Viburnum*, *Salix* and *Sambucus*, 2 Apr. 2023, E. Stöckli s.n. (M). – Grisons, Albula valley east of Tiefencastel, floodplain and spruce forest near Zinols, between Alvaneu Bad and Filisur, on the southern side of the Albula river (about 2 km WSW of Filisur), approx. 46.6949°N, 9.4838°E, alt. 980–1,000 m, on *Lewinskya speciosa* together with *Belonium coroniforme*, 1 Sept. 2005, P. Döbbeler 9195a (M).

Biological remarks

Perithecia of *B. insolens* form singly or in groups on both the abaxial and adaxial surfaces of infected host leaves, emerging from superficial mycelial webs. Leaves harboring perithecia often contained algae and other debris, suggesting weakening from necrotrophic parasitism. Often, only the tips of infected plants remained green while the lower leaves were brown.

Octospora affinis Benkert & L.G. Krieglst. (Benkert & Krieglsteiner 2006) was observed in the Belgian collections while *Belonium coroniforme* Rehm (Döbbeler et al. 2022) was observed in the Swiss collection on *Lewinskya speciosa*. Young apothecia of these *Octospora* Hedw. species superficially resemble the perithecia of *B. insolens*, but they develop specifically on the rhizoid-matted lower host stems, whereas the *Bryocentria* produces perithecia nearer to the shoot apices. *Belonium coronforme* produces larger discocarpous ascomata nestled in the shoot apices. The same host shoot may therefore harbor all three fungi, each specialized to a different host microniche.

Lewinskya affinis is one of the most common and widespread orthotrichalean mosses growing in a variety of habitats across Europe, parts of Asia and North America (Blockeel et al. 2014). *L. speciosa* is generally rarer, particularly in the British Isles where it is confined to inland, submontane localities (Blockeel et al. 2014). In the Alps and other parts of mainland Europe, it is more common, often occupying similar habitats to *L. affinis*.

Morpho-taxonomic remarks

Bryocentria insolens fits the generic concept of *Bryocentria* very well, particularly as new species have been added since the circumscription of the genus in 2004. Perithecia are small, often orange, with angular excipular cells and cyanophilous cell walls. Superficial mycelial webs are present at the bases of the perithecia (Döbbeler 2004). Fleeting apical paraphyses may or may not be apparent in mature perithecia, which produce numerous small, thin-walled asci and tiny, 1-septate ascospores. In these respects, the new species compares well with *B. brongniartii*, the type species of the genus (Döbbeler 2004). Spore morphology, including the presence of cyanophilous medial bands, is considered to be variable in *Bryocentria*, as is the propensity for perithecia to perforate through host cell layers or not (Döbbeler 2010; Nordén et al. 2015; Döbbeler & Davison 2017; Döbbeler et al. 2018).

B. insolens is ecologically similar to the two other *Bryocentria* species that parasitize mosses and develop superficial perithecia, namely *B. cyanodesma* and *B. septinensis*. *B. cyanodesma* is very similar, growing on the predominately saxicolous mosses *Grimmia pulvinata* (Hedw.) Sm., *Schistidium apocarpum* (Hedw.) Bruch & Schimp. and *Tortella tortuosa* (Hedw.) Limpr. (Döbbeler 2004). Our specimen of *B. cyanodesma* had perithecia on *T. tortuosa* and intermingled *Amblystegium serpens* (Hedw.) Schimp., a new host for that species (see specimens examined below). *B. cyanodesma* has shorter, narrower, smooth-walled ascospores that possess distinct medial cyanophilous bands – the latter being notably absent in *B. insolens*.

B. septinensis, known only from the type collection in Finland (Döbbeler 2010), forms superficial perithecia on the moss *Sanionia uncinata* (Hedw.) Loeske and differs from the new species in its distinctly doliform (barrel-shaped) rough-walled ascospores with cyanophilous bands. The two have similar peridium structures, however, with relatively strong, irregular tomenta formed of adjacent hyphae, along with short setae near the ostiole.

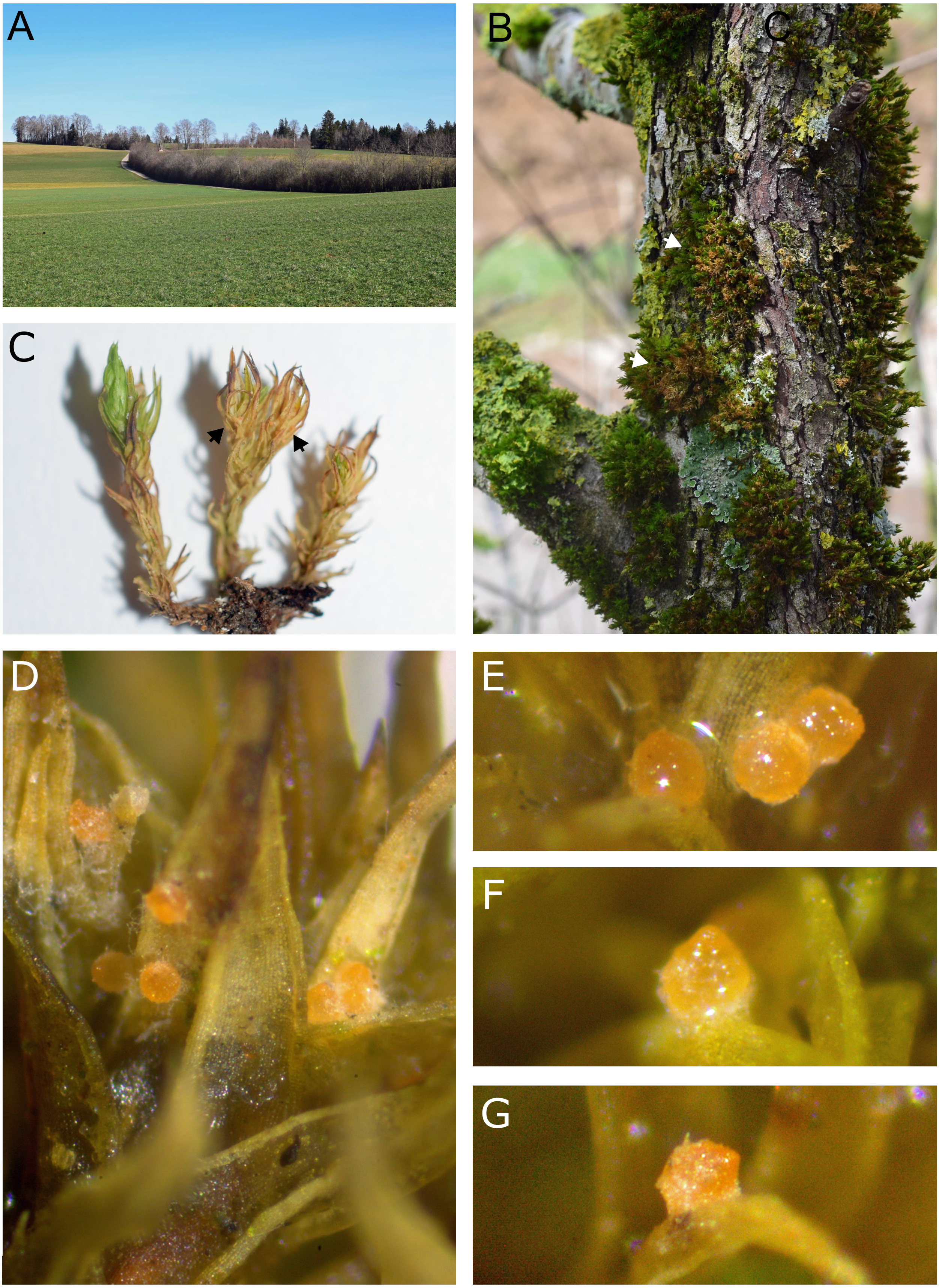

Figure 1. *Bryocentria insolens* – habitat of the holotype collection. A – hedgerow with infected host populations; B – infected host shoots with discolored basal parts and green apical parts; C – infected host shoots with perithecia indicated in the central shoot; D–G – macro-photographs of perithecia on infected host plants (photos: E. Stöckli).

The five hepaticolous *Bryocentria* species reported in Europe, *B. brongniartii*, *B. confusa* Greiff & Döbbeler, *B. hypothallina*, *B. metzgeriae* and *B. octosporelloides*, have never been recorded on mosses. Except for the enigmatic *B. octosporelloides*, these species develop leaf- or thallus-perforating perithecia and all of them have deviating ascospore morphology to the new species presented here. *B. hypothallina* is the most similar morphologically (and molecularly, see below), but has larger spores with a more conspicuously and coarsely warted epispore, as well as strict host specificity for the liverwort *Metzgeria furcata* (Nordén et al. 2015).

Outside of the nineteen other currently accepted species of *Bryocentria*, *B. insolens* is very similar to the muscicolous parasite *Nectria voratella* Döbbeler, known from a few records in France and Italy, on *Ctenidium molluscum* (Hedw.) Mitt., *Homalothecium sericeum* (Hedw.) Bruch, Schimp. & E. Guembel and *Leucodon sciuroides* (Hedw.) Schwägr. (Döbbeler 2005). *N. voratella* has pyriform, yellow to colorless (two years after collecting, color presumably fading over time) perithecia that measure 120–190 × 100–150 µm with outer peridium wall cells measuring 8–12 µm. The main differences are that the ascus walls of *N. voratella* appear to dissolve upon reaching maturity, passively releasing the ascospores. *N. voratella* ascospores deviate from *B. insolens* in their shape and size, (5.5–)6–8.5(–10) × (3–)3.5–4.5(–5) µm in the former vs (6.2–)6.5–8.5(–9.5) × 2.2–2.8(–3.3) µm in *B. insolens*. Although both species parasitize bryopsid mosses, the hosts of *N. voratella* belong to the *Hypnales*

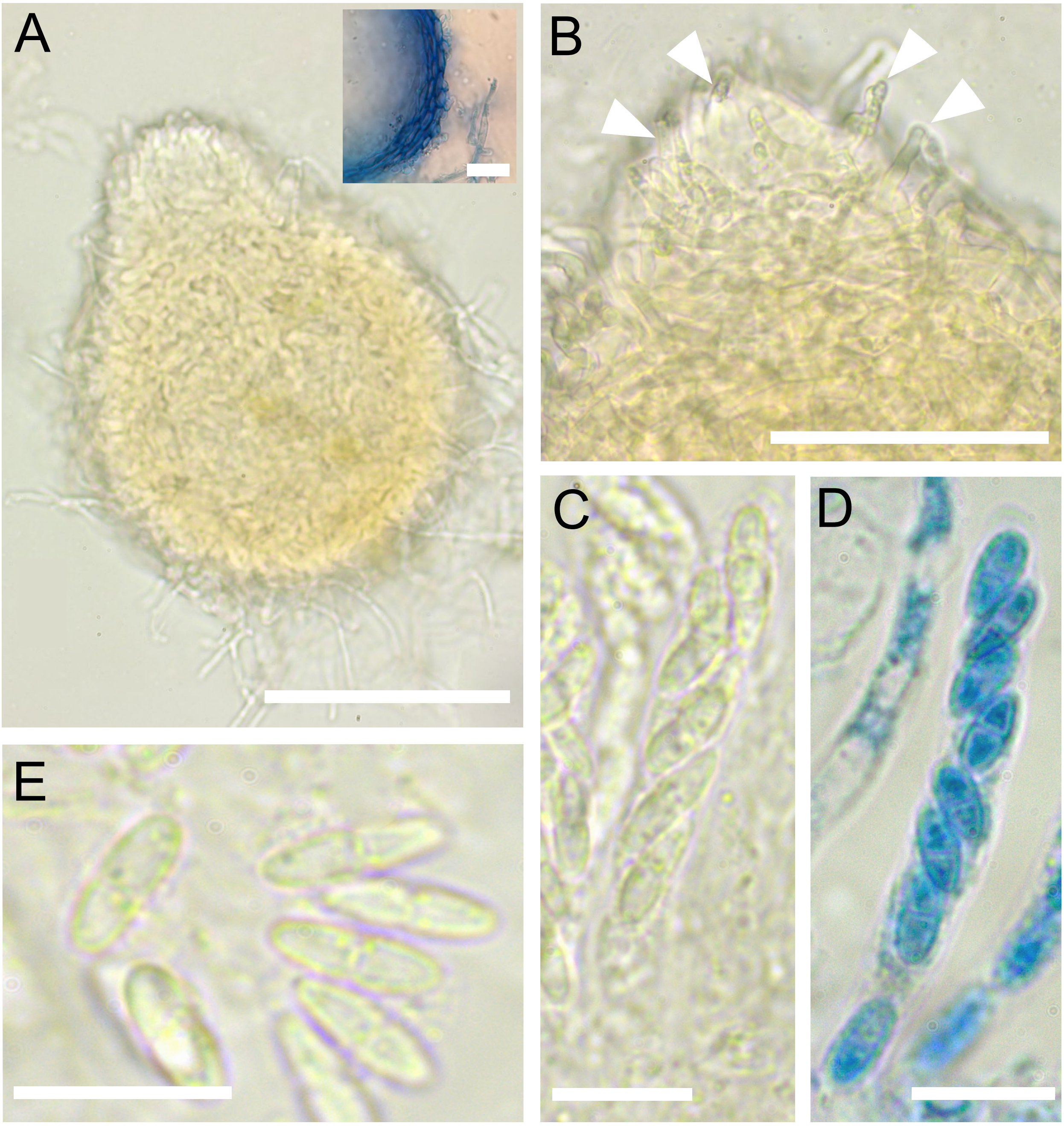

Figure 2. *Bryocentria insolens* – micro-morphology of the holotype. A – perithecium in water, inset showing section of the peridium in lactophenol cotton blue; B – apical setae (arrowed) around ostiole; C–D – mature asci in water and lactophenol cotton blue, respectively; E – ascospores in water. Scale bars: A = 60 µm (inset 10 µm), B = 50 µm, C–E = 10 µm (photos: A–E: E. Stöckli, A inset: G. R. L. Greiff).

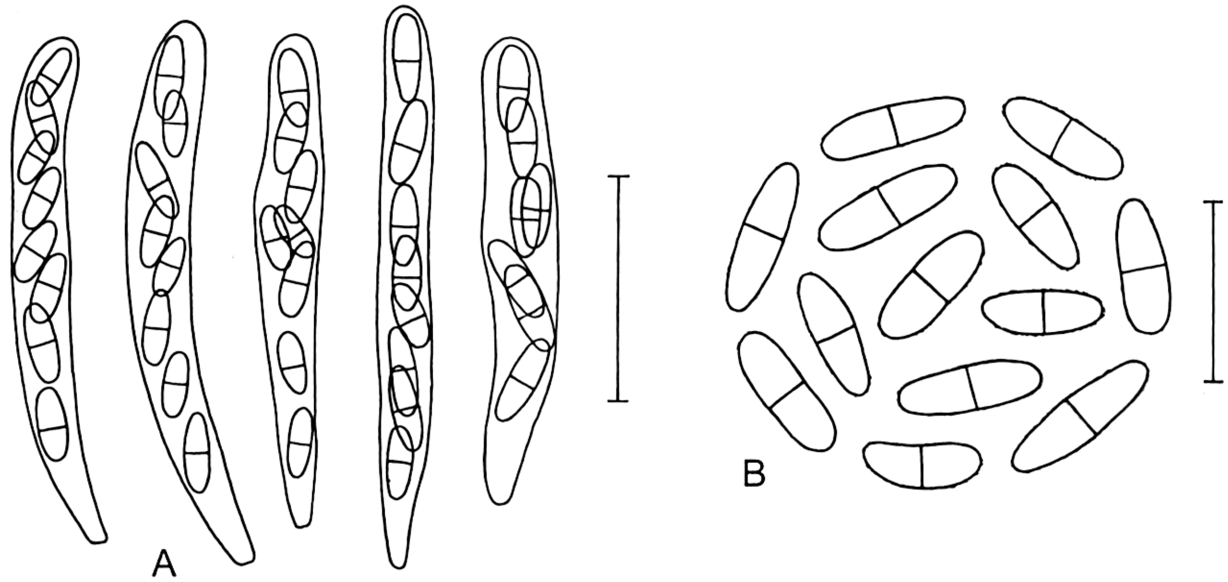

Figure 3. *Bryocentria insolens* – schematics showing asci and ascospores. Scale bars: A = 20 µm, B = 10 µm (drawn by P. Döbbeler).

while those of *B. insolens* belong to the *Orthotrichales*. *Nectria voratella* should be re-assessed in future studies as it appears to be closely related to *B. insolens*.

Molecular analysis

Since previously published molecular data from *Bryocentria brongniartii* and *B. metzgeriae* only included short sequences of the ITS2 locus (Stenroos et al. 2010), we decided to focus on generating LSU sequence data for the purposes of comparing our results with previous work. We amplified fragments of the LSU locus from fresh and herbarium material of most European *Bryocentria* species, excluding *B. septinensis*, which remains to be only known from the type collection in Finland (Döbbeler 2010). In addition to *B. insolens*, our European *Bryocentria* specimens include recently published samples of *B. brongniartii* and *B. confusa* (Greiff et al. 2024), as well as *B. cyanodesma*, *B. hypothallina*, *B. metzgeriae* and *B. octosporelloides*. Despite multiple attempts, we failed to obtain molecular data from *B. octosporelloides* due to contaminating lichens growing amongst the infected hosts.

The sequences obtained from *Bryocentria insolens* are fully identical to one another, congruent with morphological information from the collections studied. Initial analysis of the *B. insolens* LSU locus by BLASTn searches showed 95.99% similarity to *B. metzgeriae* (EU940182; Stenroos et al. 2010). When supplemented with the additional sequences generated in this study, the *B. insolens* sequences are 97.24% identical to those

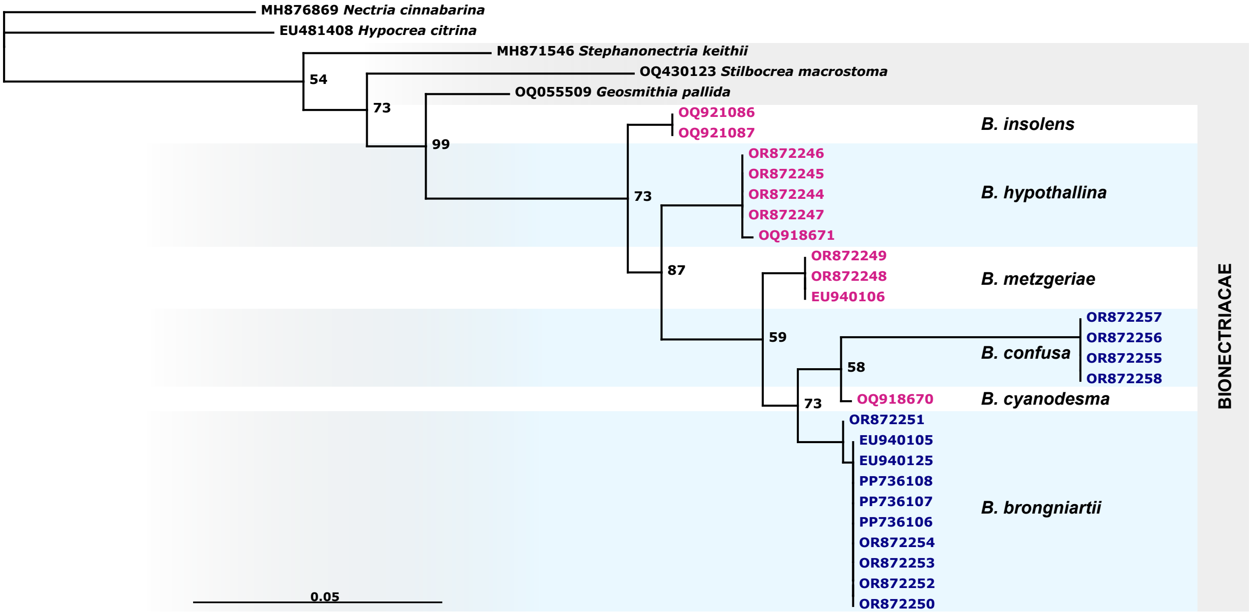

Figure 4. Maximum likelihood analysis of nuclear LSU sequences of *Bryocentria* and related *Hypocreales* based on the LSU locus with 100 bootstrap replicates. The tree is rooted on *Nectria cinnabarina* and *Hypocrea citrina*. Magenta labels indicate necrotrophic species while blue labels indicate biotrophic species.

produced from *B. hypothallina* and approximately 95–96% identical to the other species sampled. Generic placement in *Bryocentria* is therefore strongly supported by the molecular data.

We then performed a maximum likelihood phylogenetic analysis on our data to determine the relationships between species in *Bryocentria*. Two previous studies using large-scale phylogenetic datasets showed that *Bryocentria* fits within the *Bionectriaceae*, closely related to *Stephanonectria* Schroers & Samuels (Stenroos et al. 2010) and more so to *Geosmithia* Pitt (Perera et al. 2023). We focused specifically on *Bryocentria* in a small-scale analysis, using *Geosmithia pallida* (G. Sm.) M. Kolařík, Kubátová & Pažoutová, *Stephanonectria keithii* (Berk. & Broome) Schroers & Samuels and *Stilbocrea macrostoma* (Berk. & M.A. Curtis) Höhn. as representative closely related *Bionectriaceae* species based on Perera et al. (2023). Our tree broadly recapitulated the topology obtained in that study, but the sister position to *Bryocentria* was occupied by *Geosmithia* rather than by *S. macrostoma*, although with only moderate support (73%). *Bryocentria* is recovered as a strongly supported monophyletic clade containing all six sequenced species.

The relationships between the taxa within *Bryocentria* are less strongly supported, constrained by the analysis being based on a single locus. However, the following trends are evident in our dataset. Firstly, *B. brongniartii*, *B. confusa*, *B. cyanodesma* and *B. metzgeriae* form a well-supported clade. These species are unified morphologically by possessing transverse band-like structures around the septum position of their ascospores that react strongly in lactophenol cotton blue. *B. hypothallina* is sister to this clade, while *B. insolens* is sister to the clade containing the banded species plus *B. hypothallina*. The relationships between the taxa, particularly within the group containing *B. brongniartii*, are not strongly resolved and require the sampling of additional loci. The biotrophic *B. brongniartii* and *B. confusa* may have evolved from necrotrophic ancestor species as both are nested within an otherwise necrotrophic array of sampled species.

Specimens sampled for molecular analysis

Bryocentria cyanodesma

Italy, Trentino-Alto Adige, Bolzano, Franzenhöhe, 46.53156°N, 10.48214°E, alt. 2,230 m, on *Tortella tortuosa* and *Amblystegium serpens* growing together on stone wall, 14 Aug. 2022, E. Stöckli s.n. (E).

Bryocentria hypothallina

England, Isle of Wight, Parkhurst, UK grid ref. SZ 4764 9203, 50.725946°N, 1.3264231°W, alt. ±44 m, on *Metzgeria furcata* on *Fagus sylvatica*, 28 Dec. 2022, G.R.L. Greiff 283 (E). – Briddlesford Copse, UK grid ref. SZ54569030, 50.709782°N, 1.2286479°W, alt. 31 m, on *Metzgeria furcata* on dead *Fraxinus excelsior* trunk, 23 Dec. 2021, G.R.L. Greiff 274 (NMW). – Bonchurch Landslip, UK grid ref. SZ58157889, 50.606838°N, 1.1795981°W, alt. 80–100 m, on *Metzgeria furcata* also infected by *Epibryon hepaticicola* on *Fagus sylvatica*, 23 Dec. 2022, G.R.L. Greiff 414 (NMW).

Scotland, Isle of Arran, Glenashdale Falls, UK grid ref. NS03442544, 55.483140°N, 5.1122548°W, alt. 90–110 m, on *Metzgeria furcata* on large tree of *Fagus sylvatica*, 28 July 2022, G.R.L. Greiff 306 (NMW).

Wales, Monmouthshire, Bargain Wood, UK grid ref. SO524030, 51.723689°N, 2.6905158°W, alt. ±215 m, on epiphytic *Metzgeria furcata*, 4 Mar. 2023, G.R.L. Greiff 439 (NMW).

Bryocentria metzgeriae

Austria, Tyrol, Umhausen, 47.130363°N, 10.940976°E, alt. ~1,200 m, on epiphytic *Radula complanata*, 27 Aug. 2022, G.R.L. Greiff 491, L. Janošík & M. Vega (M).

Scotland, Moray, Bridge of Logie, UK grid ref. NJ002497, 57.526412°N, 3.6681615°W, alt. ±92 m, on epiphytic *Metzgeria furcata*, 17 Sept. 2023, L. Janošík, G.R.L. Greiff 525 & M. Vega (NMW).

Bryocentria octosporelloides

England, Isle of Wight, Brading Down, UK grid ref. SZ598867, 50.677257°N, 1.1550188°W, alt. ±70 m, on *Myriocoleopsis minutissima* on *Acer pseudoplatanus*, 10 Apr. 2023, G.R.L. Greiff 384 (NMW).

Discussion

This study adds to our knowledge of the bryophilous genus *Bryocentria* by describing a new muscicolous species and provides the first phylogenetic analysis of the genus based on six European species. We present new records of *B. hypothallina*, *B. metzgeriae* and *B. octosporelloides* for the British Isles, while *Amblystegium serpens* is a new host for *B. cyanodesma*.

Novel and interesting biological features of *Bryocentria* continue to be discovered, but much yet remains unknown. For example, Greiff et al. (2024) recently described an asexual morph of the type species, *B. brongniartii*, suggesting that the dispersal and/or reproduction of these fungi is more complex than previously thought. On the other hand, functions of the rather unique band-like structures in the spores of most species in the genus remain unknown. Our phylogenetic analysis suggests that these bands arose after an ancestral and probably necrotrophic species acquired a bryophilous lifestyle, but further studies are needed to understand these structures.

This first look at the systematics of *Bryocentria* can only be preliminary. New data are required from other morphologically related bryophilous *Hypocreales*, especially *Bryotria* Döbbeler & P.G. Davison and *Laniatria myxostoma* Döbbeler & P.G. Davison (Döbbeler & Davison 2017), as well as several *Nectria* s.lat. species with similar peridium morphology such as *N. voratella*, *N. hyperepiphylla* Döbbeler (Döbbeler 2005), and *N. contraria* Döbbeler (Döbbeler 1998), among others. Indeed, the sampling of *Bryocentria* itself remains very much incomplete, with multiple tropical and North American species absent from this analysis. Future studies should aim to improve the taxon sampling and the number of loci used. Nonetheless, this study shows that, thus far, the LSU locus is sufficiently variable to act as a species-specific molecular marker for *Bryocentria*, particularly the European species.

In a general sense, *Bryocentria* is homogeneous. Similar species are differentiated by specific changes in relatively few obvious variable traits, such as spore morphology, host selection, and nutritional mode. Necrotrophy is common in the group, but some species develop elaborate haustoria and live as biotrophic parasites. This study helps set the groundwork for further investigations into the evolution of fungal lifestyles, taxonomy and biology in *Bryocentria* and bryophilous *Hypocreales* more generally.

Key to the European species of *Bryocentria*

1 Ascospores distinctly fusiform or doliform 2

Ascospores cylindrical, ellipsoid or dumbbell-shaped . . . 3

2(1) Ascospores fusiform, 5.5–7.5 × 1.5–2 µm, with a distinct medial cyanophilous band. Perithecia leaf-perforating; necrotrophic on *Frullania dilatata*, *F. fragilifolia*, *Porella platyphylla*, *Porella* sp., *Lejeunea cavifolia*, *Metzgeria furcata*, *Radula complanata* (most common host) . . . . **B. metzgeriae**

Ascospores doliform, 6–7 × 3–3.5 µm, epispore with delicate cyanophilous warts (but may appear smooth), with a cyanophilous medial band. Perithecia superficial; necrotrophic on *Sanionia uncinata* . . . . . **B. septinensis**

3(1) Ascospores strongly dumbbell-shaped, 6–8 × 2–2.5 µm, with a distinct medial cyanophilous band. Perithecia leaf-perforating; biotrophic, frequently on *Frullania dilatata* . . . . **B. brongniartii**

Ascospores cylindrical, narrowly ellipsoid or weakly dumbbell-shaped 4

4(3) Ascospores rough-walled, cyanophilous band-like structures absent 5

Ascospores smooth-walled, cyanophilous band-like structures present 7

5(4) Ascospores usually shorter than 10 µm 6

Ascospores usually longer than 10 µm, 9–14 × 3.5–4.5 µm, narrowly ellipsoid, epispore minutely rugose (not easily apparent in British material). Perithecia superficial; biotrophic parasite on *Myriocoleopsis minutissima* . . . . **B. octosporelloides**

6(5) Ascospores narrowly ellipsoid, 7.5–10 × 2.5–3.5 µm, epispore minutely rugose with some larger warts. Perithecia thallus-perforating; necrotrophic on *Metzgeria furcata* . . . . **B. hypothallina**

Ascospores narrowly ellipsoid, 6.5–8.5 × ~2–3 µm, epispore minutely rugose or occasionally appearing smooth. Perithecia superficial; necrotrophic on *Lewinskya affinis* and *L. speciosa* . . . . **B. insolens**

7(4) Ascospores subcylindrical to narrowly ellipsoid, 5.5–7.5 × 1.5–2 µm, with a distinct, narrow medial cyanophilous band. Perithecia superficial, formed on a superficial white mycelium; necrotrophic on *Amlystegium serpens*, *Grimmia pulvinata*, *Schistidium apocarpum*, and *Tortella tortuosa* . . . . **B. cyanodesma**

Ascospores cylindrical to weakly dumbbell-shaped, 8–10 × 2–2.5 µm, with a distinct medial cyanophilous band. Perithecia superficial or leaf-perforating; biotrophic on *Frullania dilatata* . . . . **B. confusa**

Acknowledgements

GG thanks the British Mycological Society and Bristol Naturalists' Society for small sequencing grants, and K. Slade, N. Smith, M. Vega, L. Janošík, A. Bailey and J. Harrison for support. We thank the curators of E, M, NMW and ZT for facilitating the deposition of specimens or providing material. We also thank the anonymous reviewers for their feedback on the manuscript.

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