

Diplolaeviopsis symmictae (Helotiales, Ascomycota), a new lichenicolous fungus on *Lecanora symmicta*

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Abstract. The new *Diplolaeviopsis symmictae*, lichenicolous on *Lecanora symmicta* in the U. S. A. and in Scotland, differs from *D. ranula* by an olivaceous, K– excipular and pycnidial pigment, and by a different host selection.

1. Introduction

The genus *Diplolaeviopsis* Giralt & D. Hawksw. was described for a lichenicolous coelomycete with hyaline, 1-septate conidia with unequal cells confined to the *Lecanora strobilina* group (Giralt & Hawksworth 1991). The type species *D. ranula* Giralt & D. Hawksw. is rare, but widespread in Europe and America.

Suija et al. (2014) reported the discovery of blackish apothecia intermixed with *D. ranula* pycnidia in two collections from the Azores and Italy. These apothecia strongly resemble those of the helotialean genus *Skyttea* Sherwood, D. Hawksw. & Coppins, as circumscribed by Diederich & Etayo (2000), but are easily distinguished by a different ascus type and a different excipular pigment. Molecular data of these apothecia confirm that they belong to a helotialean species distinct from *Skyttea* (Suija et al. 2014). Although no sequences are available from the *D. ranula* pycnidia, Suija et al. (2014) hypothesized that both pycnidia and apothecia are two morphs of the same species, *D. ranula*. This hypothesis has not been confirmed by molecular results, but is based on the co-occurrence of both morphs on the same host thallus in two specimens from different geographical origins, and on the presence of the same pigment with the same reaction in K

in both the ascomatal exciple and the pycnidial wall.

In this paper, we describe from *Lecanora symmicta* a second species of *Diplolaeviopsis* from North America (Arizona) and Europe (Scotland), the type specimen of which also shows a mixture of both sexual and asexual morphs.

2. Material and Methods

Dry herbarium specimens were examined and measured under a binocular microscope Leica MZ 7.5 (magnification up to 50×). Macroscopic photographs were done using a Canon 40D camera with a Nikon BD Plan 10 microscope objective, StackShot (Cognisys) and Helicon Focus (HeliconSoft) for increasing the depth of field. Hand-made sections of ascomata were studied in water, 10% KOH (K), concentrated nitric acid (N), Congo Red and Lugol's iodine, without (I) or with (K/I) pre-treatment with KOH. Microscopic photographs were prepared using a Zeiss Photomikroskop III with a Canon PowerShot G3 camera and a Leica DMLB microscope with DIC optics and a Leica EC3 camera. Drawings were done using a Zeiss Photomikroskop III fitted with a drawing tube.

3. Results

Diplolaeviopsis symmictae Diederich & Coppins sp. nov. (Figs 1–3)

Differs from *Diplolaeviopsis ranula* by an olivaceous, K⁻ (versus brown, K⁺ purplish) excipular and pycnidial pigment, longer excipular hairs [28–35(–65) μm long, versus 15–31 μm] and by a different host selection (*Lecanora symmicta*).

Type: U. S. A., Arizona, Apache Co., Mount Baldy Wilderness, along west fork of the Little Colorado River above Sheep's crossing, 33°57'N, 109°31'W, 2830 m, spruce–fir forest, on *Lecanora symmicta*, 30 Sept. 1997, T. H. Nash III 39357 (ASU—holotype; BR, hb. Diederich—isotypes; the new species is typified on ascomata, not on pycnidia, in case both morphs were not conspecific).

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Ascomata apothecia, dispersed on the host thallus and apothecia, superficial, blackish, partly to almost entirely covered by whitish hairs, 80–160 μm diam.; margin in opened ascumata 20–40 μm thick, \pm smooth; ascumatal pore 20–80 μm diam. *Exciple* laterally pale to relatively dark olivaceous green, K⁻, N⁻ (or more yellowish), 14–30 μm thick, mainly composed of the basal parts of the hairs, basally hyaline to pale brown, K⁻, N⁻, 20–70 μm thick, composed of elongate cells of 4.5–11 \times 2.5–4 μm (in optical section);

excipular hairs abundant, hyaline to pale brown, K⁻, N⁻, aseptate, smooth, 28–35(–65) \times 2–3.5 μm , apically thin-walled, not refractive. *Hymenium* hyaline, 25–45 μm tall, I⁻, K/I⁻; epihymenium hyaline; subhymenium hyaline, 5–20 μm thick. *Paraphyses* septate, simple, 1.7–2.3 μm thick, apically not thicker. *Asci* clavate, unitunicate, wall laterally thin, apically slightly thickened, I⁻, K/I⁻, (5–)8-spored, 22–35 \times 6.5–7 μm ; ascogenous hyphae with croziers. *Ascospores* ellipsoid to narrowly ellipsoid, aseptate, smooth, hyaline, probably biguttulate (only dead spores examined), 5.5–7(–9) \times 2.3–3 μm ; ratio length/width c. 2.2–2.5; perispore absent.

Conidiomata pycnidia, arising singly, unilocular, scattered over the thallus and in the apothecia of the host, immersed to slightly erumpent, subglobose, becoming cupulate when mature, dark brown to black, 100–160 μm diam.; ostiole visible as a white or pinkish pore, enlarging at age and reaching the diameter of the entire pycnidium; conidiomatal wall hyaline to olivaceous, K⁻, 8–20 μm thick, composed of 2–6 layers of pseudoparenchymatous cells, cells \pm rounded to irregularly polyhedral. *Conidiophores* arising from the inner wall of the pycnidial cavity, subcylindrical, with a few septa,

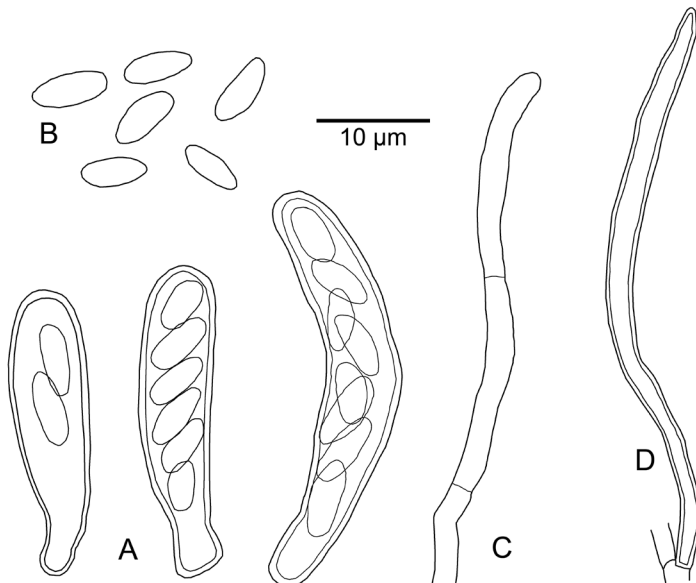


Fig. 1. *Diplolaeviopsis symmictae* (holotype). A, asci. B, ascospores. C, paraphysis. D, excipular hair.

simple or branched, hyaline, smooth-walled. *Conidiogenous cells* arising terminally from short conidiophores, 1–2 per conidiophore, cells subcylindrical, with a slight collar, hyaline, smooth, $5\text{--}6 \times 2.5\text{--}3.5 \mu\text{m}$. *Conidia* holoblastic, arising singly, acrogenous, abundant, dry, elongate soleiform to tadpole-shaped, often asymmetric, 1-septate, strongly constricted at the septum, hyaline, smooth, guttulate, $7.5\text{--}11 \times 3\text{--}4 \mu\text{m}$, upper cell \pm globose and 1-guttulate, lower cell subcylindrical, sometimes swollen in the middle part, 1–2 guttulate, base truncate without any basal frill.

Distribution and host. On thallus and apothecia of *Lecanora symmicta*, not visibly damaging the host. The species is known from Arizona (U. S. A.) and Scotland (U. K.).

Observations. The new species strongly resembles *Diplolaeviopsis ranula*. It mainly differs by an olivaceous, K– excipular and pycnidial pigment (versus brown, K+ purplish in *D. ranula*). Excipular hairs are rather variable in length: in the type material they are mainly 25–35 μm long and thus slightly longer than those of *D. ranula* (15–31 μm), but in the Scottish specimen, they may reach up to 65 μm in length. The two species furthermore differ by the host selection, *D. ranula* being confined to the *Lecanora strobilina* group, and *D. symmictae* currently known only on *L. symmicta*.

The Arizona material presents numerous ascomata intermixed with a smaller number of pycnidia. Macroscopically both morphs are easily distinguished as ascomata are covered by whitish hairs, contrarily to pycnidia. Pycnidia can hardly be distinguished from those of *Diplolaeviopsis ranula*, especially if the pycnidial wall is poorly pigmented. The co-occurrence of ascomata and pycnidia, and the same excipular and pycnidial pigment suggests that both morphs belong to the same species, and supports the hypothesis of Suija et al. (2014) that *D. ranula* pycnidia and co-occurring ascomata belong to the same species. Nonetheless, we typify the new species on the ascomata of the lichenicolous fungus, not on the pycnidia, to avoid any problems in case later studies proved that both morphs were not conspecific.

The Scottish specimen is rather reduced, and *Diplolaeviopsis* type pycnidia are missing. Instead, we observed pycnidia of *Everniicola* sp., *Lichenocodium lecanorae*, and overmature, empty pycnidia resembling those of *Diplolaeviopsis*, but with a dark brown wall. Excipular hairs in this specimen may reach up to 65 μm in length, distinctly longer than those of the Arizona specimen. Because of similar ascomata, similar morphological and anatomical characters, the same pigmentation and the same host selection, we consider the Scottish specimen as being conspecific with the Arizonan material, although typical pycnidia are missing. The discovery of more European specimens on *Lecanora symmicta* should show us if pycnidia are always missing or if they are identical to those from Arizona.

Interestingly, the pycnidia of *Everniicola* observed in the Scottish specimen are reminiscent of those of *Diplolaeviopsis*, with an enlarged ostiole showing the white conidial matrix, except that they are much smaller, c. 20 μm diam. (Fig. 3 I–J). Macroscopically, the pycnidial wall is olivaceous. Microscopically, the pycnidial wall is olivaceous, K+ violet, which suggests the same pigment as *Everniicola* material on *Nephroma* (Sweden, Härjedalen, Tännäs par., Mt. Ramundberget, eastern slope, c. 1 km S of Hotel Ramundberget, on *Nephroma arcticum*, 1970, Santesson 22668, UPS and herb. Diederich). The type species *Everniicola flexispora* D. Hawksw. is known from a single specimen from the British Isles, and Alstrup & Hawksworth (1990) stated that the material is sparse, which hardly allows re-examination and study of the pigments. As conidia of *Everniicola* are also reminiscent of *Diplolaeviopsis* (2-celled, slightly to strongly bent, constricted at the septum), but much smaller, the rediscovery of fresh material of *E. flexispora* on *Evernia prunastri* should allow obtaining DNA sequences and checking if *Everniicola* might be related or even congeneric with *Diplolaeviopsis*.

Additional specimens examined: U. S. A.: Same locality as type, Nash 39358 (ASU). Scotland: VC 82, East Lothian, Tynninghame, Binning Wood, grid: 36(NT)/599.906, alt. 15 m, on *Lecanora symmicta* on *Pinus* twigs, 16 July 2012, Coppins 24107 (E).

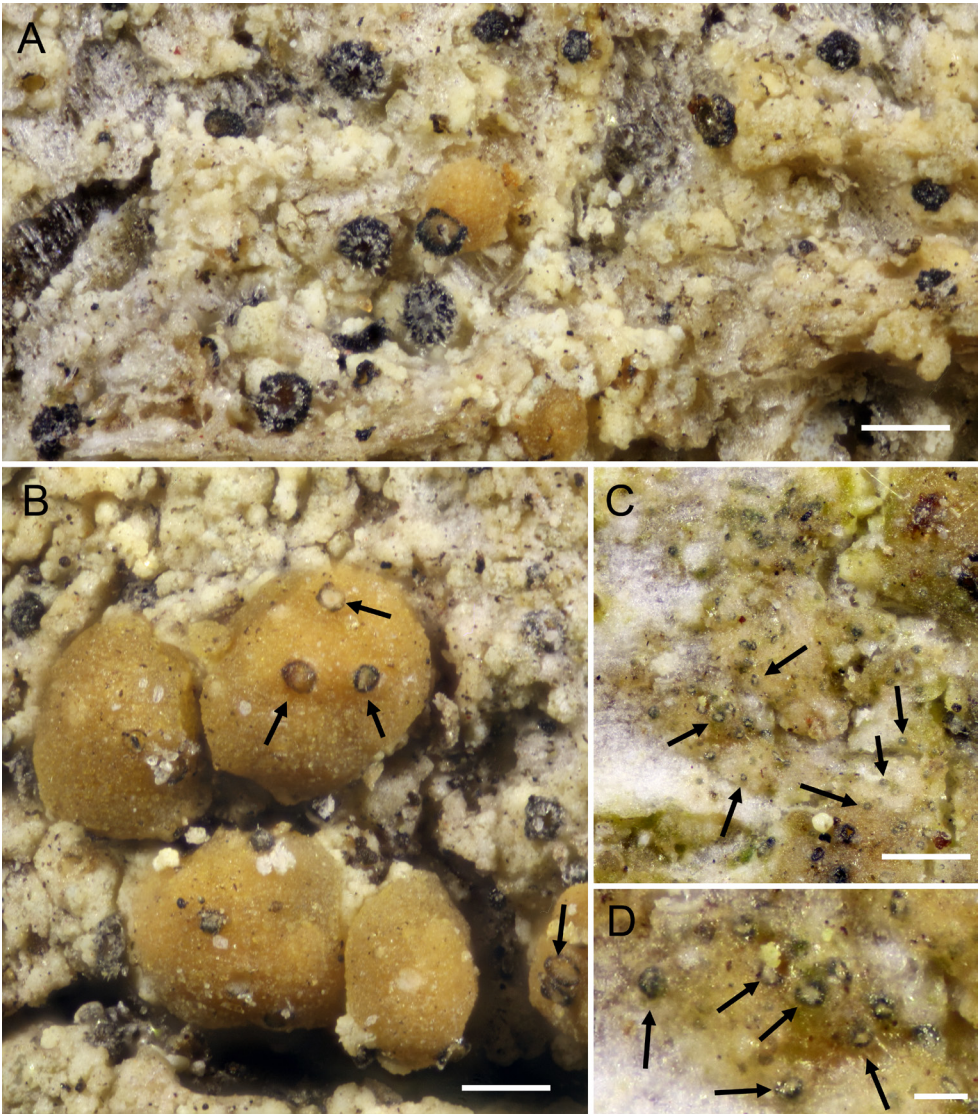


Fig. 2. A–B, *Diplolaeiopsis symmictae* (holotype), ascomata (with white superficial hairs) and pycnidia (arrows) on *Lecanora symmicta*. C–D, *Everniicola* sp. (Coppins 24107), pycnidia (arrows) on *L. symmicta* accompanying *Diplolaeiopsis symmictae* (not visible on photos). Scale bars: A–C = 200 µm; D = 50 µm.

Acknowledgements

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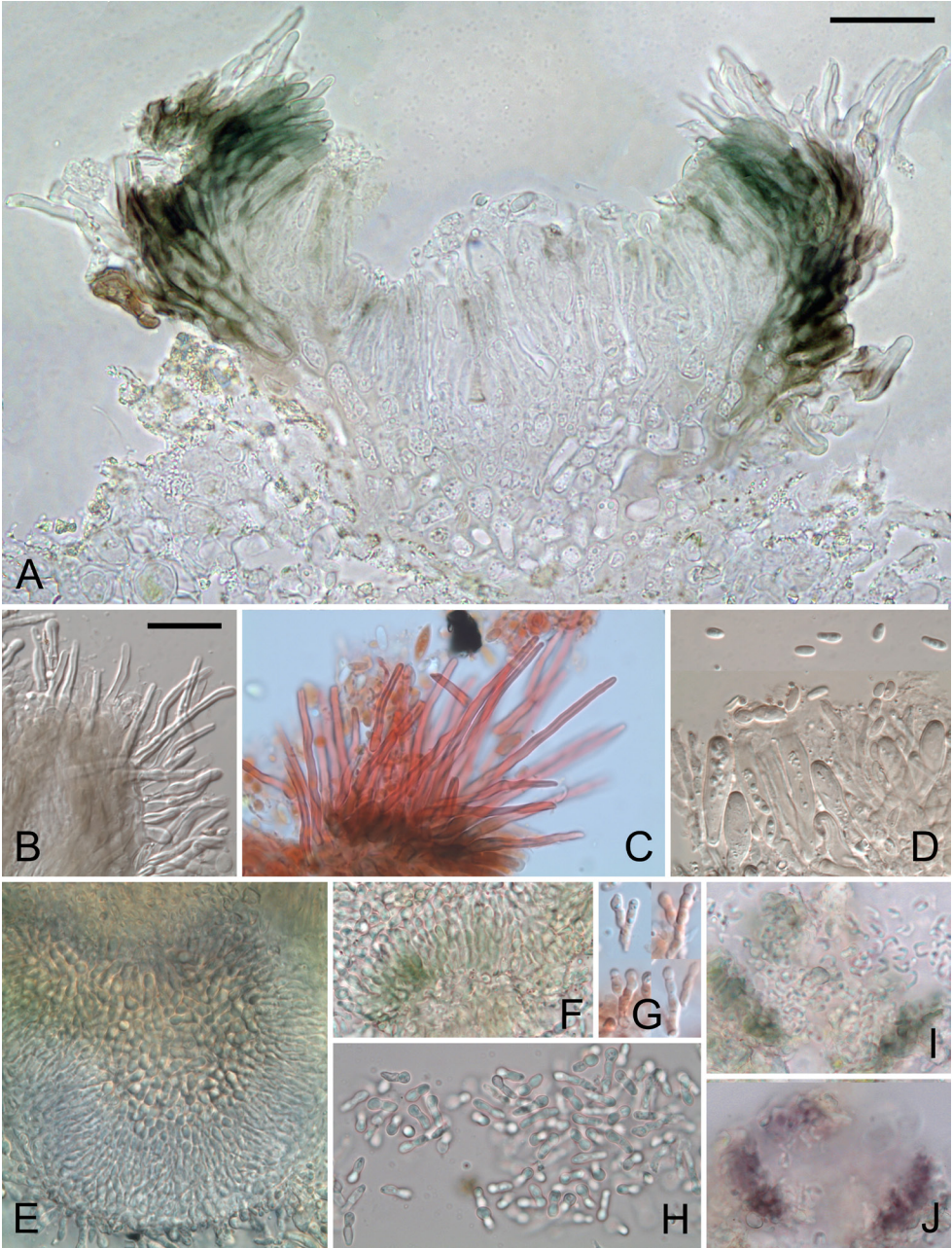


Fig. 3. A–H, *Diplolaeviopsis symmictae* (holotype, except C: *Coppins* 24107). A, Section through ascoma in water, showing olivaceous excipular pigment. B, Excipular hairs (DIC). C, Longer excipular hairs in Scottish specimen (Congo red). D, Asci and ascospores (DIC). E, Section through pycnidium in water, showing olivaceous pigment. F, Pycnidial wall with conidiophores in water, showing olivaceous pigment. G, Conidiophores (Congo red). H, Conidia (DIC). I–J, *Everniicola* sp. accompanying *Diplolaeviopsis symmictae* (*Coppins* 24107). I, Section through pycnidium in water, showing olivaceous pigment, and conidia. J, The same in 10% KOH, showing the violet reaction. Scale bars: 20 μ m (B–J are all at the same scale, see scale bar of B).