

A new species of *Llimoniella* (Ascomycota, Helotiales) on *Ramboldia cinnabarina* from Alaska

Sergio PÉREZ-ORTEGA, Javier ETAYO and Toby SPRIBILLE

Abstract: The new lichenicolous fungus *Llimoniella cinnabariniae* growing on *Ramboldia cinnabarina* from Alaska is described. The lack of excipular or epihymenial K⁺ purplish, violet or green pigments places it near the recently described *L. phaeophysciae* group. Its affinity with *Llimoniella* and other members of lichenicolous *Helotiales* is discussed.

Key words: lichenicolous fungus, lichens, North America, taxonomy

Introduction

The genus *Llimoniella* was introduced for two lichenicolous fungi growing on terricolous lichens of gypsum soils (Hafellner & Navarro-Rosinés 1993). Up to now, thirteen taxa have been recognized within the genus (Diederich & Etayo 2000; Diederich *et al.* 2010). The original concept of *Llimoniella* has recently been enlarged to include a group of taxa, referred to as the *L. phaeophysciae* group, which does not exhibit the K⁺ violet, purplish or green pigments in the epihymenium and excipulum but are otherwise typical of *Llimoniella* (Diederich *et al.* 2010). During the course of recent work on lichens and lichenicolous fungi in Alaska (e.g., Spribille *et al.* 2010), we found an undescribed species of lichenicolous fungus growing on the soralia of *Ramboldia cinnabarina* that matches the concept of the *Llimoniella phaeophysciae* group. Here we describe the new taxon and discuss its affinity to *Llimoniella* and related genera within the *Helotiales*.

Material and Methods

Specimens were examined using a Leica S8 stereomicroscope. Hand-cut sections of ascomata were studied in distilled water, lactophenol cotton blue and KOH. The amyloid reactions were tested in Lugol's iodine solution (Panreac 251774) directly (I) and after pre-treatment with KOH (K/I). Pigment reactions were observed under KOH and c. 40% nitric acid. Habit photographs were taken using a Leica S8 stereomicroscope fitted with a Leica EC3 digital camera. Microscopic pictures were taken using a Zeiss AX10 microscope fitted with 'Nomarski' differential interference contrast and an AxioCam digital camera. All measurements were made in water. Ascospore size measurements are indicated as (minimum–) $\bar{x} - \sigma_x - \bar{x} + \sigma_x$ (–maximum), with the average together with \pm standard deviation in italics; values are approximated to the nearest 0.5 μm .

Taxonomy

***Llimoniella cinnabariniae*
Pérez-Ortega, Etayo & T. Sprib.
sp. nov.**

Mycobank No.: MB 519894

Llimoniella species, in thallis *Ramboldiae cinnabariniae* vigenis, ascomatibus atris, 180–300 μm diam., excipulo et epihymenio brunneo, K⁺ brunneo-aurantiaco; hymenio non insperso; ascis subcylindricis ad clavatis, 8-sporis; ascosporis stricte ellipsoideis, (15–)16–18(–20) \times 3–4(–5) μm .

Typus: USA, Alaska, Matanuska-Susitna Borough, Denali National Park, Tokositna River below terminus of Tokositna Glacier, west bank of river, 62°38'–947' N, 150°47'–590' W, on *Ramboldia cinnabarina*, on young *Picea*, 264 m elev., 18 August 2008, T. Spribille 27868 with C. Printzen, P. Nelson & J. Walton (NY—holotypus).

S. Pérez-Ortega: Museo Nacional de Ciencias Naturales, CSIC. c/ Serrano 115 dpdo, E-28006, Madrid, Spain. Email: sperezortega@ccma.csic.es

J. Etayo: Navarro Villoslada 16, 3° dcha., E-31003, Pamplona, Spain.

T. Spribille: Institute of Plant Sciences, University of Graz, Holteigasse 6, A-8010 Graz, Austria.

(Fig. 1A–L)

Ascomata usually aggregated on the host thallus (soralia), immersed in the substratum to slightly superficial; dark brown to black, matt or slightly shiny, disc usually faintly lighter, closed when young but soon with exposed hymenium, irregularly rounded to elongated, up to 300 µm wide at the widest point; margin thin, prominent, especially when young, without hairs; disc concave to applanate. *Exciple* thin, composed of radiating to interwoven hyphae, with thick walls and usually with an enlarged and subglobose terminal cell, with an orange to light brown pigment in the upper part, disappearing in the lower part, K+ darker brown, N+ brighter orange, 17–35 µm thick in the upper part, 16–25 µm in the hyaline, lowest part; not stipitate at the base. *Subhymenium* colourless. *Hymenium* hyaline to light yellowish, not interspersed, 55–70 µm tall, I–, K/I–. *Epihymenium* with an orange, medium brown pigment, K+ dark brown, N+ brighter orange. *Paraphyses* hyaline, septate, 1.5–2.5 µm thick, thicker at the tips, up to 5–6 µm, with pigmented caps, sparingly branched. *Asci* clavate to subcylindrical, 35–55 × 8–12 µm, wall apically not thickened, I–, K/I–, 8-spored. *Ascospores* hyaline, narrowly ellipsoid to fusiform, usually slightly curved, non-septate, (15 –)16–18(–20) × 3–4(–5) µm; wall smooth, without perispore.

Conidiomata unknown.

Host. On the thallus, mostly on the soralia of *Ramboldia cinnabarina*.

Distribution. The species is so far known only from two localities in Alaska (USA). The host is very common in this region and the species can be expected to be more common than these two records would seem to indicate.

Remarks. Our new taxon is characterized by its immersed to superficial ascomata, thin exciple, paraphyses with swollen apices and pigmented caps, brownish orange excipular and epihymenial pigments that react K+ dark brown and N+ lighter orange, non-thickened ascus apex and non-septate ascospores,

which are c 16–18 × 3–4 µm. This description falls within the new, enlarged concept of *Llimoniella* (Diederich *et al.* 2010). The genus was originally conceived to include species with black, superficial apothecia, with a slightly concave to convex disc, upper exciple composed of thick, radiating cells forming a margin externally lacking hairs, a combination of characteristic pigments that usually react K+ violet or purplish, thin-walled, non-amyloid asci and simple to septate, straight to sigmoid, hyaline ascospores (Hafellner & Navarro-Rosinés 1993; Diederich & Etayo 2000). The description and inclusion of a group of species without such pigments, namely the *L. phaeophysciae* group, has broadened the concept of *Llimoniella* (Diederich *et al.* 2010). Within this group, the most similar species to the one we describe here is *L. heppiae* (Nav.-Ros., Hladun & Llimona) Diederich & Ertz. This species, growing on the terricolous lichen *Heppia despreauxii*, was originally described in *Gelatinopsis* (Navarro-Rosinés *et al.* 2008), and only later combined into *Llimoniella* (Diederich *et al.* 2010). *Llimoniella cinnabarinae* shares with *L. heppiae* the apically swollen paraphyses with a distinct pigment cap, a character exclusive to these two species, as well as a brownish orange pigment. *Llimoniella cinnabarinae* differs from *L. heppiae* in ascospore shape and size, with ascospores irregularly ellipsoid, straight to slightly curved and 10.5–13 × 4.5–5.5 µm in *L. heppiae*, and narrowly ellipsoid to fusiform, usually curved, and 16–18(–20) × 3–4 µm in *L. cinnabarinae*. Both species also differ in their ascus shape, which is apically applanate in *L. heppiae*, but not in *L. cinnabarinae*, and in their different hosts.

The new species is also reminiscent of two other genera in *Helotiales*: *Gelatinopsis* Rambold & Triebel 1990 and *Rhymbocarpus* Zopf 1896 (Rambold & Triebel 1990; Diederich & Etayo 2000). *Gelatinopsis* was described to include two fungi that were lichenicolous and fungicolous, respectively (Rambold & Triebel 1990). Further incorporations to the genus have shown that fungicolous species differ from lichenicolous species in the presence of a thicker apical

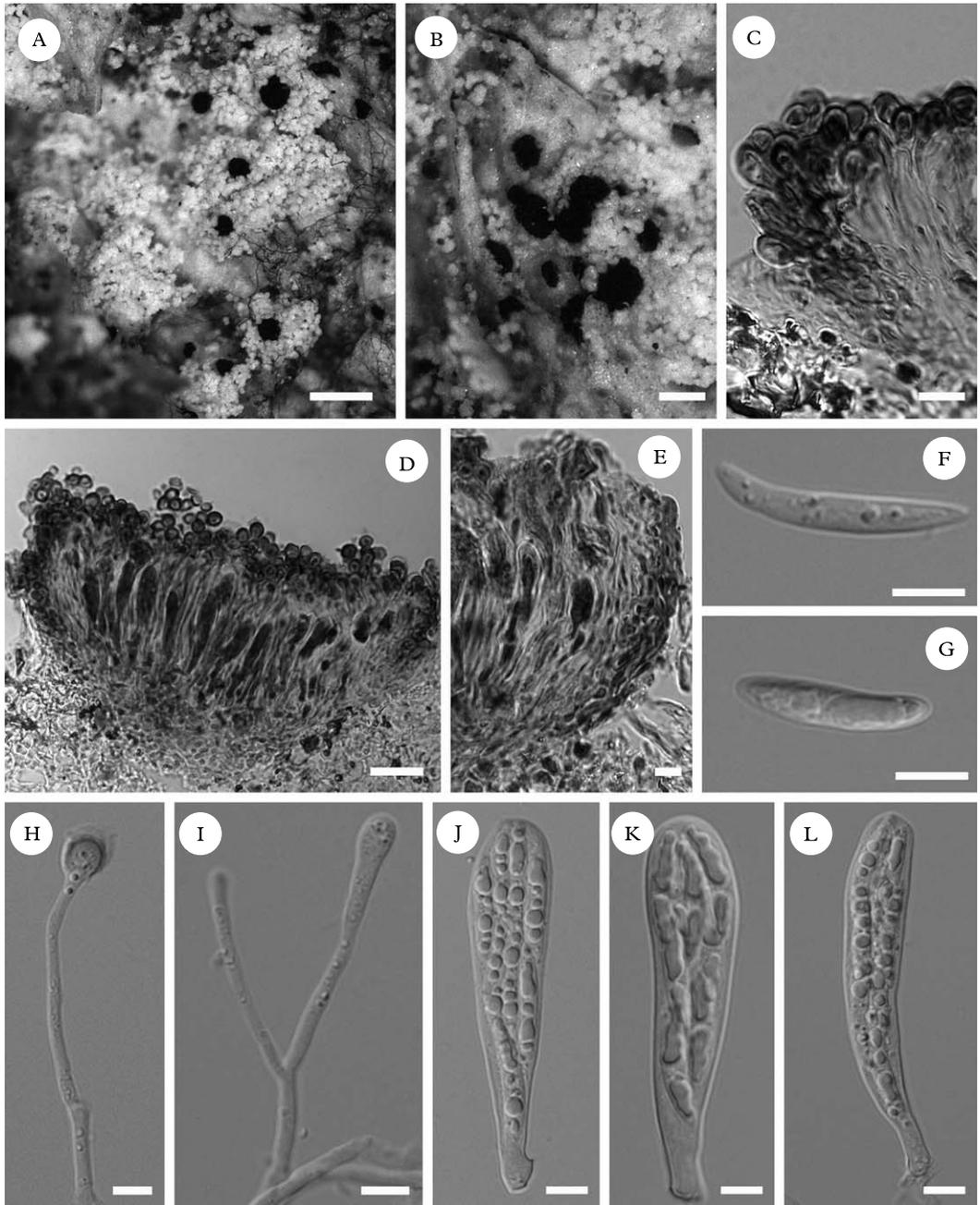


FIG 1. *Llimoniella cinnabarinae* (holotype, *Spribille* 27868, NY). A & B, habit; C & E, details of excipulum; D, longitudinal section of an ascoma; F & G, ascospores; H, paraphysis showing a swollen apex and a pigmented apical cell; I, young and branched paraphysis; J–L, asci. C, D & E: in lactophenol blue; F, G, H, I, J, K & L: in water after KOH pretreatment (differential interference contrast). Scale bars: A = 500 µm; B = 250 µm; C & E = 15 µm; D = 30 µm; F–L = 5 µm.

ascus wall and, at least in the type, strongly convex and immarginate apothecia (Baral & Marson 2001). This fact led Diederich *et al.* (2010) to combine all previously described lichenicolous *Gelatinopsis* species into *Llimoniella* or *Rhymbocarpus*. *Rhymbocarpus* is probably closely related to *Llimoniella*, but differs from it in that the ascumata are immersed and almost perithecioid when young, with a greenish, K+ olivaceous pigment present in the epihymenium, and an excipulum composed of elongate cells that are sometimes filiform in the apex or prolonged in the form of external, excipular hairs (Diederich & Etayo 2000). In *L. cinnabarinæ* the hymenium is exposed from an early stage, not appearing perithecioid, and it does not contain a greenish, K+ olivaceous pigment in the epihymenium. *Llimoniella cinnabarinæ* also possesses an exciple composed of radiating to entangled hyphae that are usually swollen in the apex, not filiform as in *Rhymbocarpus*.

Spirographa cf. *fusisporella* was found growing together with *L. cinnabarinæ* in the holotype. However, *S. fusisporella* is a very different species easily distinguishable from *Llimoniella* by its sigmoid and twisted ascospores.

Additional specimen examined. **USA:** Alaska: Klondike Gold Rush National Historic Park, Chilkoot trail just above Sheep Camp, 59°39-930'N, 135°15-912'W, on *Ramboldia cinnabarina*, 378 m elev., 2008, Pérez-Ortega 1440 *et al.* (hb. Pérez-Ortega).

SPO is supported by the program JAE-Doc (Consejo Superior de Investigaciones Científicas). We thank Dr J.

Wierzchos (CSIC, Spain) for his kind help with microscopy questions. Support for fieldwork in Klondike Gold Rush National Historical Park was provided by the U.S. National Park Service Natural Preservation Program and the Southeast Alaska Inventory and Monitoring Program, with special thanks to Dave Schirokauer. Fieldwork in Denali National Park was made possible by Peter Nelson, James Walton and Carl Roland and is gratefully acknowledged.

REFERENCES

- Baral, H.O. & Marson, G. (2001) Monographic revision of *Gelatinopsis* and *Calloriopsis* (Calloriopsidae, Leotiales). In *Micologia 2000* (G. Consiglio, ed.): 23–46. Trento: Associazione Micologica Bresadola.
- Diederich, P. & Etayo, J. (2000) A synopsis of the genera *Skyttea*, *Llimoniella* and *Rhymbocarpus* (lichenicolous Ascomycota, Leotiales). *Lichenologist* **32**: 423–485.
- Diederich, P., Ertz, D. & Etayo, J. (2010) An enlarged concept of *Llimoniella* (lichenicolous *Helotiales*), with a revised key to the species and notes on related genera. *Lichenologist* **42**: 253–269.
- Hafellner, J. & Navarro-Rosinés, P. (1993) *Llimoniella* gen. nov. - eine weitere Gattung lichenicoler Disco-myceten (Ascomycotina, Leotiales). *Herzogia* **9**: 769–778.
- Navarro-Rosinés, P., Hladun, N. L. & Llimona, X. (2008) *Gelatinopsis heppiae* sp. nov. (Leotiales, Ascomycota) un hongo liquenícolas sobre on *Heppia despreauxii* de Aragón (NE de España). *Revista Catalana de Micologia* **30**: 57–64.
- Rambold, G. & Triebel, D. (1990) *Gelatinopsis*, *Geltingia* and *Phaeopyxis*: three helotialean genera with lichenicolous species. *Notes from the Royal Botanic Garden Edinburgh* **46**: 375–389.
- Spribille, T., Pérez-Ortega, S., Tønsberg, T. & Schirokauer, D. (2010) Lichens and lichenicolous fungi of the Klondike Gold Rush National Historic Park, Alaska, in a global biodiversity context. *Bryologist* **113**: 439–515.