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***Enterographa confusa* sp. nov. (Roccellaceae, Arthoniales) discovered by chance in type material of its host lichen *Arthonia ochraceella*, described more than a century ago**

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Abstract

Enterographa confusa is described as new to science from Cuba. It is characterized by a lichenicolous habit, punctiform ascomata immersed in the host thallus, (6–)8-spored asci and 3-septate ascospores of 17–23 × (2.5–)3 µm with a distinct gelatinous sheath of 1.5(–)2 µm. The new species was discovered by chance in the thallus of type material of *Arthonia ochraceella*, which is lectotypified here. A detailed description of this latter is also provided. *Enterographa confusa* can be easily overlooked because of its tiny pale ascomata similar in colour to those of its host lichen. A key to all lichenicolous *Enterographa* species is presented.

Introduction

Enterographa Fée (1825: 57) is a heterogeneous lichen genus including lichenized and lichenicolous species and with a higher diversity in the tropics (e.g. Matzer 1996; Sparrius 2004; Ertz & Tehler 2011). Nine lichenicolous *Enterographa* species have been described so far, all in the past two decades (Matzer 1996; Ertz *et al.* 2005; Sparrius & Aptroot 2007; Yeshitela *et al.* 2009; Morse 2013; Seavey *et al.* 2017; Etayo 2017). In the framework of taxonomic revisions of the genus *Arthonia*, an unknown lichenicolous fungus was discovered on type material of *Arthonia ochraceella*, a corticolous, crustose lichen described from Cuba in 1890 by H. Willey. The aim of the present study is to describe this new lichenicolous species and to provide a detailed description of *Arthonia ochraceella* from type material.

Material and Methods

Material of the new species has been found in type material of *Arthonia ochraceella* deposited in G. Detailed morphological descriptions of the new lichenicolous species as well as its host are provided. Colour reactions of thallus and apothecial structures were made with C (commercial bleach), K (10% water solution of potassium hydroxide) and PD (*para*-Phenylenediamine) (Orange *et al.* 2001). The presence of calcium oxalate crystals was investigated using 25% H₂SO₄ and the colour of the thallus and apothecia was checked in ultraviolet light (UV). Hand sections and squash mounts were examined in tap water. The amyloidity of asci and hymenium was studied using Lugol's solution, without (I) or with (KI) pre-treatment with K. All measurements were made in tap water.

Results

Enterographa confusa Ertz & Van den Broeck *spec. nov.* (Fig. 1)

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Diagnosis: A lichenicolous species of *Enterographa* characterized by punctiform ascomata immersed in the host thallus, (6–)8-spored asci and 3-septate ascospores of $17\text{--}23 \times (2.5\text{--})3 \mu\text{m}$ with a distinct gelatinous sheath of $1.5\text{--}(2) \mu\text{m}$.

Type:—CUBA. *C. Wright*, #Graphid. Cub. 121c (holotype G!; barcode G00290277).

Thallus absent (lichenicolous). *Ascomata* punctiform, immersed in the host thallus, emarginate, discrete or rarely two confluent, $60\text{--}115 \mu\text{m}$ diam.; hymenial disc exposed, pale yellowish to pale brown. *Excipulum* hyaline, rudimentary, not well separated from the hymenium and the epihymenium. *Epihymenium* hyaline to pale yellowish, I+ immediately red, KI+ blue. *Hymenium* hyaline, not interspersed, $60\text{--}80 \mu\text{m}$; hymenial gel I+ immediately dark red, KI+ blue. *Subhymenium* hyaline, c. $10\text{--}15 \mu\text{m}$. *Paraphysoids* branched, anastomosed, $1\text{--}2 \mu\text{m}$, not or slightly enlarged at the apex. *Asci* ellipsoid to clavate, (6–)8-spored, $40\text{--}48 \times 13\text{--}16 \mu\text{m}$; wall with a distinct I+ red, KI+ blue ring around a tiny ocular chamber. *Ascospores* hyaline, fusiform, 3-septate, $17\text{--}23 \times (2.5\text{--})3 \mu\text{m}$ (N=20); gelatinous sheath distinct, hyaline, $1.5\text{--}(2) \mu\text{m}$ in water. *Conidiomata* not seen.

Etymology:—The specific epithet refers to the possible confusion of the ascomata of the lichenicolous fungus with younger ones of the host lichen.

Distribution and ecology:—The species is only known from the type locality of its host, *Arthonia ochraceella*, in Cuba, on bark.

Remarks:—The new species is lichenicolous on the thallus of *Arthonia ochraceella* and can be easily overlooked because the ascomata are tiny, pale and of similar colour than those of the host. It was discovered by chance by examining a type specimen of *Arthonia ochraceella* from herbarium G (G00290277). At first, we did not notice the presence of a lichenicolous fungus on the specimen. By doing a cross section of what we believed to be a young ascoma or pycnidium of *Arthonia ochraceella* and by its examination under the microscope, it became immediately clear that the fungus was not related to Arthoniaceae because of the combination of ellipsoid to clavate asci with a thin upper wall and the fusiform narrow ascospores having a thick gelatinous sheath. Once detected, the ascomata of the lichenicolous fungus were rather easy to distinguish from those of the host that are much larger when mature, often slightly darker and elongated. The thallus of the host becomes also slightly paler when the lichenicolous fungus is present. Moreover, *A. ochraceella* is very different microscopically having *Arthonia* type asci and much larger macrocephalic ascospores (see description below). The new species fits well with the genus *Enterographa* by emarginate ascomata, ellipsoid to clavate asci with a KI+ blue ring and transversally septate, isolocular, and fusiform ascospores usually having a distinct gelatinous sheath. Among the lichenicolous *Enterographa* species, *E. punctata* Ertz & Diederich in Ertz *et al.* (2005: 128) known from Sri Lanka, is the most similar and differs by gall-inducing ascomata, slightly larger ascomata (up to $170 \mu\text{m}$ diam.), a taller hymenium that is I+ persistently blue above, 6-spored asci and a different host lichen genus (an unidentified *Lobaria* species with a green photobiont). *Enterographa bagliettoae* F. Seavey & J. Seavey in Seavey *et al.* (2017: 221) is very different by ascomata immersed in raised white galls on the thallus of saxicolous Verrucariaceae. *Enterographa osagensis* C.A. Morse (2013: 234) on saxicolous *Bacidina* has shorter and wider spores, $12\text{--}15\text{--}(17.5) \times 3.5\text{--}4.5 \mu\text{m}$. *Enterographa mazosiae* R. Sant. ex Matzer & R. Sant. in Matzer (1996: 54) is lichenicolous on *Mazosia* and differs by distinctly lirelliform, stellately branched ascomata having a dark reddish to blackish hymenial disc (Matzer 1996; Sparrius 2004). *Enterographa brezhonega* Sparrius & Aptroot (2007: 315) is very different by shortly lirelliform ascomata immersed in pseudostromata, 4-spored asci, and (4–)6-septate ascospores. *Enterographa epiphylla* (Sérus.) Ertz, Diederich & Sparrius in Ertz *et al.* (2005: 126), *E. fellhaneroides* Yeshitela, Eb. Fisch., Killmann & Sérus. in Yeshitela *et al.* (2009: 18) and *E. meklitia* Yeshitela, Eb. Fisch., Killmann & Sérus. in Yeshitela *et al.* (2009: 20) are all lichenicolous on foliicolous *Coenogonium* species and differ by 4 or 4–6-spored asci and more than 3-septate ascospores. *Enterographa epigraphis* Etayo & Sipman in Etayo (2017: 190) is very unusual for a lichenicolous *Enterographa* species because of the large ascospores with an ontogeny reminding the genus *Fulvophyton* (Ertz & Tehler 2011).

Specimen G00290277 is both, the lectotype of *Arthonia ochraceella* and the holotype of *Enterographa confusa*. Amazingly, *A. ochraceella* was described in 1890, but 128 years were necessary before the lichenicolous fungus present on the same type material was detected and described.

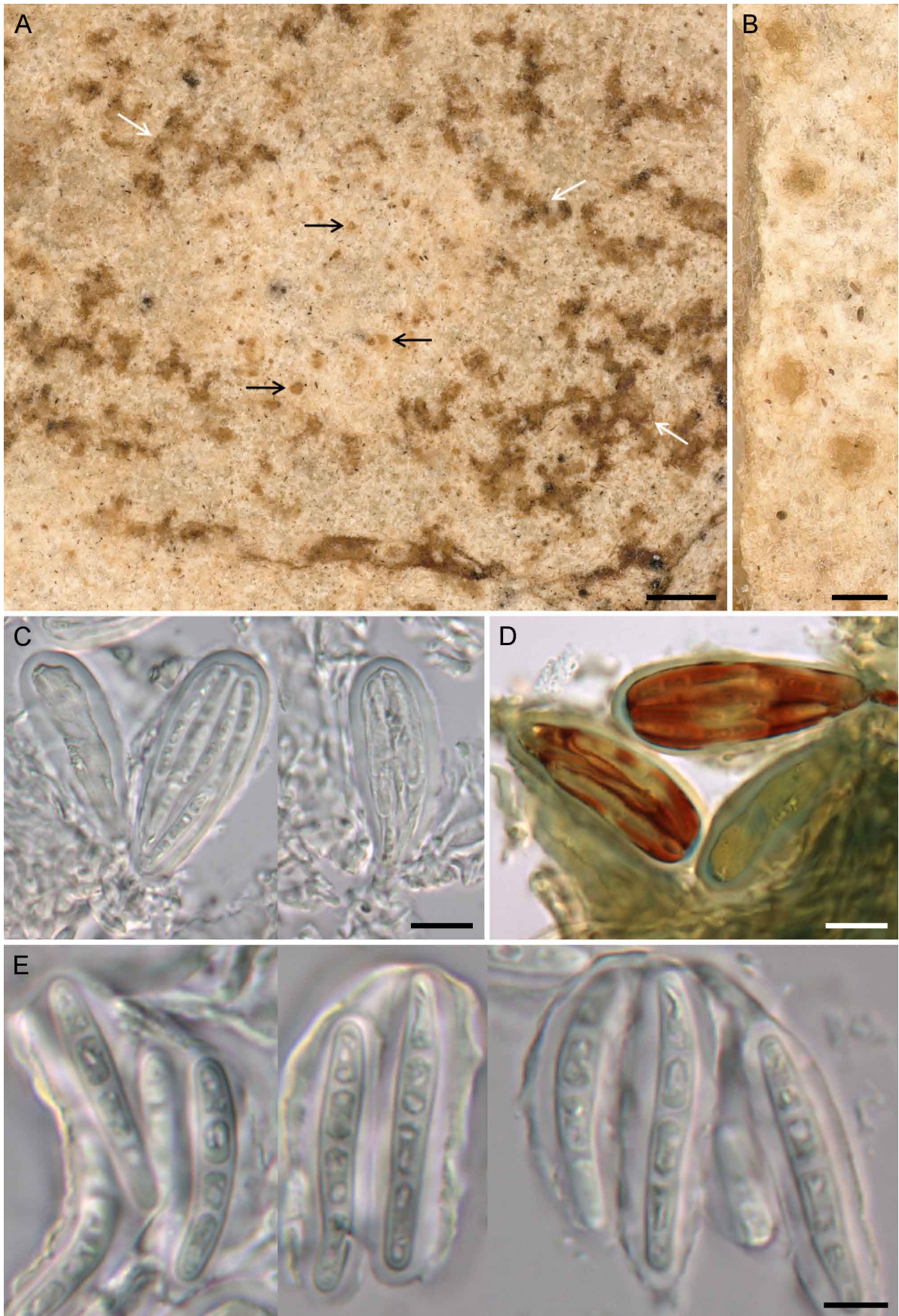


FIGURE 1. *Enterographa confusa* (A–E, holotype, G). **A.** *E. confusa* (black arrows point to ascomata of the lichenicolous fungi) forming a paler, whitish area in the thallus of its host, *Arthonia ochraceella* (white arrows point to ascomata of the host lichen). **B.** Ascomata of *E. confusa*. **C.** Asci in water. **D.** asci in KI showing the KI+ blue apical ring. **E.** Ascospores in water. Scale in A = 500 μ m, in B = 100 μ m, in C–D = 10 μ m, in E = 5 μ m. (Photos D. Ertz).

Arthonia ochraceella Nyl. ex. Willey

Synopsis of the genus *Arthonia*: 10 (1890)

Type:—CUBA. C. Wright, #Graphid. Cub. 121c (lectotype G!, here designated; barcode G00290277)

Thallus corticolous, crustose, greenish white, discontinuous, thin, the periderm ± visible through the thallus, in section ca. 3–10 µm thick, largely endophloeodal; UV± pale orange with white to yellowish patches; thallus hyphae and prothallus not observed. *Photobiont* trentepohlioid, cells 8–13 × 7–12 µm, rounded, solitary or in short chains. *Ascomata* rounded to lirelliform to stellate, immersed, irregularly spread on the thallus, emarginate, numerous, 0.2–1.4 × 0.07–0.1 mm; in section 85–100 µm thick; disc brown, epruinose, translucent, wet orange and transparent, flat to concave; calcium oxalate crystals absent. *Excipulum* inconspicuous. *Epihymenium* yellowish, 20–25 µm, composed of intermingled tips of paraphysoids, I– to I+ orange. *Hymenium* hyaline to slightly yellowish, 45–55 µm, strongly conglutinated; hymenial gel I+ blue, KI+ blue. *Subhymenium* yellowish, c. 16–18 µm, not well separated from hymenium, gel I+ blue, KI+ blue. *Paraphysoids* richly branched and anastomosed, 1.7–2.2 µm, distinct, embedded in dense gelatinous matrix, hyaline, without dark walled tips or caps, ± parallel between the asci. *Asci* globose to broadly clavate, 2–6-spored; 40–54 × 36–45 µm (N = 4); a KI blue ring like structure in the tholus or an ocular chamber not observed; stipe not observed; walls thin, 0.6–1.5 µm; ascogels I+ orange to red. *Ascospores* hyaline, ovoid, (3–)4(–5)-septate, with two enlarged end cells, 28–34 × 9.5–14.5 µm (N = 12), constricted at septa; spore ontogeny macrocephalic, unidirectional; gelatinous sheet hyaline, 1.0–2.5 µm in water. *Conidiomata* not seen. Thallus and ascomata K–, C–, KC–, PD–.

Distribution and ecology:—The species is only known from the type locality in Cuba, on bark.

Remarks:—The examined specimen of *Arthonia ochraceella* (Graphid. Cub. 121c, barcode G00290277) is selected as lectotype because two type specimens are present in G. The other one with barcode G00290276 is also infected by *Enterographa confusa* (photo present on JSTOR). The specimen G00290277 is well-developed and representative of the species. As stated by Müller (1894), Willey described the species and therefore we prefer to add « ex. Willey » as author of the species.

Identification key of lichenicolous *Enterographa* species

- 1. Ascospores 3-septate 2.
- Ascospores more than 3-septate 5.
- 2. Ascomata gall-inducing 3.
- Ascomata not gall-inducing 4.
- 3. Ascomata 30–70 µm diam.; galls 0.3–0.65 mm diam.; ascospores 18–19 × 4–4.5 µm; on *Bagliettoa* *E. bagliettoae*
- Ascomata 75–170 µm diam.; galls 0.5–1.2 mm diam.; ascospores 17–21 × 3–4 µm; on *Lobaria* *E. punctata*
- 4. Ascomata without a distinct white margin; ascospores up to 3 µm wide; on corticolous *Arthonia* *E. confusa*
- Ascomata with a thick white outer margin at least when young; ascospores 3.5–4.5 µm wide; on saxicolous *Bacidina* *E. osagensis*
- 5. Ascospores 9–10-septate; on *Graphis* *E. epigraphis*
- Ascospores with fewer septa 6.
- 6. Asci 8-spored; on foliicolous *Mazosia* *E. mazosiae*
- Asci 4-spored or 4–6-spored; not on foliicolous *Mazosia* 7.
- 7. Pseudostromata white pruinose; on corticolous lichen with trentepohlioid photobiont (mainly on *Porina rosei*) *E. brezhonega*
- Pseudostromata not pruinose; on foliicolous *Coenogonium* 8.
- 8. Ascomata apothecioid, raised *E. fellhaneroides*
- Ascomata punctiform or shortly elongate 9.
- 9. Ascomata brown; ascospores 15–17 × 2.5–3 µm *E. meklitiae*
- Ascomata blackish; ascospores 17–22 × 3–4.5 µm *E. epiphylla*

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