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A contribution to the study of *Helotiales* and *Rhytismatales* in Turkey

Makbule Erdoğdu[1*], Gökhan Doğan[1],
Elşad Hüseyin[1] & Zekiye Suludere[2]

[1] *Ahi Evran University, Faculty of Science and Literature, Department of Biology, Bağbaşı, Kırşehir, Turkey*

[2] *Gazi University, Faculty of Science, Department of Biology, Teknikokullar, Ankara, Turkey*

[*] Correspondence to: *merdogdu@ahievran.edu.tr*

Abstract—*Naemacyclus fimbriatus*, *Lophodermium juniperinum*, and *Marssonina daphnes* have recently been discovered in Turkey. This is the first record of *Naemacyclus* from Turkey. Morphological data obtained by light and scanning electron microscopy of these fungi are presented.

Key words—acervular anamorph, *Ascomycota*, new host, new records, SEM

Introduction

Rhytismatales are an order of endophytic, parasitic, or saprotrophic fungi in *Leotiomycetes* (*Ascomycota*), the inoperculate discomycetes. Especially common on conifers, grasses and members of *Ericaceae,* they are also found on other vascular plants. The species disperse by ascospores and at least in temperate regions usually infect their hosts in spring/summer to develop fruiting bodies the next year on dead material (Lantz et al. 2011). The order includes plant parasitic fungi causing serious needle cast, such as *Lophodermium seditiosum* Minter et al. on *Pinus sylvestris* (Minter 1981b)., Rhytismatalean fungi the members of *Rhytismatales* are poorly known in Turkey and have not been yet intensively studied.

Within *Leotiomycetes, Helotiales* represents the largest order of inoperculate discomycetes—an ecologically and morphologically highly diverse group of

ascomycetes that also includes lichen-inhabiting (lichenicolous) species (Suija et al. 2015). At present the order comprises c. 4000 species of saprophytes, mycorrhizal fungi, root endophytes, and plant and fungal (including lichens) parasites (Schoch et al. 2009).

Materials & methods

Plant specimens infected with microfungi were collected from Erciyes Mountain in Kayseri province of Turkey. The host specimens were prepared following conventional herbarium techniques. Host plants were identified using the Flora of Turkey and East Aegean Islands (Davis 1965–85). Thin fungal sections prepared from host tissue were examined under a Leica DM E light microscope and measured from mounts in 5% KOH or tap water. Close-up photographs of infected host surface were done via Leica EZ4D stereomicroscope. The fungi were identified using relevant literature (for *Naemacyclus* – DiCosmo et al. 1984, Hou et al. 2006; for *Lophodermium* – Dennis 1981, Ellis & Ellis 1987; for *Marssonina* – Grove 1937, Ellis & Ellis 1987, Ignatavičiūtė & Treigienė 1998). All examined specimens were deposited in the Mycology Laboratory of Ahi Evran University, Department of Biology, Kırşehir, Turkey (C), under Gökhan Doğan (GD) collection numbers.

For scanning electron microscopy (SEM), 8–10 mm square pieces of infected leaf or cone scale were mounted on aluminium stubs with double-sided adhesive tape, coated with gold using Polaron SC 502 Sputter Coater, and examined using a Jeol JSM 6060 scanning electron microscope operated at 5–10 kV in the Electron Microscopy Unit, Gazi University (Turkey).

Taxonomy

Naemacyclus fimbriatus (Schwein.) DiCosmo, Peredo & Minter, Eur. J. For. Path. 13(4): 207 (1983) Pl. 1

Apothecia scattered, immersed to erumpent, sessile, circular to subcircular, 300–430 × 230–285 µm, dark brown to black. Asci cylindrical, short-stalked, thin-walled, 85–95 × 8.5–9.5 µm, rostrate at the apex, without circumapical thickening, discharging spores through a small apical pore, 8-spored. Ascospores fasciculate, filiform, (50–)54–80 × 2–2.2 µm, rounded at both ends, hyaline, (2–)5-septate, rarely aseptate, with mucous sheath. Paraphyses filiform, unbranched, septate, 90–105 × 1 µm, hyaline, covered by a thin mucous sheath.

Specimen examined—**TURKEY**, **Kayseri**, Erciyes mountain, Turkish World Forest, 38°36′12″N 35°30′56″E, 1850–1900 m asl., on fallen female cone scales of *Pinus nigra* L. (*Pinaceae*), 31.05.2010, G. Doğan (AEUT GD1069).

Notes: While DiCosmo et al. (1983) resolved the nomenclatural problems surrounding *N. fimbriatus,* the systematic position of this species is still

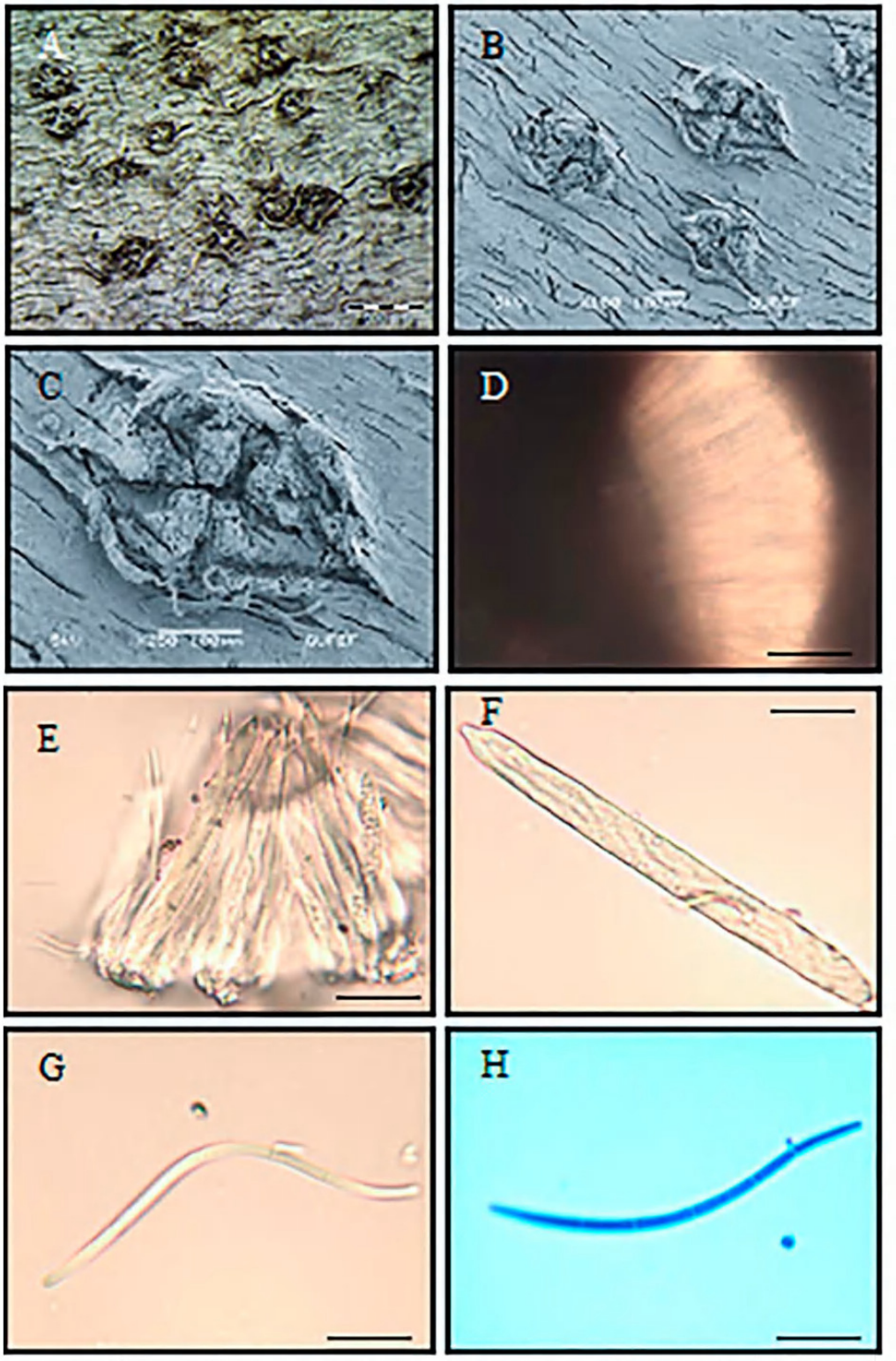

Plate 1. Microscopic characters of *Naemacyclus fimbriatus*. A: apothecia on cone scales; B, C: apothecia on cone scales (SEM); D: vertical section of an apothecium; E: asci and ascospores; F: ascus and ascospores; G: ascospore; H: lacto phenol cotton blue mount showing ascospore. Scale bars = D: 100 µm; E: 20 µm; F–H: 15 µm.

controversial. *Naemacyclus fimbriatus* was once placed in the *Phacidiaceae,* but DiCosmo (1979) suggested that it is more closely related to *Rhytismataceae* because of J– apical ring and ascospores covered by a mucous sheath (Kirk et al. 2001, Cannon & Kirk 2007). The recent analysis of the partial small subunit rDNA (Hou et al. 2006) indicates that *N. fimbriatus* is closely related to taxa in the *Helotiales*.

Although our Turkish specimen of *Naemacyclus fimbriatus* is morphologically similar to specimens described in literature (DiCosmo et al. 1984, Vujanovic et al. 1998, Hou et al. 2006), it does differ slightly in some aspects, particularly with respect to ascospore size and number of septa: 75–90 × 2–2.5 µm and 7-septate (DiCosmo et al. 1984); 78–91 × 2–2.5 µm and 7-septate (Vujanovic et al. 1998); 65–95 × 1–1.5 µm and (2–)4–6-septate (rarely aseptate) (Hou et al. 2006). The ascospores in the specimen from Turkey are shorter and (2–)5-septate (rarely aseptate).

Naemacyclus fimbriatus is widely distributed on *Pinus* spp. in Asia (China), Europe, and North America (Cannon et al. 1985; Dennis 1981; Dudka et al. 2004; Eriksson 2014; Gremmen 1960, as *Lasiostictis fimbriata*; Hanlin 1963, as *Stictis fimbriata*; Lin 2012; Minter 1981a; Sherwood 1979, as *Lasiostictis fimbriata*; Vujanovic et al. 1998). The genus *Naemacyclus* and *N. fimbriatus* are reported for the first time from Turkey.

Lophodermium juniperinum (Fr.) De Not., G. Bot. Ital. 2(7–8): 46 (1847) Pl. 2

Apothecia hysterioid, elliptical, blister-like, strongly raised above the surface of needles, 600–650 × 200–220 µm, blackish; disc soft, whitish. Asci cylindrical-clavate, short-stalked, thin-walled, 95–110 × 13.5–15.5 µm, 8-spored; Ascospores fasciculate, filiform, attenuated at both ends, 65–87 × 1.5–3 µm, guttulate, hyaline, with a thin hyaline gelatinous coating. Paraphyses slender, filiform, hyaline, swollen and curled at the tip.

Specimen examined—**TURKEY**, **Kayseri**, Erciyes mountain, Develi, 2000–2050 m asl., conifer plantation area, on dead leaves of *Juniperus communis* L. (*Cupressaceae*), 25.07.2011, G. Doğan (AEUT GD1106).

Notes: Ten rhytismataceous ascomycetes from the genera *Coccomyces*, *Colpoma*, *Hypoderma*, *Lophodermium*, *Pseudophacidium*, *Soleella*, and *Tryblidiopsis* have been reported on species of *Juniperus*; those on juniper needles are mostly pathogenic, causing needle cast (Hou et al. 2005). *Lophodermium juniperinum* is probably weakly parasitic, capable of endophytic growth in needles for part of its life cycle, like most species of *Lophodermium* (Minter 1981b).

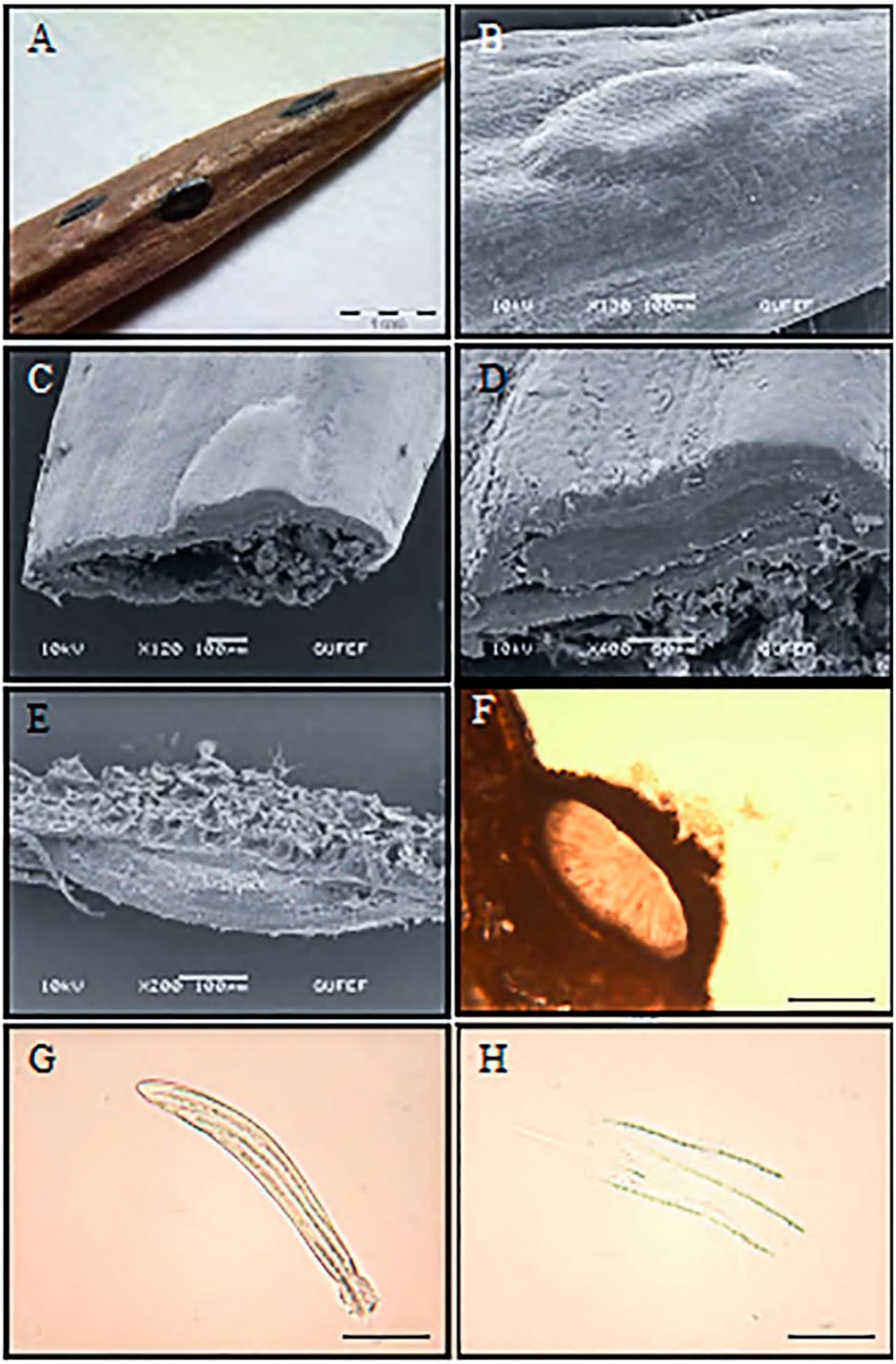

Plate 2. Microscopic characters of *Lophodermium juniperinum*. A: apothecia on leaf; B: apothecium on leaf (SEM); C-E: vertical section of an apothecium (SEM); F: vertical section of an apothecium; G: ascus and ascospores; H: ascospores. Scale bars = F: 200 µm; G,H: 25 µm.

The Turkish specimen of *L. juniperinum* agrees with other reports of the species in ascomata, asci and ascospores morphology; the only observable difference being the smaller dimensions of ascomata, asci, and ascospores. Dennis (1981) describes 1 × 0.4 mm apothecia, 130 × 17 µm asci, and 60–100 × 2 µm ascospores; Ellis & Ellis (1987) cite 1 mm long apothecia and 60–90 × 2 µm ascospores.

Lophodermium juniperinum is known from Asia (Dudka et al. 2004), Europe, and North America (Hou et al. 2005). This species is reported for the first time from Turkey.

Marssonina daphnes (Roberge ex Desm.) Magnus, Hedwigia 45: 89 (1906) Pl. 3

Leaf spots amphigenous, irregularly rounded, 1–3 mm diam., greenish and then brownish. Conidiomata acervular, amphigenous, at first immersed in host tissue, later erumpent, small, pale brown. Conidia ovoid to pyriform, slightly curved, 15–20 × 6.5–8 µm, when mature with transverse septum near the base, not constricted, obtuse at the apex, truncate at the base, with numerous guttulations, hyaline.

Specimen examined—**TURKEY**, **Kayseri**, Erciyes mountain, Kayseri memorial forest, 38°36′13″N 35°30′58″E, 1870 m asl., on living leaves of *Daphne oleoides* Schreb. subsp. *oleoides* (*Thymelaeaceae*), 25.07.2011, G. Doğan (AEUT GD1080).

Notes: *Marssonina daphnes* is the causal agent of the daphne anthracnose, which usually causes little damage. The Turkish specimen of *Marssonina daphnes* is morphologically similar to specimens described in literature (Grove 1937, Ellis & Ellis 1987, Ignatavičiūtė & Treigienė 1998). However, the conidia of the Turkish samples are wider than the British collection (12–20 × 4–5 µm; Grove 1937), while those reported by Ignatavičiūtė & Treigienė (1998) are slightly narrower and shorter than our specimen.

Marssonina daphnes is distributed on *Daphne* spp. in Asia (Japan), Australasia, Europe, and North America (Conners 1967; Ginns 1986; Cook & Dubé 1989; Kobayashi 2007; Pennycook & Galloway 2004; Piątek & Wołczańska 2004; Shaw 1973). The species is reported for the first time from Turkey, and *Daphne oleoides* Schreb. subsp. *oleoides* represents a new host record.

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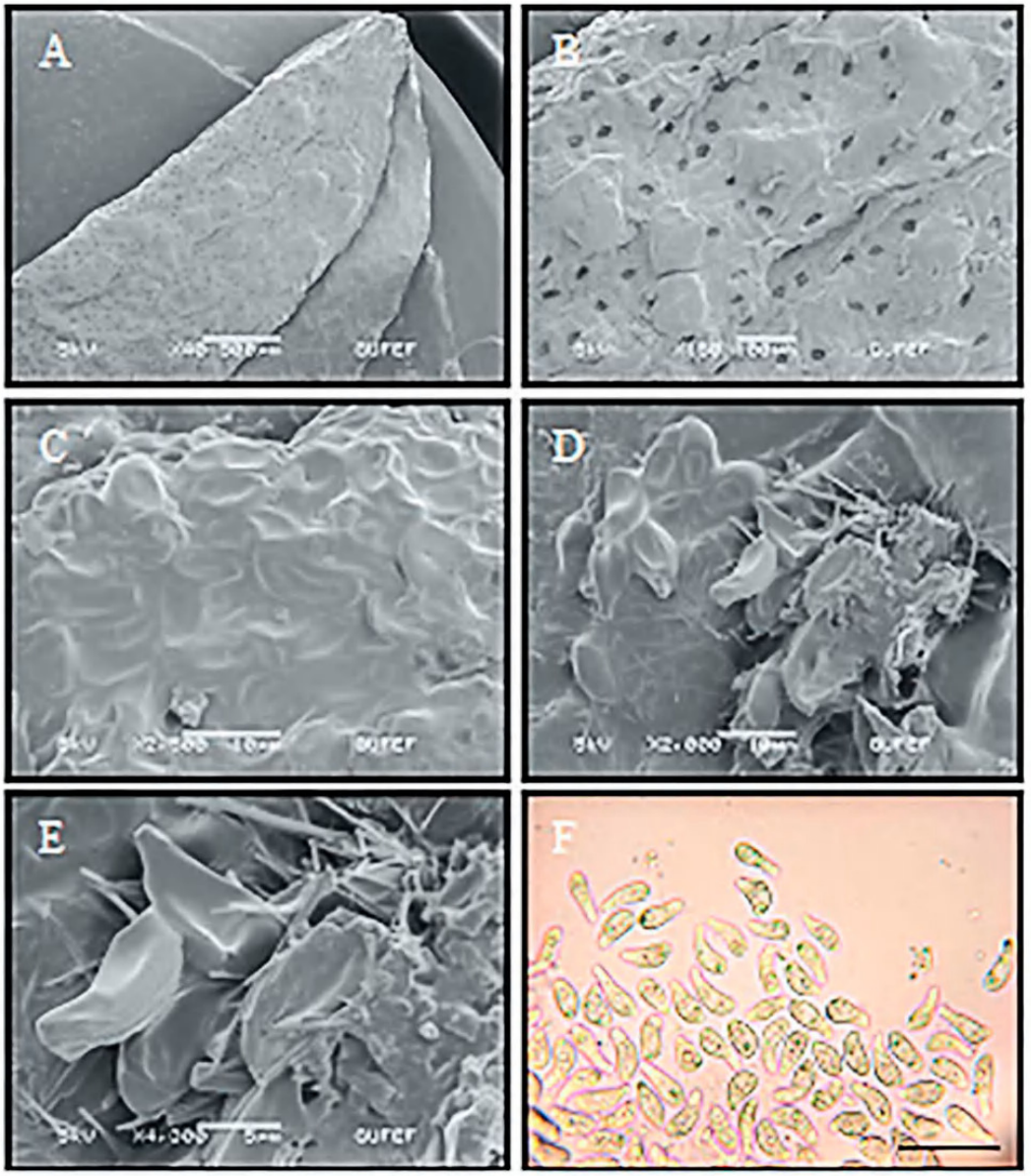

Plate 3. Microscopic characters of *Marssonina daphnes*. A,B: acervulus on leaf (SEM); C–E: Conidia (SEM); F: Conidia. Scale bar = 10 μm.

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