

New records of *Trochila* (*Cenangiaceae*, *Helotiales*) from the Balkans

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Abstract. *Trochila laurocerasi* is recorded in North Macedonia, while *T. craterium* is reported from Greece and studied from Turkey. They are presented with concise descriptions and illustrations. Comparison with Bulgarian specimens is provided on the basis of ascospore measurements. The known distribution in the Balkans is also given.

Key words: discomycetes, Greece, North Macedonia, *Trochila*, Turkey

Introduction

The present work describes two species of *Trochila* Fr., newly recorded from Greece, North Macedonia and Strandzha Mts (in Turkey). Each of them is associated with dead leaves of a different plant genus: *Trochila laurocerasi* (Desm.) Fr. on leaves of Cherry Laurel (*Laurocerasus officinalis* M. Roem.) and *T. craterium* (DC.) Fr. on ivy leaves (*Hedera helix* L. and *H. algeriensis* Hibberd). The apothecia of the *Trochila* species develop beneath the epidermis, more or less erumpent, asci react positively in iodine, ascospores are ellipsoid, hyaline and one-celled; paraphyses are usually up to 7 µm wide at the apex (Nauta & Spooner 2000).

Trochila craterium was reported for the first time from the Balkans in Albania, by Petrak (1922) on *Hedera helix* leaves. In Bulgaria, the first reports came from the Western Rhodopi and Belasitsa mountains, on leaves of the same host plant (Dimitrova 1997a, b). It has been recently reported on *H. helix* leaves from Turkey (Selçuk & al. 2013) and Serbia (Savić & Karaman 2016). A comprehensive study of the Bulgarian species of *Trochila* was made by Stoykov & Assyov (2009), including new records of *T. ilicina* (Nees : Fr.) Courtec. and *T. laurocerasi*. Subsequently, *T. ilicina*

was recorded in Sofia region, Vrana Park (Pencheva & al. 2009; Dimitrova & Gyosheva 2010). Recently, *Trochila craterium*, along with *T. laurocerasi*, were found in the Balgarka Nature Park (Gyosheva & al. 2016). *Trochila ilicina* is known also from Greece (Pantidou 1973; Zervakis & al. 1999), Romania (Stoykov & Assyov 2009) on *Ilex aquifolium* L. and Turkey (Stoykov & Denchev 2007; Stoykov & Assyov 2009; Selçuk & al. 2013) on *I. colchica* Pojark. *Trochila laurocerasi* was reported recently from Serbia by Savić & Karaman (2016) on leaves of Cherry Laurel.

Material and methods

Dried specimens were examined in water mounts, or in aqueous Cotton Blue in lactic acid. The studied materials were stored at the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia (SOMF). Color photographs were taken by Canon PowerShot A460, Canon PS A1400 HD or Canon IXUS 175 HD on Boeco BM-180/T/SP microscope. Asci and ascospores were measured from the LM color images in water mounts, with the help of specialized software for

digital images Carnoy 2.0 © Peter Schols 2001 (Schols & al. 2002). Identification generally follows Greenhalgh & Morgan-Jones (1964), Dennis (1978), Ellis & Ellis (1997) and Medardi (2012). The genus *Trochila* is accepted in the *Cenangiaceae* Rehm (*Helotiales* Nannf., *Leotiomyces* O.E. Erikss.), in accordance with the latest edition of *Outline of Ascomycota: 2017* (Wijayawardene & al. 2018). The known distribution in the neighbouring countries is applied. Comparison was made with the Bulgarian materials of *T. craterium* and *T. laurocerasi* on the basis of morphometric features of the ascospores. The measurements of the ascospores are presented in the text as ranges, calculated from the mean \pm standard deviation, along with minimum and maximum values outlined in brackets, length/width (L/W) ratio. The number of measured asci and ascospores is denoted below with 'n'.

Results and discussion

Trochila laurocerasi was recorded on dead leaves of *L. officinalis* in North Macedonia, near Lake Ohrid, while *T. craterium* was studied from Turkey, Kırklareli, on overwintered leaves of *Hedera helix*, and reported from Greece on the Chalkidiki Peninsula, on dead leaves of *H. algeriensis*. Comparison has been made between these specimens of *T. craterium* and *T. laurocerasi* and the selected Bulgarian ones (Table 1, Table 2).

Trochila craterium (DC.) Fr. (Figs 1-2, Table 1).

Apothecia numerous, immersed in leaf tissue, sessile, densely disposed, initially globose, subsequently expanding, opening by a variable number of irregularly torn teeth, discoid. **Disc** up to 400 μ m, dark-brown. **Asci** (45-)50–60(-65) \times (8-)8.5–9(-10) μ m, n=17, clavate, arising from croziers, with a small pore blued

by iodine, 8-spored. **Ascospores** (5-) 7.1 \pm 0.7 (-9.1) \times (3-) 4.3 \pm 0.6 (-6) μ m, n=75, L/W ratio usually <2, (1.2-) 1.57 \pm 0.2 (-2.1) (n=90), broadly elliptical occasionally to ovoid, unicellular, biseriate, hyaline. **Paraphyses** cylindrical, septate, clavate at the top, up to 3.5 μ m wide.

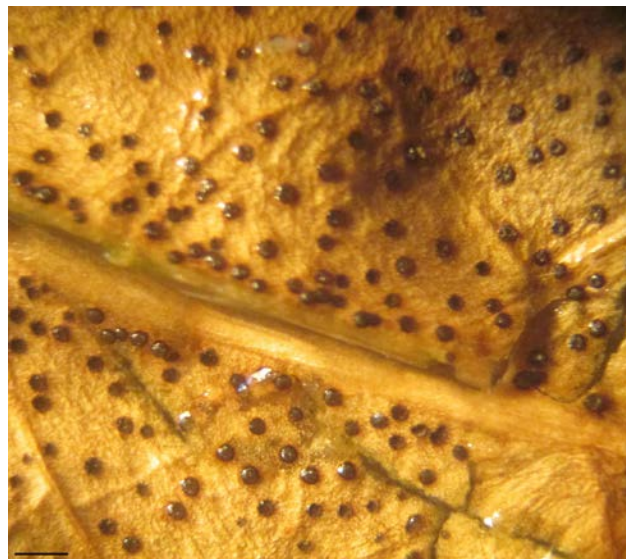


Fig. 1. *Trochila craterium*, apothecia on wet leaf surface of *Hedera algeriensis*. Scale bar = 1 mm.



Fig. 2. *Trochila craterium*, ascus in water. Scale bar = 10 μ m.

Table 1. Comparison between Bulgarian, Greek and Turkish specimens of *Trochila craterium*.

Specimen/locality, country	Ascospores (μ m) (mean; s.d.)	L/W ratio (mean; s.d.)
SOMF 21490 (BG) ^a	6–9 \times 3.5–4.5 (7.8 \times 4; \pm 1.0 \times \pm 0.4)	1.5–2.2 (2 \pm 0.2)
SOMF 26376 (BG)	5.5–8.5 \times (2.5-)3–4 (6.7 \times 3.1; \pm 1.1 \times \pm 0.5)	1.8–2.2 (2.1 \pm 0.4)
SOMF 26383 (BG)	5.5–9.5 \times (2.5-)3–5 (7.5 \times 4; \pm 1.1 \times \pm 0.9)	1.4–2.8 (2 \pm 0.4)
SOMF 26384 (BG)	5–8 \times 3.5–5 (6.7 \times 4.3; \pm 1.0 \times \pm 0.6)	1.2–2 (1.6 \pm 0.2)
SOMF 26391 (BG)	4.5–10 \times 3–5 (6.6 \times 4.1; \pm 1.5 \times \pm 0.6)	1.1–2.3 (1.6 \pm 0.3)
SOMF 26393 (BG)	5.5–9.5 \times 2.8–5.5 (7.4 \times 4; \pm 1.0 \times \pm 0.5)	1.4–2.4 (1.8 \pm 0.3)
Chalkidiki Peninsula, Greece	5.7–8.5 \times 3.1–4.2 (6.9 \times 3.8; \pm 0.6 \times \pm 0.3)	1.5–2.1 (1.84 \pm 0.2), n=15
Strandzha Mts, Turkey	5–9.1 \times 3–6 (7.1 \times 4.3; \pm 0.6 \times \pm 0.7)	1.2–2.1 (1.65 \pm 0.2), n=75

^aData about the selected Bulgarian specimens of *T. craterium* were taken from Stoykov & Assyov (2009: 356).

Specimens examined: Greece, Chalkidiki Peninsula, Stratoni village, on dead leaves of *Hedera algeriense*, 01.01.2016, leg. B. Assyov., det. D. Stoykov, SOMF 30177; Turkey, Strandzha Mts, Demirköy distr., Kirklareli, 5–6 km SW of Sarpdere village, in the vicinities of Dupnisa Mağarası natural landmark, 10.04.2007, on dry leaves of *Hedera helix*, D. Stoykov, SOMF 30175.

Known distribution in the Balkans. Albania – Shkodër (Petrak 1922), Sarandë distr. (Stoykov 2019); Bulgaria – Blagoevgrad, Burgas, Lovech, Gabrovo, Petrich, Plovdiv, Sofia, and Varna districts (Dimitrova 1997a, b; Stoykov & Assyov 2009; Dimitrova & Gyosheva 2010; Gyosheva & al. 2016); Romania (Bontea 1985), Serbia – Mt Fruška Gora, Brankovac (Savić & Karaman 2016), Turkey – Strandzha Mts (Selçuk & al. 2013).

Notes. Both asci and ascospores of the examined specimens were observed in squash mounts prepared with tap-water. *Trochila craterium* from the Balkans was studied with Romanian and Bulgarian collections by Stoykov & Assyov (2009). Dimitrova (1997a) reported apothecia on *H. helix* leaves with ascospores $7.5\text{--}8 \times 5 \mu\text{m}$, and paraphyses up to $5 \mu\text{m}$ wide at the apex. According to Ellis & Ellis (1997: 143), *T. craterium* possesses ascospores about $7\text{--}9 \times 4\text{--}5 \mu\text{m}$, while Medardi (2012: 296) describes discoid apothecial ascoma up to 0.5 mm in diam., and ellipsoid spores $7\text{--}8.5 \times 4\text{--}5 \mu\text{m}$ in size. The Greek and Turkish specimens conformed well to the selected Bulgarian ones (Table 1).

***Trochila laurocerasi* (Desm.) Fr.** (Figs 3-4, Table 2).

Apothecia numerous, scattered, subcuticular, discrete, circular, occasionally confluent, immersed in dead-leaf tissue, opening by shedding a circular patch of host epidermis. **Disc** circular or seldom irregular in confluent ascomata, up to $500 \mu\text{m}$ in diam, dark-grey. **Asci** $45\text{--}60 \times 6\text{--}10 \mu\text{m}$, $n=17$, clavate, arising from croziers, with a small pore blued by iodine, 8-spored. **Ascospores** $(6.3\text{--}) 7.7 \pm 0.7 \text{ (-}9.2) \times (2.5\text{--}) 3.3 \pm 0.4 \text{ (-}4.6) \mu\text{m}$, L/W ratio usually >2 , $(1.85\text{--}) 2.4 \pm 0.27$

(-3) , $n=50$, hyaline, broadly ellipsoid to slightly ovoid, non-septate, biseriate (occasionally tending to uniseriate) in the ascus. Paraphyses cylindrical, septate, up to $3.5 \mu\text{m}$ at the top.

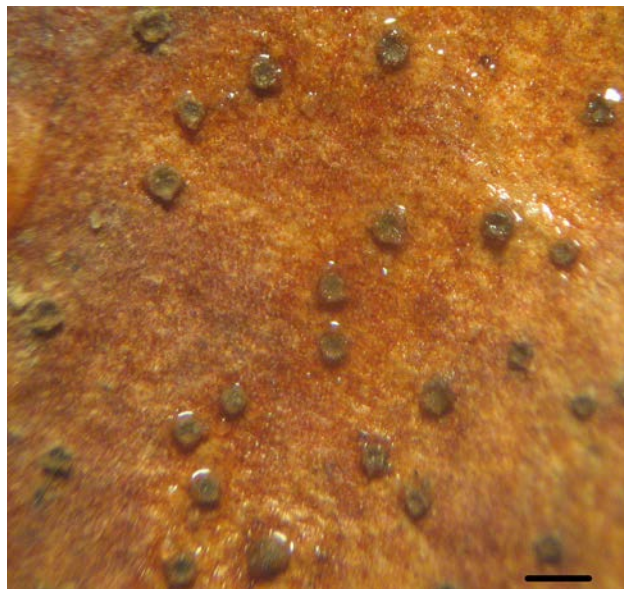


Fig. 3. *Trochila laurocerasi*, apothecia on wet leaf surface of *Laurocerasus officinalis*. Scale bar = 1 mm.



Fig. 4. *Trochila laurocerasi*, ascus, in water solution of Cotton Blue. Scale bar = $10 \mu\text{m}$.

Table 2. Comparison between Bulgarian and North Macedonian specimens of *Trochila laurocerasi*.

Specimen/locality, country	Ascospores (μm) (mean; s.d.)	L/W ratio (mean; s.d.)
SOMF 25462 (BG) ^a	$4.5\text{--}9 \times 2.5\text{--}5.5$ (6.9 ± 3.8 ; $\pm 1.2 \times \pm 0.7$)	$1.4\text{--}2.3$ (1.8 ± 0.3), $n=50$
SOMF 26389 (BG)	$5\text{--}10 \times 2.5\text{--}4.5$ (7.8 ± 3.3 ; $\pm 1.2 \times \pm 0.5$)	$1.2\text{--}3.3$ (2.4 ± 0.4), $n=50$
SOMF 26919 (BG)	$6\text{--}8.5 \times 2.2\text{--}3.5$ (7.5 ± 2.6 ; $\pm 0.7 \times \pm 0.4$)	$2.2\text{--}3.3$ (2.7 ± 0.4), $n=50$
Stara Planina Mts, Balgarka Nature Park (BG)	$6\text{--}9.4 \times 2.2\text{--}3.4$ (7.6 ± 2.8 ; $\pm 0.95 \times \pm 0.3$)	$2.2\text{--}3.3$ (2.7 ± 0.3), $n=35$
Lake Ohrid, North Macedonia	$6.3\text{--}9.2 \times 2.5\text{--}4.6$ (7.7 ± 3.3 ; $\pm 0.7 \times \pm 0.4$)	$1.85\text{--}3$ (2.4 ± 0.3), $n=50$

^a Data about the Bulgarian specimens with SOMF numbers were taken from Stoykov & Assyov (2009: 356).

Specimen examined: North Macedonia, Lake Ohrid, in the vicinity of Metropol Hotel, on fallen dead leaves of *Laurocerasus officinalis*, 20.10.2016, leg. D. Stoykov & B. Assyov, SOMF 30179.

Additional specimen examined: Bulgaria, Stara Planina Mts, Gabrovo distr., Balgarka Nature Park, Suhata Cheshma locality, 21.06.2012, D. Stoykov, on dead leaves of *L. officinalis*, SOMF 28676.

Known distribution in the Balkans. Bulgaria – Sofia region, Sofia city; Stara Planina Mts, Gabrovo distr., Balgarka Nature Park; Mt Strandzha, Marina Ryaka protected area (Stoykov & Assyov 2009; Gyosheva & al. 2016), Serbia – Mt Fruška Gora, Iriški Venac (Savić & Karaman 2016).

Notes. According to Ellis & Ellis (1997: 196), *T. laurocerasi* possesses spores ranging between 8–9 × 4 µm, which corresponds to the studies of Medardi (2012: 296). Generally, the Bulgarian and North Macedonian specimens were identical, when comparing the ascospore data, with an insignificant deviation of the minimal spore width (2.2 µm) in the two Bulgarian specimens (Table 2).

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