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The genus *Trochila* in Bulgaria

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Abstract — Two species of *Trochila* occurring on leaves of relict plants (*T. ilicina* on *Ilex* spp. and *T. laurocerasi* on *Laurocerasus officinalis*) are reported for the first time from Bulgaria, while *T. craterium* is recorded from a number of new localities. The three species are described, illustrated, and their known distribution in the Balkans and neighbouring countries noted. A morphology-based key to *Trochila* in Bulgaria is also provided. The possibly threatened status of the species in Bulgaria is also addressed.

Key words — discomycetes, *Ascomycota, Dermateaceae, Helotiales*

Discomycetes have been studied in Bulgaria for quite a long time, as reflected by the 420 species of *Helotiales, Pezizales,* and *Rhytismatales* recorded in the most recent overview (Denchev & Bakalova 2002). However, new species are constantly found and there is still need for further research.

The present work describes three species of *Trochila*, each attached to the dead leaves of an apparently different plant genus in Bulgaria: *T. ilicina* on holly (*Ilex*), *T. laurocerasi* on cherry laurel (*Laurocerasus*), and *T. craterium* on ivy (*Hedera*).

Materials and methods

Dried specimens were examined in lactophenol with cotton blue, Melzer's reagent (Kirk et al. 2008), and distilled water. Apothecia were rehydrated before being described and measured. The studied specimens are held in the mycological collections (SOMF) of the Institute of Botany, Bulgarian Academy of Sciences, Sofia. Collector abbreviations include DYS (Stoykov) and BA (Assyov). The descriptions and key are based on Bulgarian specimens. Additional specimens from the United Kingdom, Turkey, Poland, Romania and Hungary were studied for comparison with those from Bulgaria (see Tables 1, 2 & 3, p. 356). Line drawings were prepared by tracing digital photograph images onto transparent paper. The photographs were taken from slides in

lactophenol with Canon PowerShot A630 on Boeco BM-180/T/SP microscope. Species concepts follow Greenhalgh & Morgan-Jones (1964), Dennis (1978), Coste & Rey (1994), and Zioło et al. (2005). The taxonomy of the genus is in accordance with the latest *Ascomycota* outline (Lumbsch & Huhndorf 2007).

Results

Trochila craterium (DC.) Fr., Summa Veg. Scand., p. 367, 1849 Fig. 1, Table 1

Apothecia immersed in leaf tissue, sessile, densely disposed, at first globose later expanding, opening by a variable number of irregularly torn teeth, discoid. Disc 150–300(–400) μm, dark-brown. Asci (45–)65–80(–85) × 7.5–12(–15) μm, clavate, arising from croziers, with a small pore blued by iodine, 8-spored. Ascospores (4.5–)7–9(–10) × (2.5–)3–5(–6), mean 7.1 × 3.9 (±1.1 × ±0.6) μm, L/W ratio usually <2 (n = 500), broadly elliptical, unicellular, biseriate, hyaline. Paraphyses cylindrical, septate, clavate at the top, 2–3.5(–4) μm wide.

Distribution in the Balkans — Bulgaria: Blagoevgrad, Burgas, Lovech, Petrich, Plovdiv, Sofia, & Varna districts; Romania (Bontea 1985).

Specimens examined: **BULGARIA.** On dry leaves of *Hedera helix* L.: **Black Sea coast (north): Varna distr.** Albena, Seaside Resort 1.VI.2006 DYS (SOMF 26384); **Dobrich distr.** Balchik town, Botanical Garden (43°25'N 28°10'E) alt. ca 20 m 4.VI.2006 DYS (SOMF 26799); **Varna distr.** Kamchiya river 12.IX.2006 BA (SOMF 26378), Flooded forests 29.VII.2008 BA (SOMF 26727); **Black Sea coast (south): Burgas distr.** Arkutino locality 8.VI.2008 BA (SOMF 26393); Ropotamo Nature reserve, on the right river bank, 10.IX.2008 DYS & BA (SOMF 26741); Primorsko village, along trail to Maslen Nos cape, mixed oak-ash forests (42°16'N 27°46'E) alt. ca 0 m 9.IX.2008, BA & DYS (SOMF 26742); **Forebalkan: Lovech distr.** Patreshko village, Dalevska mahala (42°55'N 24°46'E) alt. ca 571 m 2.VII.2006 DYS (SOMF 26376), Predela locality 2.V.2008 DYS (SOMF 26392), near new water power building 4.V.2008 DYS (SOMF 26382); Golyama Zhelyazna village, Promkombinat locality 5.V.2008 (42°58'N 24°28'E) alt. ca 503 m DYS (SOMF 26383), 13.V.2008 DYS (SOMF 26387), 21.VI.2008 DYS (SOMF 26391); **Sofia distr.** along the road to Milanovo village (43°11'N 23°39' E) alt. ca 550 m 7.VII.2006 DYS (SOMF 26374); **Stara Planina Mts: Lovech distr.** Oreshak village (42°53'N 24°46'E) alt. ca 473 m 1.VII.2006 DYS (SOMF 26379); town of Troyan (42°53'N 24°43'E) alt. ca 510 m 2.V.2008 DYS (SOMF 26390); **Sofia region: Sofia distr.** Sofia, Geo Milev estate 14.IV.2008 DYS & BA (SOMF 26380); Borisova Gradina park (42°41'N 23°19'E) alt. 592 m 2.VI.2008 DYS (SOMF 26388); **Belasitsa Mt: Petrich distr.** above Samouilovo village 10 May 1994 V. Fakirova (SOMF 21490; see also Dimitrova 1997a); **Pirin Mts (southern): Blagoevgrad distr.** town of Melnik (41°31'N 23°24' E) alt. 438 m 16.V.2008 BA (SOMF 26385); **Rhodopi Mts (central): Asenovgrad distr.** Bachkovski monastery 22.VII.1992 V. Fakirova (SOMF 21007; 21300; see also Dimitrova 1997b).

Extralimital specimens examined: **UNITED KINGDOM.** London, Southwark, between Jamaica Road and Chambers Str. 19.I.2008 DYS (SOMF 26381), on dry leaves of *Hedera algeriensis* Hibberd; Cotebrooke, Cheshire 18.VI.2007 BA (SOMF 26373), on dry leaves of *H. helix*; **HUNGARY.** Szekszárd cemetery 24.VI.1928 L. Hollós (SOMF

6163), on dry leaves of *H. helix*; Dédesvár, Bükk Hegyaség Mt 17.VIII.1960 S. Tóth (SOMF 6164), on dry leaves of *H. helix*; **POLAND.** Kraków, Rakowicki cemetery 4.X.2008 DYS (SOMF 26800), on dry leaves of *H. helix*; **ROMANIA. Transylvania:** Cluj 5.VI.1961 M. Bechet (SOMF 9854), on dry leaves of *H. helix*.

Trochila ilicina (Nees : Fr.) Courtec., in Courtecuisse et al., Doc. Mycol. 16(62): 5, 1986 Fig. 2, Table 2

Apothecia often with remnants of covering attached as lid, scattered, on the upper side of the leaves, immersed in leaf tissue, discrete, circular, sometimes confluent in dead leaf tissues, black, opening by shedding a circular patch of host epidermis. Disc circular or irregular in confluent ascomata, 350–500(–700) µm in diam, dark greyish. Asci (50–)65–75(–90) × (5–)8–10(–11) µm, clavate, arising from croziers, with a small pore blued by iodine, 8-spored. Ascospores (4.5–)9–13.5 × 3.5–5.5, mean 11.6 × 4.5 (±1.0 × ±0.4) µm, L/W ratio usually >2 (n = 100), hyaline, elliptical to ovoid, non-septate, biseriate (sometimes tending to uniseriate) in the ascus. Paraphyses cylindrical, septate, clavate at the top, up to 4.5 µm wide at the apex.

Distribution in the Balkans — Bulgaria: Burgas & Petrich districts, Greece (Pantidou 1973), Romania (Bontea 1985), Turkey (Stoykov & Denchev 2007).

Specimens examined: **BULGARIA.** On fallen leaves of *Ilex aquifolium* L.: **Belasitsa Mt: Petrich distr.** between Belasitsa hut and the waterfall (41°36'84"N 23°18'52"E) 15.IX.2003 BA (SOMF 25408); On fallen leaves of *I. colchica* Pojark: **Strandzha Mt: Burgas distr.** Marina Rjaka protected area 27.V.2005 BA (SOMF 25720).

Extralimital specimens examined: **UNITED KINGDOM. Cheshire:** Sandymere, Oakmere reservoir 20.VI.2007 BA (SOMF 26354), on fallen leaves of *I. aquifolium*; **ROMANIA. Ilvof distr:** Bucharest, Botanical Garden 7.II.2007 BA (SOMF 26386), on fallen leaves of *I. aquifolium*; **TURKEY. Strandzha Mt:** Kuru Dere ravine 1.VI.2007 DYS (SOMF 26314), on fallen leaves of *I. colchica*.

For the nomenclature of this species the reader is referred to Courtecuisse et al. (1986).

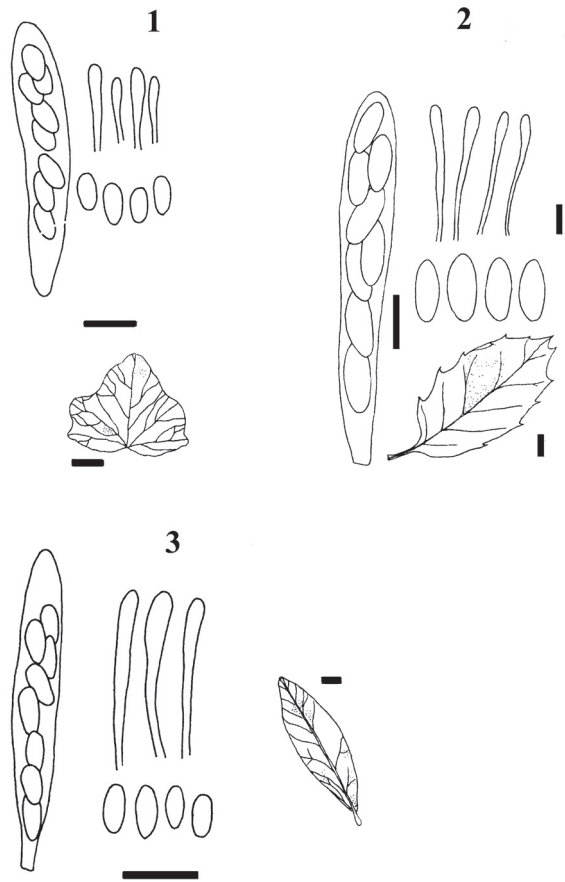
Trochila laurocerasi (Desm.) Fr., Summa Veg. Scand., p. 367, 1849 Fig. 3, Table 3

Apothecia numerous, scattered, subcuticular, discrete, circular, sometimes confluent, immersed in dead leaf tissue, opening by shedding a circular patch of host epidermis. Disc circular or rarely irregular in confluent ascomata, (220–)260–350(–500) µm in diam, dark grey. Asci 45–70(–85) × 5.5–10(–13) µm, clavate, arising from croziers, with pore ca 1 µm long and blued by iodine, 8-spored. Ascospores 4.5–10(–11) × (2.2–)2.5–5.5, mean 7.7 × 3.6 (±1.2 × ±0.6) µm, L/W ratio usually >2 (n = 200), hyaline, broadly ellipsoid to slightly ovoid, non-septate, biseriate (sometimes tending to uniseriate) in the ascus. Paraphyses cylindrical, septate, clavate at the top, up to 4.5 µm wide at the apex.

Distribution in the Balkans — Bulgaria: Burgas & Sofia districts.

Specimens examined: **BULGARIA.** On fallen leaves of *Laurocerasus officinalis* M. Roem. (≡ *Prunus laurocerasus* L.): **Sofia region: Sofia distr.** Sofia, Borisova Gradina Park alt. 592 m (42°41'N 23°19'E), 2.VI.2008 DYS (SOMF 26389), 16.VII.2008 DYS (SOMF 26400); Lozenetz Estate—19 Gorski Patnik str. 13.XII.2008 BA (SOMF 26919); **Strandzha Mt: Burgas distr.** Marina Rjaka protected area 27.V.2005 BA (SOMF 25462).

Extralimital specimen examined: **UNITED KINGDOM. Lancashire:** Lancaster, Ashton Memorial 18.VIII.2007 BA (SOMF 26375), on fallen leaves of *Laurocerasus lusitanica* (L.) M. Roem. (≡ *Prunus lusitanica* L.).



Figures 1–3: Ascus, paraphyses, spores and outline of the leaf.
1. *T. craterium* (SOMF 26393); 2. *T. ilicina* (SOMF 25408); 3. *T. laurocerasi* (SOMF 25462).
Scale bars = 10 μm for asci, spores and paraphyses, and = 1 cm for the leaves.

Key to *Trochila* species in Bulgaria

1a. On leaves of *Ilex*. Ascomata epiphyllous. Ascospores 9–13.5 × 3.5–5.5, mean 11.6 × 4.5 μm, length/width ratio (L/W) usually >2 ..... ***T. ilicina***

1b. On leaves of *Hedera* and *Laurocerasus*. Ascomata usually hypophyllous, rarely epiphyllous **2**

2a (1b). On *Hedera* leaves. Ascospores 4.5–9(–10) × 2.5–5, mean 7.1 × 3.9 μm, L/W ratio usually <2 ..... ***T. craterium***

2b. On *Laurocerasus* leaves. Ascospores 4.5–10.5 × (2.2–)2.5–5.5, mean 7.7 × 3.6 μm, L/W ratio usually >2 ..... ***T. laurocerasi***

Discussion

Trochila species are usually (Dennis 1978) characterised by a biseriate arrangement of ascospores in the asci. However, in Bulgarian specimens the arrangement of spores in some asci appeared uniseriate, possibly resulting from studying dead asci (Baral 1992).

Although easily separated by their host specificity, the three *Trochila* species are relatively difficult to distinguish microscopically (Tables 1–3, Figure 4). Spore widths are not diagnostic, as the mean values are very similar and there is considerable overlap in the M±1σ area for the three species. More useful is the spore length. In *T. ilicina* the mean ascospore length is greater than in the two other species. We have not found any data in the literature regarding the ascospore length/width ratio for European *Trochila* species. Although the ratio might well be helpful — it is usually <2 in *T. craterium* and >2 in *T. ilicina* and *T. laurocerasi* — it appears only secondarily diagnostic in view of the variation noted for all three species. In general the ascus and ascospore measurements in *T. ilicina* and *T. laurocerasi* resemble those given by Coste & Rey (1994), Nauta & Spooner (2000), and Zioło et al. (2005), and are somewhat similar to those pointed out in Phillips (1893), Dennis (1978), and Medardi (2006). The values for the Romanian specimen (SOMF 26386), however, are considerably lower. One possible explanation is that the ascomata of the Romanian collection are immature. Ascospore measurements of *T. craterium* more or less agree by those cited by Dennis (1978), Greenhalgh & Morgan-Jones (1964), Nauta & Spooner (2000), and Medardi (2006). Only in the specimen on *Hedera algeriensis* from London (SOMF 26381) are the ascospores noticeably larger. Further study is needed to explain the variability of this fungus, as also noted by Greenhalgh & Morgan-Jones (1964).

Trochila craterium is apparently not a rare fungus in Bulgaria, considering the number of the above records and the distribution of the host, which is a common native and popular ornamental plant. Instead, *T. ilicina* seems to be rather uncommon in this country. Both hosts (i.e., *Ilex aquifolium*, *I. colchica*) are rare relict species and thus protected by the law. They are relatively rarely

Table 1. Comparison of Bulgarian and extralimital *Trochila craterium* specimens

Specimens	Ascospores (µm) mean (s.d.)	L/W ratio	Host species (*Hedera*)
21490 (BG)	6–9 × 3.5–4.5 7.8 × 4 (±1.0 × ±0.4)	**1.5–2.2** (2±0.2)	*H. helix*
26376 (BG)	5.5–8.5 × (2.5–)3–4 6.7 × 3.1 (±1.1 × ±0.5)	**1.8–2.2** (2.1±0.4)	*H. helix*
26383 (BG)	5.5–9.5 × (2.5–)3–5 7.5 × 4 (±1.1 × ±0.9)	**1.4–2.8** (2±0.4)	*H. helix*
26384 (BG)	5–8 × 3.5–5 6.7 × 4.3 (±1.0 × ±0.6)	**1.2–2** (1.6±0.2)	*H. helix*
26391 (BG)	4.5–10 × 3–5 6.6 × 4.1 (±1.5 × ±0.6)	**1.1–2.3** (1.6±0.3)	*H. helix*
26393 (BG)	5.5–9.5 × 2.8–5.5 7.4 × 4 (±1.0 × ±0.5)	**1.4–2.4** (1.8±0.3)	*H. helix*
9854 (ROM)	4–7 × 2–3.5(–4) 5.5 × 3 (±1.0 × ±0.5)	**1.2–2.4** (1.9±0.3)	*H. helix*
6163 (HUN)	6.5–10 × 3.5–5(–6) 7.8 × 4.6 (±1.0 × ±0.9)	**1.4–2.1** (1.7±0.2)	*H. helix*
26381 (UK)	8.5–12 × 4.5–6 9.7 × 5.1 (±1.1 × ±0.6)	**1.6–2.2** (1.9±0.2)	*H. algeriensis*
26385 (BG)	4.5–7(–8) × (2.5–)3.5–4 5.6 × 2.8 (±0.8 × ±0.4)	**1.7–2.3** (2.1±0.2)	*H. helix*

Table 2. Comparison of Bulgarian and extralimital *Trochila ilicina* specimens

Specimens	Ascospores (µm)	L/W ratio	Host species (*Ilex*)
25408 (BG)	9–13.5 × 3.5–5.5 11. 6× 4.5 (±1.0 ×±0.4)	**2–3.3** (2.6±0.4)	*I. aquifolium*
26354 (UK)	7–11 × 3–5(–5.5) 9.1 × 4 (±1.7 × ±0.8)	**2–2.5** (2.3±0.2)	*I. aquifolium*
26314 (TUR)	7–16 × 3.5–5.5 11.2 × 5.1 (±2.1 × ±0.4)	**1.5–3** (2.2±0.4)	*I. colchica*
26386 (ROM)	(3.5–)4–7 × (2–)2.5–4(–4.5) 5.8 × 2.9 (±0.8 ×±0.5)	**1.2–2.8** (2±0.3)	*I. aquifolium*

Table 3. Comparison of Bulgarian and United Kingdom *Trochila laurocerasi* specimens

Specimens	Ascospores (µm)	L/W ratio	Host species (*Laurocerasus*)
25462 (BG)	4.5–9 × 2.5–5.5 6.9 × 3.8 (±1.2 × ±0.7)	**1.4–2.3** (1.8±0.3)	*L. officinalis*
26375 (UK)	5–11 × 3–5.5 8.5 × 3.9 (±1.3 × ±0.7)	**1.8–2.9** (2.2±0.3)	*L. lusitanica*
26389 (BG)	5–10 × 2.5–4.5 7.8 × 3.3 (±1.2 × ±0.5)	**1.2–3.3** (2.4±0.4)	*L. officinalis*
26919 (BG)	6–8.5 × 2.2–3.5 7.5 × 2.6 (±0.7 × ±0.4)	**2.2–3.3** (2.7±0.4)	*L. officinalis*

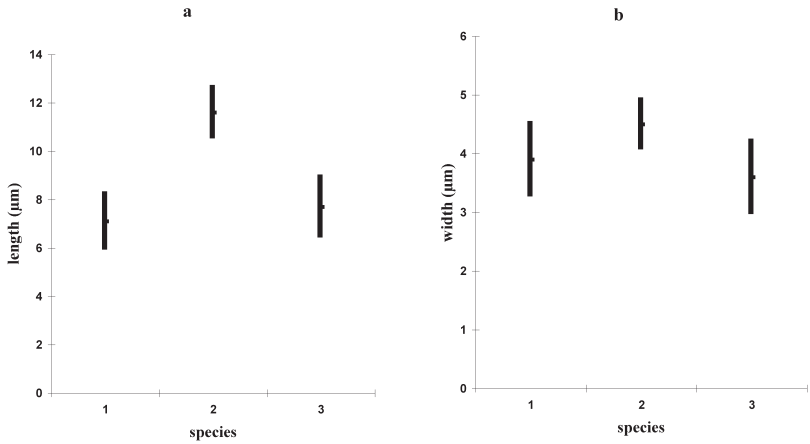


FIGURE 4. Variation of length (a) and width (b) measurements of the ascospores: 1. *T. craterium;* 2. *T. ilicina;* 3. *T. laurocerasi*.

found in gardens and the authors know of few places where they are successfully grown. Furthermore *T. ilicina* has not been seen every year even in holly's natural habitats. Denchev & Bakalova (2002) discussed the need for evaluating the threatened status of micromycetes, pointing out that fungi on rare host plants may or may not also be rare. However, in view of the available data on *T. ilicina,* it seems likely that the fungus is also a threatened species.

Trochila laurocerasi, does not appear equally threatened. Its host plant is also a preglacial relict, but although quite rare in wild in Bulgaria, it is extensively planted nearly everywhere. Though *T. laurocerasi* has been found only once in wild and twice on planted cherry laurel, it seems logical to think it is more widespread, given the numerous records on *Laurocerasus officinalis* in Slovakia (Juhásová et al. 2003) and on both *L. officinalis* and *L. lusitanica* in the United Kingdom (Ainsworth 1950, Greenhalgh & Morgan-Jones 1964, Nauta & Spooner 2000). Both host plants are non-native to those countries, so one could conclude that *T. laurocerasi* is probably a somewhat adaptable species. It is difficult to say at this point whether *T. laurocerasi* should qualify as threatened in Bulgaria.

It seems probable that *Trochila* species are sometimes overlooked. *Trochila craterium* could be relatively widespread and thus should be looked for in other neighbouring Balkan countries such as Albania, Greece, Turkey, FYR Macedonia, and Serbia. *Trochila ilicina* (as well as the two other species) tends to follow the distribution of the host plants (*Ilex aquifolium* and *I. colchica*),

and in Europe *I. aquifolium* extends to northern Germany and Austria. In the Balkans this plant is known to grow in the neighbouring countries and the fungus could be expected from Albania, FYR Macedonia, Serbia. The other European host, *I. colchica* is found in Bulgaria and Turkey. *Trochila laurocerasi* may thus also be more widely distributed in the Balkan Peninsula and could be expected elsewhere in Albania, Greece, Serbia, FYR Macedonia, Turkey, and Romania.

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