

New records of *Ophiognomonia* (*Gnomoniaceae*, *Diaporthales*) from Bulgaria, Greece and Turkey

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Received: June 24, 2016 ▷ Accepted: October 25, 2016

Abstract. *Ophiognomonia melanostyla* is reported from Bulgaria (Vitosha region) and Turkey (Mt. Strandzha), while *O. setacea* represents a new find from Greece (Epirus region). *Quercus castaneifolia* and *Q. trojana* are recorded as new host plants of *O. setacea*. Morphological descriptions, color illustrations and additional information about the new finds are included. Data on the *Gnomoniaceae* from Bulgaria and the adjacent Balkan countries are summarized.

Key words: Balkan Peninsula, fungal diversity, *Gnomoniaceae*, new host, *Ophiognomonia*

Introduction

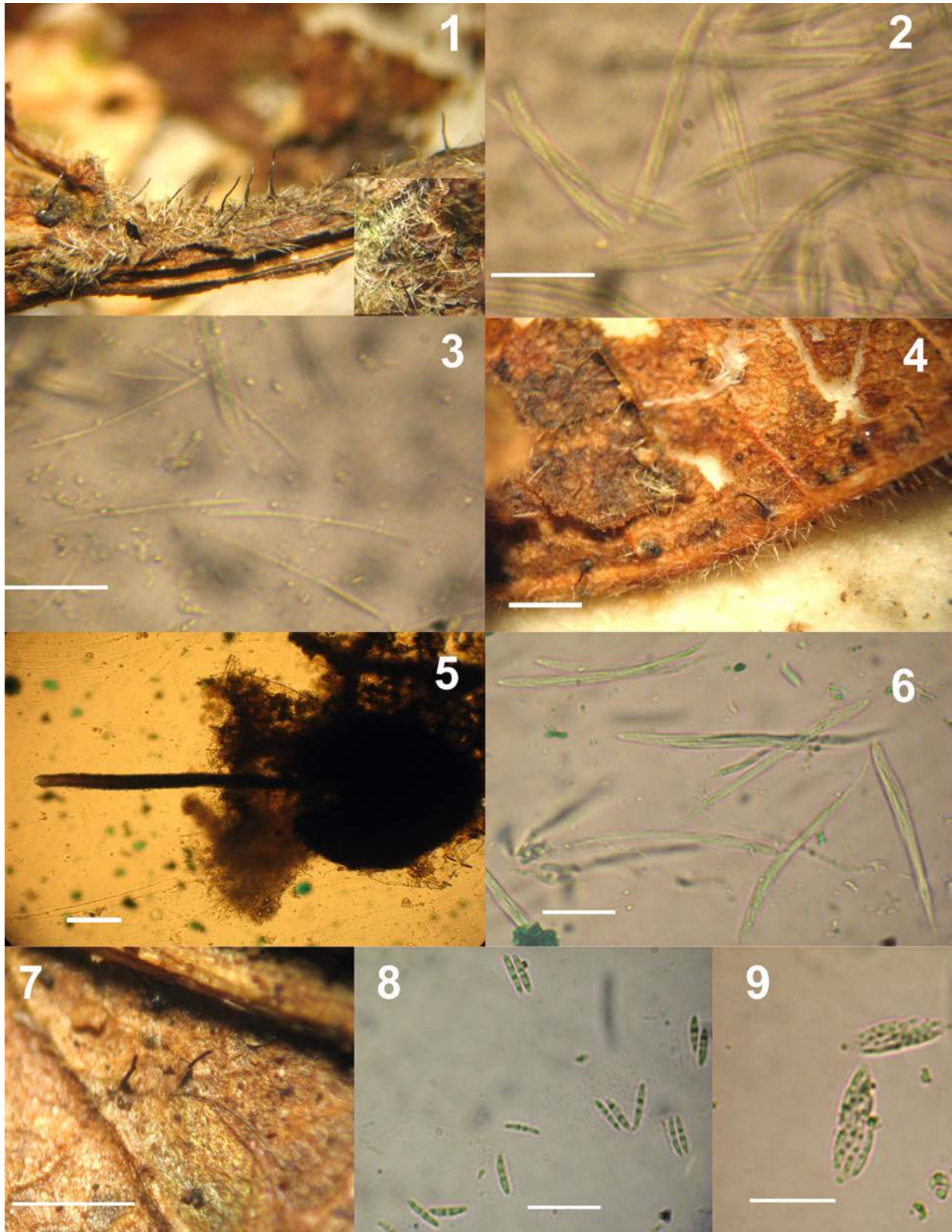
The widest accepted concept of the *Gnomoniaceae* (*Diaporthales*) before the recent molecular studies (Castlebury & al. 2002, Mejia & al. 2008, 2011; Sogonov & al. 2008; Walker & al. 2010, etc.) was that of the Canadian researcher Prof. Margaret E. Barr-Bigelow. She recognised two families (*Gnomoniaceae* G. Winter and *Valsaceae* Tul. & C. Tul.) within the suborder *Gnomoniineae*, distinguished by the placement of the perithecial beak (Barr 1978). The members of *Gnomoniaceae* were defined with upright perithecia, central or seldom eccentric beaks, erumpent separately, and having nonseptate, 1-septate or occasionally several septate ascospores (Barr 1978: 18). This family, according to Margaret Barr, included three subfamilies, one of which was the *Gnomonioideae*, comprising four genera along with the genus *Ophiognomonia* (accepted afterwards within the *Gnomoniaceae*).

The genus *Ophiognomonia* (Sacc.) Sacc. is typified by the species *Ophiognomonia melanostyla* (DC. : Fr.) Berl. (Monod 1983; Sogonov & al. 2008). It is usually known as common host-specific fungus developing

on overwintered leaves of *Tilia* spp., while *O. setacea* (Pers. : Fr.) Sogonov is attached to the overwintered leaves of *Castanea* Mill. and *Quercus* L. (in Northern hemisphere) and *Nothofagus* Blume (in Southern hemisphere). The perithecia of both fungi are usually seen from the underside of the leaves, immersed in leaf tissues or in the leaf veins, where their long necks are easy to observe (Plate I, Figs 1, 4, 7).

Diversity of *Gnomoniaceae* family in the adjacent Balkan countries (excluding Bulgaria) is still scanty. *Gnomoniaceous* fungi in Bulgaria comprise 51 species, including the recent reports of *Gnomoniopsis guttulara* (Stårback) D. M. Walker on *Agrimonia eupatoria* L., *Gnomoniella vagans* Johans. on *Dryas octopetala* L., *Linospora capreae* (DC. : Fr.) Fuckel on *Salix caprea* L., etc. (Walker & al. 2010; Denchev & al. 2011; Stoykov 2012; etc.). Among them, the genus *Ophiognomonia* is represented by six species within the *Gnomoniaceae* (Stoykov 2000; Stoykov 2005, 2012; Stoykov & Assyov 2006; Sogonov & al. 2008 etc.): *O.alni-viridis* (Podl.-Růž. & Svrček) Sogonov, *O. gei-montani* (Ranoj.) Sogonov, *O. ischnostyla* (Desm.) Sogonov, *O. leptostyla* (Fr. : Fr.) Sogonov, *O. rosae* (Fuckel) Kirschst., and *O. setacea*.

Plate I



Ophiognomon melanostyla: Fig. 1. Perithecia on petiole and leaf of *Tilia platyphyllos*; Fig. 2. Asci. Scale bar = 22.5 μ m; Fig. 3. Ascospores. Scale bar = 22.5 μ m; Fig. 4. Perithecia on leaf of *Tilia* sp. Scale bar = 1 mm; Fig. 5. Perithecium in water solution of Cotton Blue. Scale bar = 100 μ m; Fig. 6. Asci and spores. Scale bar = 25 μ m; *O. setacea*: Fig. 7. Perithecia on a leaf of *Quercus trojana*. Scale bar = 1 mm; Fig. 8. Ascospores. Scale bar = 22.5 μ m; Fig. 9. Asci. Scale bar = 22.5 μ m.

In Romania, the *Gnomoniaceae* is represented by six species (see Stoykov 2004 and the references therein), comprising *Ophiognomonia ischnostyla* on leaves of *Carpinus betulus* L. and *O. setacea*, known on *Castanea sativa* Mill. and *Quercus petraea* (Matt.) Liebl.

Recently, *Ophiognomonia leptostyla* was recorded, studied and documented in detail from the eastern part of the Republic of Macedonia (Karov & al. 2014).

Four species of *Gnomoniaceae* are known to us from Serbia (Arsenjević 1979; Arsenjević & al. 2005; Milijašević 2007; Karaman & al. 2012; etc.), of these, *O. leptostyla* is known as important plant parasite on walnuts (Arsenjević 1979; Karov & al. 2014).

Nine gnomoniaceous fungi were registered by Stoykov & Denchev (2007) in the region of Mt. Strandzha (Turkey), including *O. leptostyla* on leaves of *J. regia* and *O. setacea* on *Quercus cerris* L.

Six species of *Gnomoniaceae* are known from Greece (according to the data published in Pantidou 1973 and Zervakis 2001). Of these, only *O. leptostyla* on *Juglans regia* L. was reported (Pantidou 1973).

Material and methods

The collected specimens studied herein are stored in the Mycological Collection, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOMF). The size of the perithecia, beaks, asci, and ascospores is presented below, usually in the form of minimum-maximum values; where indicated as [(min-) mean \pm 1 standard deviation (-max), *n*], 'n' – denotes the number of asci/spores used. Measurements under LM were always taken in water with the help of software Carnoy 2.0 (© Peter Schols, 2001) for digital images. The microscopic features were examined in water, or in water solution of Cotton Blue. Color photographs were taken by means of Olympus E330 digital camera under Olympus BX-41 LM and by Canon PS A460 under Boeco 180/T/SP LM and Boeco BOE3500 dissecting microscope.

Results

Diaporthales

Gnomoniaceae

Ophiognomonia melanostyla (DC. : Fr.) Berl., Icon. Fung., 2: 146, 1899 (Plate I, Figs 1-6)

Perithecia 160–190 (-240) \times (240-) 260–300 (-335) μm , black, solitaire, nonstromatic, immersed in

the leaf blade, nervatures or petioles; on the underside of the leaf blade, occasionally epiphyllous (seen on the upper part of the petioles), at first immersed, later partly erumpent at maturity, depressed globose to subglobose when moist, convex to occasionally concave when dry. **Beaks** 380–620(-950) \times 30–40 μm , about 25 μm wide at the apex, central to eccentric, never truly lateral, usually curved. **Asci** (45-) 50–70 (-80) \times (3.5-) 4.5–5 (-5.5) μm [(45-) 62.5 \pm 8.8 (-81) \times (3.0-) 4.7 \pm 0.6 (-6.5) μm , *n*=50], narrowly fusiform, apical annulus *ca* 1.5 μm , 8-spored, with \pm parallel spores. **Ascospores** 36–40 (-45.5) \times (1.3-) 1.5–2 μm [(36-) 41.3 \pm 2.1 (-45.5) \times (1.3-) 1.6 \pm 0.2 (-2.0), *n*=30, μm , hyaline, needle shaped, straight, 2-celled, septum suprmedian, slightly constricted at the septum; ends rounded, lower cell usually slightly narrower than the upper one; guttulate; appendages visible only with the help of differential interference contrast microscope (Sogonov & al. 2008: 3).

Specimens examined: Bulgaria: Vitosha region, Mt. Vitosha, Vitosha Nature Park, along the track to Boyanski Vodopad waterfall, 04.06.2016, on overwintered leaves of *Tilia platyphyllos* Scop., 42°38'03.8"N, 23°15'32.7"E, *ca* 1065 m, D. Stoykov (SOMF 26652), Plate I, Figs 1-3; Turkey: Strandzha Mt., Demirköy distr., Kırklareli vilayet, 5-6 km SW of Sarpdere village, in the vicinities of Dupnisa Mağarası natural landmark, 10.04.2007, on overwintered leaf of *Tilia* sp., D. Stoykov (SOMF 26637), Plate I, Figs 4-6.

Additional material examined: United Kingdom: England, the city of London, Hyde Park, 28.01.2008, on overwintered leaves of *Tilia* sp., D. Stoykov.

Note. The morphological description of *O. melanostyla*, based on the specimens from Bulgaria and Turkey, conforms well with the description of the fungus given in Barr (1978).

Habitat. On fallen overwintered leaves of: *Tilia americana* L., *T. cordata* Mill., *T. platyphyllos* Scop., *Tilia* spp. (*Tiliaceae*).

Known distribution. Europe: Austria, Bulgaria (Vitosha region), Czech Republic, Germany, Italy, Switzerland, Turkey (Mt. Strandzha), Ukraine, United Kingdom (England); North America: Canada, United States of America (U.S.A.).

For a thorough account on the habitat and the distribution see Barr (1978: 63), Monod (1983: 159), Sogonov & al. (2008: 48), and Walker & al. (2012).

Comments. Stoykov & Denchev (2006: 181) listed the record of *O. melanostyla* on *Tilia* sp. from Pirin

Mts, named originally in Fakirova (1985) as *Cryptoderis melanostyla* (DC. : Fr.) G. Winter. The authors point out there that the thorough revision of the corresponding specimen and its host plant (Stoykov 2012: 112, SOMF 20322) revealed only the presence of *Gnomonia gnomon* (Tode : Fr.) J. Schröt. on a dry leaf of *Corylus avellana* L. *Gnomonia gnomon* is another common long-beaked gnomoniaceous fungus possessing: perithecia collapsed inwards when dry; shorter beaks; smaller asci; and quite shorter (about 15–25 × 1–1.5 µm) symmetric filiform ascospores with median septum. It is known exceptionally on leaves of *Corylus* L., only occasionally on leaves of *Populus* L. (Barr 1978; Monod 1983). Thus, we present the first reports of *O. melanostyla* on overwintered leaves of *Tilia* L. from Bulgaria and Turkey.

Ophiognomonia setacea (Pers. : Fr.) Sogonov, in Sogonov, Castlebury, Rossman, Mejía & White, Stud. Mycol., 62: 64, 2008. (Plate I, Figs 7-9)

Perithecia up to 300 µm in diam, black or yellowish-brown, globose to depressed-globose, single, without stroma, immersed in the leaf blade and near the leaf veins. **Beaks** (215-) 250–550 × 25–45 µm, black, when mature whitish at the top, straight or slightly curved, usually central, narrowed at the apex, near the base widened up to 55 µm in diameter, cylindrical. **Asci** (25-) 26.5–31 (-35) × 5.5–9(-10) µm, cylindrical-fusoid, 8-spored, with parallel and at different height or in fascicle in the ascus, apical annulus ca 1 µm. **Ascospores** (9.3-) 11.8 ± 1.0 (-14.5) × (1.3-) 2.1 ± 0.3 (-2.6) µm, *n*=50, length/width ratio (4.6-) 5.6 ± 0.85 (-8.1), hyaline, narrowly-fusoid, straight, septum median, occasionally slightly constricted at the septum, with hyaline appendages at both ends. Each cell with two small guttules.

Specimen examined: Greece: Epirus region, Delvinaki, Ioanina prefecture, 39°54'42.50"N, 20°27'31.83"E, 12.03.2016, on overwintered leaves of *Quercus trojana* Webb, leg. B. Assyov, det. D. Stoykov (SOMF 26641).

Additional material examined: United Kingdom: England, Surrey, Royal Botanic Gardens, Kew, along the walk from Climbers and clippers to Magnolia zone, 27.01.2008, on overwintered leaf of *Quercus castaneifolia* C.A. Mey., D. Stoykov.

Habitat. On overwintered leaves of: *Castanea dentata* L., *C. sativa* Mill., *Castanea* spp., *Quercus alba* L., *Q. bicolor* Willd., *Q. castaneifolia*, *Q. cerris* L., *Q. dalechampii* Ten., *Q. macrocarpa* Michx., *Q. mon-*

tana Willd., *Q. palustris* Münchh., *Q. phellos* L., *Q. pedunculiflora* C.Koch, *Q. pubescens* Willd., *Q. rubra* L., *Q. robur* L., *Q. thracica* Stef. & Nedjalkov, *Q. trojana*, *Quercus* spp. and *Nothofagus obliqua* (Mirb.) Blume (Fagaceae).

Known distribution. Europe: Austria, Bulgaria, Greece (Epirus region), Germany, Italy, Montenegro, Romania, Sweden, Switzerland, United Kingdom (England); Asia: Japan; North America: Canada, U.S.A.; South America: Chile.

For more details on the habitat and the distribution see Kobayashi (1970), Barr (1978), Monod (1983), Sogonov & al. (2008), Stoykov (2004, 2012), and Walker & al. (2012).

Comments. The Macedonian oak (*Quercus trojana*) appears to be a new substratum of *O. setacea*, according to the known published data. This is the first report of this fungus from Greece. The multi-gene phylogeny published in Sogonov & al. (2008) revealed that *O. setacea* is closely allied with *Ophiognomonia* species, rather than the genus *Gnomonia* Ces. & De Not. Another morphologically similar species of *Ophiognomonia* (*O. alni-viridis*), known in Bulgaria only on overwintered leaves of *Alnus viridis* (Chaix) DC., possesses ascospores about 9–16.5 × 1.5–2.5 µm (Stoykov 2012).

Acknowledgements. The field trip in Turkey has been carried out within the framework of the project 'Conservation and sustainable use of biodiversity in Mt. Strandzha' (MAB, UNESCO Contract, no. 8759406/2006) of the former Institute of Botany, Bulgarian Academy of Sciences. The author is indebted to Chief Assist. Dr B. Assyov (Institute of Biodiversity and Ecosystem Research, Sofia) for the materials of *O. setacea*, placed at his disposal.

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