Daldinia vernicosa from the Eastern Forebalkan (Bulgaria)

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Abstract. *Daldinia vernicosa* was found in the Eastern Forebalkan on the bark of *Carpinus orientalis*, which is the first report on this host. Identification is based on molecular analysis and morphological data. A description and illustrations of the specimens found are provided.

Key words: Bulgarian mycota, *Carpinus*, *Xylariaceae*

Introduction

The genus *Daldinia* Ces. & De Not. is known to be taxonomically difficult and, presently, seventeen species of it are accepted in Europe (Ju & al. 1997; Rogers & al. 1999; Johannesson & al. 2000; Stadler & al. 2001, 2004; Wollweber & Stadler 2001; Stadler & al. 2014). Of these, only *D. concentrica* (Bolton : Fr.) Ces. & De Not., the type species, seems to have been recorded from the countries of the Balkan Peninsula, although different larger ascomycetes, including some interesting and uncommon xylarialean fungi, have been mentioned in a number of publications from Bulgaria and the adjacent regions (e.g. Læssøe 1997; Zervakis & al. 1999; Karadelev & al. 2007; Sesli & Denchev 2009; Stoykov 2011). During field work, the authors have repeatedly collected a small species of *Daldinia*, growing on dead branches of *Carpinus orientalis* Mill., which is subject to morphological and genetic studies here.

Material and methods

Air-dried specimens of the fungal stromata have been conserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research of the Bulgarian Academy of Sciences (SOMF). The microscopic features were studied in water, KOH and Melzer's reagent. Measurements of the microstructures were always taken in water. The size of ascospores is presented below in the following format: (min-) mean ± 1 s (-max), n; where 'n' – denotes the number of measured spores, and 's' represents the standard deviation. Scanning electronic microphotographs (SEM) were taken with a JEOL JSM-6390 device at 10 kV. Spores for SEM observations were obtained from pieces of stromata, mounted on metal stubs with double-sided adhesive tape and sputter-coated with gold.

For genetic analysis, total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona 1987) included 35 cycles with annealing temperature of 54 °C. Primers ITS1F and ITS4 (White & al. 1990; Gardes & Bruns 1993) were employed to amplify ITS rDNA region. PCR products were checked in 1 % agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were searched for putative reading errors, and these were corrected.

Results and discussion

Daldinia vernicosa Ces. & De Not., Comment. Soc. Crittog. Ital. **1:** 198, 1863 (Figs 1-3)
Syn. *D. fissa* Lloyd, Mycol. Writ. **7**: 1313, 1922.

Stromata turbinate, stipitate, solitary or in groups, up to 1.2 cm in diameter and up to 1.5 cm high, vinaceous-brown, subsequently blackening, varnished when old; KOH-extractable pigments livid; tissue between perithecia greyish, pithy to woody; tissue below the perithecial layer composed of alternating darker and lighter zones; darker zones blackish-grey to black, pithy to woody; lighter zones whitish, initially gelatinous, subsequently disintegrating and becoming loculate. **Perithecia** tubulate, 530–1050 µm high and up to 215–400 µm in diameter; ostioles slightly papillate. **Asci** not seen in the Bulgarian specimens. **Ascospores** blackish to dark-brown, ellipsoid, equilateral or slightly inequilateral, with broadly rounded ends, (11.5-) 12.2 ± 0.6 (-13.5) × (5.5-) 6.6 ± 0.2 (-7) µm, n=100, length/width ratio (1.6-) 1.9 ± 0.1 (-2.1), with straight germ slit spore-length on the more convex side, perispore indehiscent in 10 % KOH, epispore smooth in SEM.

Specimens examined. Bulgaria: Lovech distr., Golyama Zhelyazna village (Troyan Municipality), 43°0'1.5"N, 24°29'42.2"E, alt. *ca* 445 m, on dead branches of *Carpinus orientalis*, 15.08.2008, D. Stoykov & B. Assyov (SOMF 28163, GenBank MN535762); idem., 13.05.2011, D. Stoykov (SOMF 28164).

Notes. So far, *D. vernicosa* was known only from a single locality at the Southern Black Sea Coast, Burgas distr., Sozopol town, on *Celtis* cf. *australis* L. (Stadler & al. 2014: 87).

The newly-collected Bulgarian specimens match the morphological concept of *Daldinia fissa* as circumscribed in Ju & al. (1997) and Wollweber & Stadler (2001), but this species is considered an aberrant form of *D. vernicosa* on the basis of genetic evidence (Stadler & al. 2014). The specimens analyzed in the present study have an ITS rDNA 100 % identical with GenBank NR152501, obtained from culture CBS 119316, which comes from specimen KR 0026316, collected on the same site of the epitype (specimen KR 0026318, culture BCRC 34048, GenBank EF026146). Some differences between NR152501 and EF026146 can be observed, but they are accepted as part of intraspecific variability. The size of ascospores generally agrees with that reported by Ju & al. (1997), Wollweber and Stadler (2001) and Chlebicki (2008), although we have recorded a somewhat lower value for the minimal spore width. This difference can be interpreted as intraspecific variability until further samples from new localities are studied, or insignificant (derived from measurements of only two collections from single locality). The spores of the studied specimens have a smooth epispore under SEM (Fig. 3), thus closely resembling the illustrations in Stadler & al. (2001) and Stadler & al. (2014).

Because of its small-sized stromata and livid to purplish extractable pigments, *D. vernicosa* seems to resemble to some extent the species *D. caldariorum* Henn. and *D. singularis* Y.-M. Ju, Vassilyeva & J.D. Rogers, which are present in Europe. The latter has been reported to date only from the Russian Far

Fig. 1. *Daldinia vernicosa*, stromata *in situ*.

Fig. 2. *D. vernicosa*, cross-section of stroma, *ex situ*.

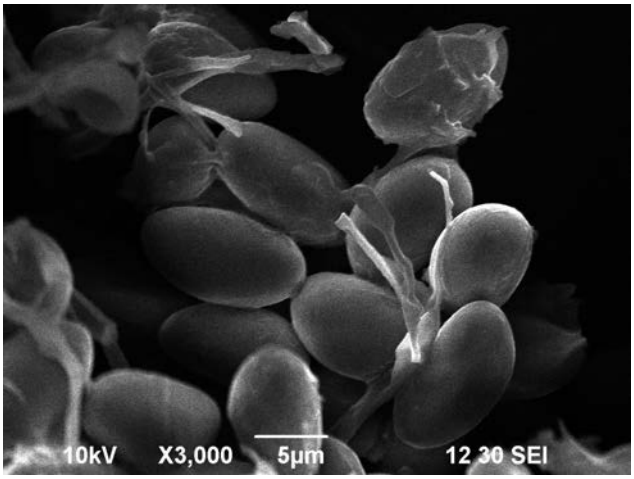

Fig. 3. *D. vernicosa*, ascospores under SEM.

East, where it occurs in association with *Carpinus* (Stadler & al. 2014). The two species can be distinguished by the presence of lighter zones on the stromata, and by their distinctly smaller ascospores, not exceeding 11 µm in length and 5.5 µm in width. In external morphology, *Daldinia vernicosa* can resemble to some extent *D. childiae* J.D. Rogers & Y.-M. Ju (Stadler & al. 2014: 74), mainly by the short stipitate to turbinate stromata. However, it differs clearly in the constitution of lighter zones on the stromata, greater spore length (up to 17 µm), and transversal striations of spores observed with SEM.

Rhoads (1918) considered *D. vernicosa* as a pyroxylophilous fungus. The size of ascospores in the collections studied by Rhoads (1918: 281) usually varied within 10-14.5 × 7-7.5 µm, and thus conform well with the current concept of Stadler & al. (2014). These authors stated that *D. vernicosa* was mostly found on 'freshly felled wood or on damaged, still living trees'. Wollweber & Stadler (2001), Stadler & al. (2001: 176) and Stadler & al. (2014: 84) consider *D. vernicosa* a pyrophilic species, often found on burnt or fire-damaged woody hosts. However, both Bulgarian collections studied in the present work were attached to dead, but not burnt large branches of *Carpinus orientalis,* which seems to be also a new substrate of this species.

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References

Chlebicki, A. 2008. Some overlooked and rare xylariaceous fungi from Poland. – Pol. Bot. J., **53**: 71-80.

Gardes, M. & Bruns, T.D. 1993. ITS primers with enhanced specificity for Basidiomycetes—application to the identification of mycorrhizae and rusts. – Mol. Ecol., **2**: 113-118.

Johannesson, H., Læssøe, T. & Stenlid, J. 2000. Molecular and morphological investigation of *Daldinia* in northern Europe. – Mycol. Res., **104**: 275-280.

Ju, Y.-M., Rogers, J.D. & Martín, F.S. 1997. A revision of the genus *Daldinia.* – Mycotaxon, **61**: 243-293.

Karadelev, M., Kost, G. & Rexer, K. 2007. New macromycetes species (*Ascomycetes* and *Basidiomycetes*) for mycota of the Republic of Macedonia. – In: Collection of papers dedicated to Academician Kiril Micevski, pp. 311-327. Macedonian Academy of Sciences and Arts, Skopje.

Læssøe, T. 1997. *Entonaema cinnabarina* – an exotic fungus. – Svampe, **36**: 21-22 (in Danish).

Mullis, K. & Faloona, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. – Meth. Enzymol., **155**: 335-350.

Murray, M.G. & Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. – Nucleic Acid Res., **8**(19): 4321-4325.

Rhoads, A.S. 1918. *Daldinia vernicosa*–a pyroxylophylous fungus. – Mycologia, **10**(6): 277-284.

Rogers, J.D., Ju, Y.-M., Watling, R. & Whalley, A.J.S. 1999. A reinterpretation of *Daldinia concentrica* based upon a recently discovered specimen. – Mycotaxon, **72**: 507-519.

Sesli, E. & Denchev, C.M. 2009. Checklists of the myxomycetes, larger ascomycetes and larger basidiomycetes in Turkey. – Mycotaxon, **106**: 65-68.

Stadler, M., Baumgartner, M., Wollweber, H., Ju, Y.-M. & Rogers, J.D. 2001. *Daldinia decipiens* sp. nov. and notes on some other European *Daldinia* spp. inhabiting *Betulaceae.* – Mycotaxon, **80**: 167-177.

Stadler, M., Wollweber, H., Jäger, W., Briegert, M., Venturella, G., Castro, J.M. & Tichy, H.V. 2004. Cryptic species related to *Daldinia concentrica* and *D. eschscholzii,* with notes on *D. bakeri.* – Mycol. Res., **108**: 257-273.

Stadler, M., Læssøe, T., Fournier, J., Decock, C., Schmieschek, B., Tichy, H.-V. & Peršoh, D. 2014. A polyphasic taxonomy of *Daldinia* (*Xylariaceae*). – Stud. Mycol., **77**(1): 1-143.

Stoykov, D.Y. 2011. *Xylaria longipes* (*Xylariaceae*) in Bulgaria. – In: **Denchev, C.M.** (ed.), New records of fungi, fungus-like organisms, and slime moulds from Europe and Asia: 28–29. – Mycol. Balcan., **8**: 173-175.

White, T.J., Bruns, T.D., Lee, S. & Taylor, J.W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: **Innis, M.A., Gelfand, D.H., Sninsky, J. & White, T.J.** (eds), PCR Protocols: a Guide to Methods and Applications. Academic, San Diego.

Wollweber, H. & Stadler, M. 2001. Zur Kenntnis der Gattung *Daldinia* in Deutschland und Europa. – Z. Mykol., **67**: 3-53.

Zervakis, G., Lizoň, P., Dimou, D. & Polemis, E. 1999. Annotated checklist of the Greek macrofungi. II. *Ascomycotina.* – Mycotaxon, **72**: 487-506.