

Didymella curtisii (Didymellaceae) on *Pancratium maritimum* in Bulgaria and Greece

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Abstract. *Didymella curtisii* is recorded on dry leaves of *Pancratium maritimum* in the coastal regions of the Southern Black Sea and Chalkidiki Peninsula. These finds are presented with brief description and color illustrations on the basis of studied specimens. Our findings are compared with the examined extralimital materials.

Key words: Balkan Peninsula, *Didymella*, new host, *Pancratium*, *Stagonospora*

Introduction

Didymella curtisii (Berk.) Q. Chen & L. Cai, commonly known under the names of *Stagonospora curtisii* (Berk.) Sacc., *Stagonosporopsis curtisii* (Berk.) Boerema and *Phoma narcissi* (Aderh.) Boerema & al., is a worldwide fungal pathogen on various plants of the *Amaryllidaceae*: *Amaryllis* L., *Hippeastrum* Herb., *Narcissus* L., etc. (Boerema 1993; Boerema & al. 2004; Punithalingam & Spooner 2005; Raabe & al. 2009). It displays itself with characteristic bright-red patches, which may affect any part of the infected plants from roots to flowers and, therefore, is also named Leaf Scorch, Red Leaf Spot, Neck Rot, Red Blotch, Leaf Blotch, or Red Fire. These names are applied because of the red staining or yellowish colouration of the leaves caused by red pigments (Saniewska & Budzianowski 1997; Plate I, Figs 1-4). In spite of the fact that the disease was generally considered of insignificant economic importance, it has been reported in single cases as prevalent (Punithalingam & Spooner 2005: 155). It is widely known as most active during cool and damp weather, and is usually spread by airborne spores. Plant hosts infested by *D. curtisii* always

develop red spots on the foliage and even on stems causing deformation of growth, generally known as Leaf Scorch.

In the present paper, *Didymella curtisii* is recorded from Bulgaria on dry leaves of *Pancratium maritimum* L. (*Amaryllidaceae*) from the sandy coastal dunes in the protected areas of Silistar and Arkutino, and from the sandy dunes of Chalkidiki Peninsula, Greece. The host is included in the *Red Data Book of the Republic of Bulgaria* (Apostolova 2015) under the category Endangered (EN).

Material and methods

The cited specimens, originating from Bulgaria and Greece, are kept at the Mycological Collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOMF). Other extralimital materials of *S. curtisii* used for comparison were examined in 2015, as a result of collaboration with Mrs. Begoña Aguirre-Hudson and Mrs. Angela Bond, K(M), Fungarium Collections, Royal Botanic Gardens, Kew, England, U.K. The size of the macro-

and microscopic characters is presented with minimum and maximum values. Measurements of Bulgarian specimens under LM were taken in tap water and aqueous Cotton Blue, with the help of specialized software for digital images Carnoy 2.0 (© Peter Schols, 2001). The microscopic features of conidia were examined in water mounts on semi-permanent slides (*P. maritimum*), and measured additionally in ready-made permanent slides stained with Cotton Blue (*Agapanthus* sp., *Narcissus* sp.). Colour photographs were taken with the help of Olympus E330 and Canon PS A460 digital cameras under Olympus BX-41, Boeco BM-180/T/SP LM and Boeco 3500 dissecting microscope. The known distribution of *D. curtisii* is given according to Punithalingam & Spooner (2005) and Chen & al. (2017). Synonyms generally follow Boerema (1993), Boerema & al. (2004) and Punithalingam & Spooner (2005).

Results and discussion

Didymella curtisii (Berk.) Q. Chen & L. Cai, in Chen, Jiang, Zhang, Cai & Crous, *Stud. Mycol.*, **82**: 175 (2015), Plate I, Figs 1-6.

Stagonospora curtisii (Berk.) Sacc., *Syll. Fung.*, **3**: 451 (1884).

Spots on leaves, initially (0.2-)0.4-0.6(-0.8) × (0.6-)0.8-1(-1.5) mm, later widening up to (1-)1.5-2(-3) × (3-)6-10 mm, yellowish, buff to purplish, ± coalescing to form large blotches. **Conidiomata** pycnidia, mostly epiphyllous, immersed to partly erumpent, pale yellowish brown, subglobose, ostiolate. **Conidiophores** absent. **Conidia** (4-)6-27(-28) × (2-)3-7(-7.5) µm, length : width (l:w) ratio generally 2.5-4.5 [(2-)2.8-4.3(-5)], n=170, hyaline, smooth, straight or slightly curved, cylindrical with rounded ends to oblong ellipsoid, constricted at the septa; truncate or rounded at the base, at times slightly narrowed at the apex, (0-)1-3-septate. Conidia very variable in size: aseptate conidia about (4-)4.5-6(-10) × 2-3.5 µm, 1-septate (10-)11-21(-23) × 3-5(-6) µm, 2-septate (13-)14-21(-22) × (3.5-)4-6(-7) µm, and 3-septate (19.5-)21-27(-28) × (5-)5.5-7(-8) µm (Plate I, Figs 5-6).

Specimens examined. Bulgaria: Southern Black Sea Coast, Burgas distr., Silistar protected site, on sandy coastal dunes, alt. ca 0 m, 25.05.2007, D.Y. Stoykov, SOMF 26620, on dry leaves of *Pancratium maritimum* (*Amaryllidaceae*), as *S. curtisii*; Black Sea

Coast (Southern), Burgas distr., Arkutino Reserve, on sandy dunes, alt. ca 0 m, 16.09.2011, D.Y. Stoykov, SOMF 26653, on dry leaves of *P. maritimum*, as *S. curtisii*; Greece: Chalkidiki Peninsula, 2 km southeast from the town of Olympiada, 15.10.2016, leg. B. Assyov, SOMF 28622, on dry leaves of *P. maritimum*.

Additional specimens examined. United Kingdom: England, Surrey, Kew, Royal Botanic Gardens, nr Palace, on 'scorched' leaf tips, 22.04.2004, leg. & det. B.M. Spooner, K(M) 122185, on leaves of *Narcissus* sp., as *Stagonospora curtisii*; England, Devon, Dartmouth, Galmpton, Greenway National Trust Gardens, 31.08.2003, leg. B.M. Spooner, det. E. Punithalingam, K(M) 123938, on fading leaves of *Agapanthus* sp. (?*A. praecox* Willd.), as *S. curtisii*; Spain: Cantabria, Duñas de Liéncres, 02.11.2017, leg. B. Assyov, SOMF 29794, minute red leaf spots on dry leaves of *P. maritimum*.

Known distribution: Africa (Egypt, Ethiopia, Malawi, South Africa, Tanzania, Zambia), Asia (India, Iraq), Australasia (New Zealand), Europe (Bulgaria, Czech Republic, Netherlands, U.K. and Channel Islands), North America (U.S.A.), South America (Brasil), and West Indies (Cuba, Puerto Rico). Widely distributed on various members of *Amaryllidaceae* (*Agapanthus* L'Hér., *Amaryllis*, *Clivia* Lindl., *Crinum* L., *Eucharis* Planch. & Linden, *Hippeastrum* Herb., *Hymenocallis* Salisb., *Galanthus* L., *Leucojum* L., *Lycoris* Herb., *Narcissus*, *Nerine* Herb., *Pancratium* L., *Sternbergia* Waldst. & Kit., *Sprekelia* Heist., *Vallota* Salisb. ex Herb., *Zephyranthes* Herb.), *Chlorophytum* Ker Gawl. (*Anthericaceae*) and *Mimusops* L. (*Sapotaceae*).

Note. Data about conidial morphology, given in the description of *D. curtisii*, are based on the author's collection from the Silistar protected site (SOMF 26620).

Comments. *Didymella curtisii*, as a rule, infests the tips of young and developing leaves, causing the symptoms of leaf blotch and leaf scorch in *Agapanthus*, *Amaryllis*, *Hippeastrum*, *Narcissus*, *Nerine*, *Pancratium* (*Amaryllidaceae*) (Vanev & al. 1996: 179; Punithalingam & Spooner 2005; Blake & al. 2008).

According to Boerema & al. (1997: 369), the conidia of *Phoma narcissi* [viz. *Stagonosporopsis curtisii*] are with normal phomoid size *in vitro* and vary within the ranges: 4-7.5(-8) × (2-)2.5-3.5(-4) µm for aseptate fungal cells and 8-15 × 3-5.5 µm for septate ones. However, according to Boerema & al. (1997), *in vivo* pycnidia on *Amaryllidaceae* contain mainly aseptate or, exceptionally, 1-septate conidia of *Stagonosporopsis curtisii*-type:

Plate I.

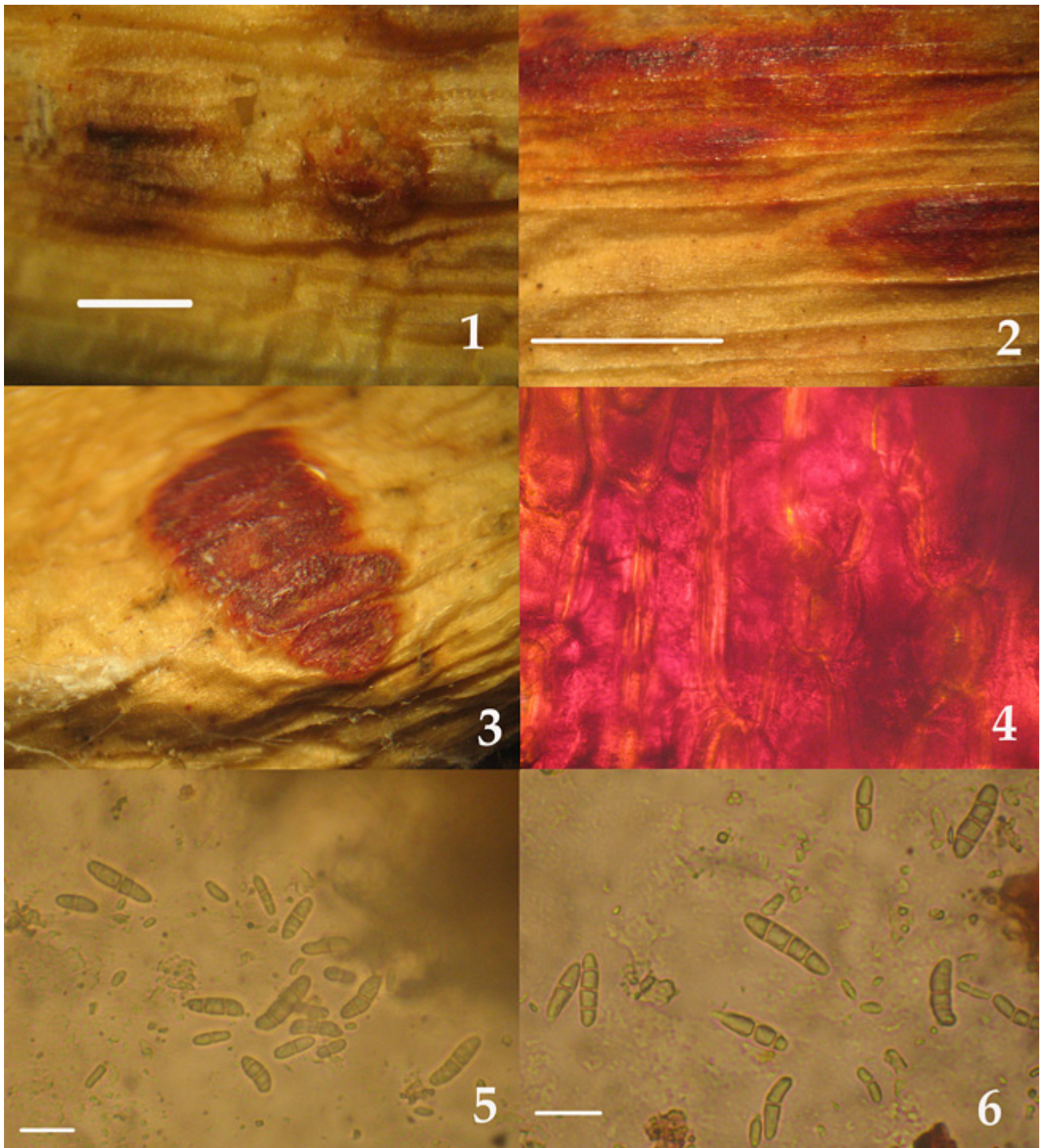
***Didymella curtisii*:**

Fig. 1. Leaf spots on *Pancratium maritimum* (SOMF 26620). Scale bar = 2 mm;

Fig. 2. Red leaf spots on *P. maritimum* (SOMF 26653). Scale bar = 1 mm;

Fig. 3. Leaf spot on *P. maritimum* (SOMF 28622);

Fig. 4. Red-colored plant cells from the epidermis of *P. maritimum* (SOMF 28622);

Figs 5-6. Aseptate, 1-septate, 2-septate and 3-septate conidia, in water (SOMF 26620). Scale bars = 15 μ m.

4.5–8(-10) × 2.5–4(-5) µm (av. 6.8–7.5 × 3–3.8 µm). This conforms with the data about aseptate conidia derived from the studied material from Chalkidiki Peninsula, Greece (Table 1). The larger conidia in the cited description (mostly 3-septate) are about 13.5–28 × 5–8 µm (av. 21 × 6.5 µm), and the aseptate ones are observed occasionally, while 1-septate conidia vary within 8–16 × 3–6.5 µm (av. 11.5 × 4.5 µm).

The disease is known in Bulgaria under the name Red Leaf Scorch on *Amaryllis* and appears as punctate to large angular or linear spots with red, cherry red to brown coloration, which cause visible deformation of leaves and stems (Christoff 1972). It also occurs on the leaves of *Narcissus* (Vanev & al. 1996). According to Saccardo (1884), mature conidia of *Didymella curtisii* derived from the host plant material of *Narcissus* in North America are 2-septate and 17–21 × 7.5 µm in size.

Punithalingam & Spooner (2005) reported *Didymella curtisii* (= *Stagonospora curtisii*) in Herb. IMI and K(M), with collections studied from different countries in Europe, Africa, Asia, and Australasia. As a result of their work, both authors concluded that *D. curtisii* is a 'polymorphic species, producing conidia which vary widely in size and septation, usually in the range 6–28 × 3–7 µm, and (0-)1–3(-4) septa, developed within thin-walled pycnidia on leaves of host plants, growing naturally in fields and gardens. In culture on agar media, conidia are usually aseptate, 1-septate or 2-septate' (Table 1). Mention deserves the fact that the name *Stagonospora curtisii* was the one that has been most often used by plant pathologists and taxonomists over the years. The examined materials of *Didymella curtisii*, originating from Bulgaria and Greece, agree with Punithalingam & Spooner (2005: 155), Boerema & al. (1997, 2004), Table 1.

Table 1. *Didymella curtisii* based on morphology on different hosts of *Amaryllidaceae*.

Author/Specimen	Spots	Conidia (µm)	Host (Origin)
SOMF 26620, as <i>Stagonospora curtisii</i> , Bulgaria	Yellowish, buff to purplish, coalescing to form large blotches, (0.2-)0.4–0.6(-0.8) × (0.6-)0.8–1(-1.5) mm, later up to (1-)1.5–2(-3) in width, (3-)6–10 mm in length. Pycnidia single, mostly epiphyllous, partly erumpent, ostiolate (Plate I, Figs 1-2).	(4-)6–27(-28) × (2-)3–7(-7.5), n=170, l:w ratio generally 2.5–4.5 [2–5], hyaline, (0-)1–3-septate, cylindrical, with rounded ends, slightly narrowed at apex, straight or slightly curved, constricted at septa.	<i>Panocratium maritimum</i> (Southern Black Sea Coast, Silistar protected site, sandy dunes)
SOMF 28622, as <i>S. curtisii</i> , Greece	Purplish, about 4.5–10 × 1.3–2.5 mm; pycnidia contain single, ellipsoid, hyaline aseptate conidia (Plate I, Fig. 3).	(5-)6–8(-10) × (2-)2.5–3(-4), aseptate (phomoid type).	<i>P. maritimum</i> , (Chalkidiki Peninsula, SE from Olympiada town, sandy dunes)
Boerema & al. (1997), as <i>Stagonosporopsis curtisii</i> , Netherlands	Pycnidia subepidermal in dead leaf tips, and in spots on leaves and scales.	0–1-septate: 4.5–8(-10) × 2.5–4(-5) (av. 6.8–7.5 × 3–3.8); 1–3-septate (8-) 13.5–16 (-28) × (3-)5–6.5(-8), (av. 11.5–21 × 4.5–6.5).	<i>Nerine</i> , the Netherlands
Boerema & al. (2004), as <i>Phoma narcissi</i> (syn. <i>Stagonosporopsis curtisii</i>)	Pycnidia subepidermal in dead leaf tips; on leaves and scales; more variable in the field; with mostly 3-septate larger conidia. Multicellular chlamydospores occur.	mostly 4–7.5 × 2.5–3.5; aseptate: 4.5–8(-10) × 2–4(-5) (av. 6.8–7.5 × 3–3.8); 1-septate: 8–16 × 3.2–6.4 (av. 11.5 × 4.5); 3-septate: 13.4–28 × 4.8–8 (av. 21 × 6.5).	<i>Narcissus</i> , <i>Hippeastum</i> , various <i>Amaryllidaceae</i>
K(M) 122185, as <i>Stagonospora curtisii</i> , United Kingdom	Yellowish, light-brown, usually elongate, continuous, with numerous small, punctiform, scattered, mostly epiphyllous, pycnidia.	(11.5-)22.5–26(-28.5) × (4-)5.5–7.5(-8.5), l:w ratio 2.7–4.6, n=20, 1–3-septate, oblong ellipsoid.	<i>Narcissus</i> sp. (England, Surrey, Kew)
K(M) 123938, as <i>S. curtisii</i> , UK	Yellowish to buff or purplish, 0.4–1(-1.3) × (1-)2–3.5 mm, often coalescing to form large blotches; pycnidia of 3-layered thin-walled cells, 140–190 µm in diam.	(7-)9–24(-28) × (2.3-)3.5–6.5(-8.5), l:w ratio 2.5–4.5, n=178, (0-)1–3(-4)-septate, cylindrical, constricted at septa.	<i>Agapanthus</i> sp. (England, Devon)
Punithalingam & Spooner (2005), as <i>S. curtisii</i> , UK	Yellowish, buff to purplish. Pycnidia composed of 3-layered thin-walled cells, 140–190 µm in diam., mostly epiphyllous, immersed becoming partly erumpent, yellowish to pale yellowish-brown, solitary.	(4-)6–22(-25) × (2-)4–4.5(-6), (0-)1–3(-4)-septate, aseptate ca 4 × 2; 1-septate 6–9 × 4–4.5; 2-septate 10–14 × 4–4.5; 3-septate 16–22(-25) × 4–6; 4-septate 23–25 × 4.5–6; cylindrical, truncate or rounded at the base, slightly narrowed at apex, constricted at septa.	? <i>Agapanthus praecox</i> (England, Devon)

Stagonospora pancratii Vanev & Bakalova (with 1–3-septate, hyaline, smooth, cylindrical with rounded ends, rarely oblong-ellipsoid, 10–27.5 × 4–7.5 µm, straight or slightly curved, constricted at septa conidia) is known as a causal agent of dark red spots on living leaves from *Pancreatium maritimum* (Vanev & Bakalova 1988; Vanev & al. 1997). Both authors stated clearly that by the time of its description ‘there are no fungi known from *Stagonospora* genus on the species of *Pancreatium*’ (Vanev & Bakalova 1988: 170, 172). However, occurrence of *S. curtisii* on *Pancreatium* has been reported (Punithalingam & Spooner 2005: 152).

It is not unlikely, that *D. curtisii* might occur in the same fungus-host association in similar habitats of Europe and Asia, where the populations of *Pancreatium maritimum* naturally exist (Elibol & Bilgen 2017: 569).

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