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New record of the fungus *Coniochaeta prunicola* on peaches from Slovakia

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Abstract: The first record of *Coniochaeta prunicola* Damm & Crous (Coniochaetales, Sordariomycetes, Ascomycota) as a pathogen of *Persica vulgaris* Mill. described and illustrated from Nitra district, Slovakia. *C. prunicola* was isolated from symptomatic leaves of host tree and based on morphological attributes identified for the first time as a causative agent of peach tree damage. *C. prunicola* is characterized by dark brown ascomata clothed with setae, the fasciculate, unitunicate, cylindrical asci and broadly almond-shaped, ellipsoidal ascospores with a longitudinal germ slit.

Key words: Ascomycota; morphological attributes; *Persica vulgaris*; Sordariomycetes

Introduction

Coniochaetaceae are characterized by dark brown to black ascocarps with an ostiolate peridium that may or may not be setose, and forming the dark brown, discoid, nearly globose or ellipsoidal ascospores which are variable in shape and size but most commonly ellipsoid to fusoid or nearly globose, 1–celled, smooth, brown to black, with a distinct germ slit (Mahoney & LaFavre 1981; Hawksworth & Yip 1981; Hanlin 1990; Weber 2002). The genus *Coniochaeta* (Sacc.) Cooke is generally described as a large and highly diversified ascomycetous genus with non-stromatic, globose or subglobose perithecia with a short neck and usually broad ostiole (Mahoney & LaFavre 1981; Asgari & Zare 2006).

Species of the genus *Coniochaeta* and their *Lecythophora* anamorphs occur on different plant material: on wood or bark of different trees, on leaves and leaf litter (Kale 1967; Rao 1970; Mahoney & LaFavre 1981; van der Linde 1991; Yuan & Mohammed 1997), on dung of various animals (Mahoney & LaFavre 1981; Richardson 1998), on soil (Mahoney & LaFavre 1981; Kamiya et al. 1995) and in water with extremely low pH and high concentrations of heavy metals (Eriksson 1992; Kamiya et al. 1995; López-Archilla et al. 2004; Asgari et al. 2007). Some *Coniochaeta* species have been found to exhibit useful biochemical properties. Species are knowed to produce antibacterial and antifugal metabolites (Wang et al. 1995; Segeth et al. 2003).

Several *Coniochaeta* species have been isolated from different parts of *Prunus*. In Moldavia *Coniochaeta ambigua* (Sacc.) Cooke was isolated on dry twigs of apricot and cherry and *Coniochaeta calva* Tode on twigs of cherry and plum (Popushoi 1971). *Coniochaeta ligniaria* (Grev.) Massee was isolated from decaying bark of *Prunus avium* L. in the Netherlands (CBS 178.75). Different species of *Coniochaeta* were isolated and identified on *Prunus*: *Coniochaeta velutina* (Fuckel) Munk on *Prunus* sp., *Coniochaeta africana* Damm & Crous on wood of *Prunus salicina* Lindl., *Coniochaeta prunicola* Damm & Crous on wood of *Armeniaca vulgaris* L. and *Prunus salicina* Lind. in South Africa (Damm et al. 2010) and on leaves and twigs of *Laurocerasus officinalis* L. in Slovakia (according to results of Ivanová & Bernadovičová, yet unpublished). From the order Coniochaetales (family Coniochaetaceae) the new record of the *Coniochaeta prunicola* causing leaf blight of *Persica vulgaris* was confirm. This is the first record of this fungus as a pathogen of *P. vulgaris* in Slovakia. The disease was of sporadic distribution but infested trees were enough affected. The aim of this study was to identify the fungus associated with the disease and to determine the distinctive morphological features of isolated *Coniochaeta* species as a causal factor involved in vitality weakening of peach trees.

Material and methods

From spring to autumn of 2009 and 2010 and from spring of 2011, leaves of *Persica vulgaris* (Redhaven) with blight symptoms were sampled from the plants growing in private gardens of the town Nitra – Zobor. Altogether 30 leaf samples were used for isolation from one location. Age of evaluated trees varied from 20 to 35 years. The samples of plant material have been deposed in Herbarium at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plant Biology in Nitra.

Classical phytopathological approaches were used to isolate and obtain pure cultures. The leaf parts cut from the diseased plants were surface-sterilized by immersion in sodium hypochlorite solution (1% available chlorine) for 20 minutes, rinsed twice or three times in sterile distilled water and then dried carefully with filter paper. After that, the plant samples were cut to fragments of 3–5 mm which were placed on potato-dextrose agar (3% PDA) in Petri dishes. This was followed by cultivation at 24 ± 1°C and 45% humidity in dark conditions in a versatile environmental test chamber MLR-351H (Sanyo) and subsequent isolation on PDA medium. Pure fungal cultures were obtained using multiple purifications. The obtained isolates were transferred on PDA medium (3%) to induce sporulation. Study of fungal structures was performed with a clinical microscope BX41 (Olympus) under a 400× and 1000× magnification.

The isolated fungus was identified by microscopic analyses based on morphological characteristics of the fruiting bodies (perithecia), spore bearing organs (asci), reproduction organs (conidia and ascospores). The identification was performed using morphological keys according to Hawksworth & Yip (1981), Ellis & Ellis (1987), Checa et al. (1988), Romero et al. (1999), Asgari et al. (2007) and morphological studies in Mahoney & LaFavre (1981), Hanlin (1990), Weber (2002) and Damm et al. (2010).

Results and discussion

Genus *Coniochaeta* belongs to three phylogenetically distinct genera of the *Lecythophora*-like fungi isolated from *Prunus* wood. Isolates from *Prunus* sp. identified as *Coniochaeta* include besides *C. prunicola* and *C. africana*, also *C. velutina* (Damm et al. 2010). Basic morphological features of *Coniochaeta* species identified in *Prunus* are shown in Table 1.

Concerning all morphological characteristics and determined differences, the fungus under investigation in our study isolated from blighted leaves of peach trees was identified as *Coniochaeta prunicola*. Table 1 includes the morphological characteristics of *C. prunicola* isolated from examined peach leaf samples.

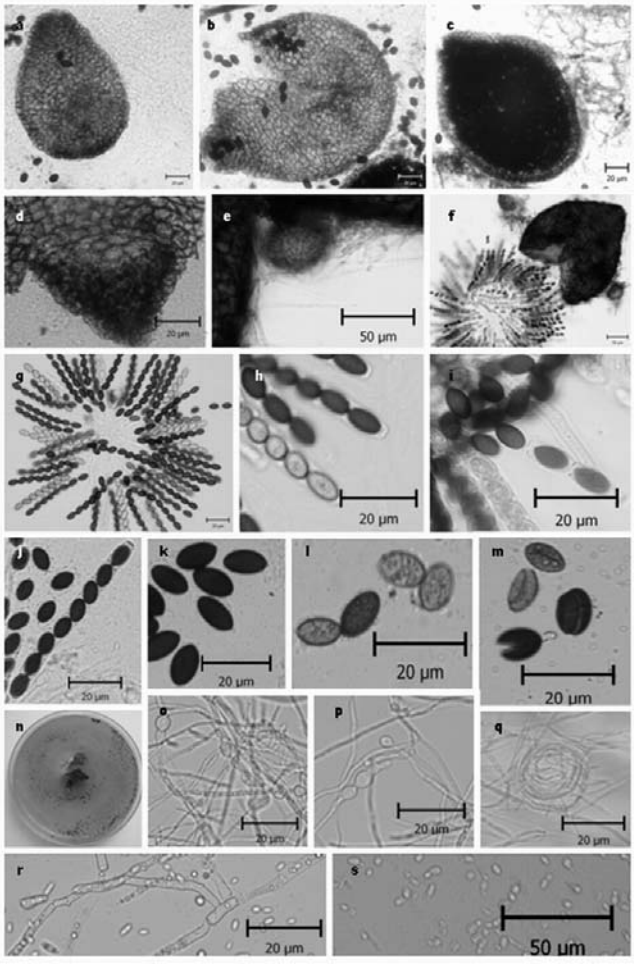

Fig. 1. *Coniochaeta prunicola* on *Persica vulgaris*. Teleomorph (a–m): a–c – ascocarps with neck; d – peridial cells; e – peridial setae; f–g – rosettes of asci; h – apical rings of asci; i – paraphysis; j – 8–spored ascus; k – ascospores; l – granular content of ascospores; m – germ slits. Anamorph (n–s): n – colony on PDA 24 days after inoculation; o – p. mycelium; q – hyphal coil; r – hyphas with collarettes; s – conidia. Scale bars: a–d, g–r = 20 µm; e, f, s = 50 µm.

Coniochaeta prunicola Damm & Crous *on Persica vulgaris* Mill. (Fig. 1)

Taxonomy: Anatomical-morphologically characteristic. Ascomata immersed or superficial on PDA developing after about 8–10 weeks were perithecial, solitary, subglobose to pyriform, 125–173(265) × 95–145(229) µm with a central ostiole 17–26 µm (Fig. 1a). Neck of ascomata was 31–42 µm long (Fig. 1b). Peridium wall were thick 8–10 µm, more-layered, outer layer composed of brown, thick-walled angular cells (Fig. 1c, d) with brown or hyaline smooth walled, straight or bent setae with globose or subglobose apices, 3–4.5 µm wide and 21–29 µm long, sometimes a little shorter and relatively infrequent (Fig. 1e). Review of the literature shows that although asci and ascospores characteristics are very important, setae are still prominent feature of the most *Coniochaeta* species. Most of the described setae are dark brown to black, straight or bent, unbranched, rigid hairs with a sharp apex. They may by scattered over the perithecial wall or concentrated on its upper portion (Mahoney & LaFavre 1981). Some species are described as lacking setae (Romero et al. 1999). According to Damm et al. (2010) fungus *C. prunicola* isolated from branches of stone fruit (*Prunus* sp.) produced subglobose to pyriform ascomata, 200–250 µm in diameter with neck, 50–60 µm long. Peridium was pseudoparenchymatous, 20–25 µm (5–8 layers), outer wall consists of dark brown textura angularis, with setae. Setae were brown (or hyaline), straight, cylindrical, tapering to a round tip, smooth-walled or granulate, 2–3.5 µm wide, up to 80 µm long.

The fasciculate, unitunicate, cylindric asci with a truncate apex and a small apical ring long 4–5 µm (Fig. 1h) form rosettes (Fig. 1f, g). Less numerous paraphyses which are hyaline, septate, 63 × 3–4 µm in size are formed between the asci (Fig. 1i). The formation of unitunicate, cylindrical asci, 58–68(94) × 8–10 µm with eight ascospores/ascus was observed (Fig. 1j). Brown ascospores were one-celled, ellipsoidal (Fig. 1k), smooth-walled without ornamentation of the ascospore

Table 1. Comparison of morphological characteristics of different *Coniochaeta* species identified previously in *Prunus* and examined material from Slovakia.

Authors	Sampled plant material under examination	Ivanová, Bernadovičová (yet unpublished)	Damm et al. (2010)		
Host plant	*Persica vulgaris*	*Laurocerasus officinalis*	*Armeniaca vulgaris, Prunus salicina*	*Prunus* sp.	*Prunus salicina*
Plant part	Leaves	twigs, leaves	wood	–	wood
Causal agent	*C. prunicola*	*C. prunicola*	*C. prunicola*	*C. velutina*	*C. africana*
Ascomata	perithecial, solitary, subglobose to pyriform, 125–173(265) × 95–145(229) µm, neck 31–42 µm	perithecial, solitary, 162–221 × 119–159 µm, subglobose to pyriform, neck 38–42 µm	perithecial, solitary, subglobose to pyriform with a central ostiole, 200–250 µm diam., setose, neck 50–60 µm	–	perithecial, solitary, 140 µm, setose with a central ostiole, remaining immature
Setae	hyaline or brown setae, smooth walled, 3–4.5 × 21–29 µm	hyaline or brown setae, smooth walled, 3–4.5 × 35–51 µm	brown or hyaline setae, straight, cylindrical, tapering to a round tip, smooth-walled or granulate, 2.5–3.5 µm wide, 80 µm long	–	brown, cylindrical, tapering to a round tip, generally straight, aseptate, smooth-walled or verruculose, 2–3 × 40 µm
Paraphyses	hyaline, septate, 63× 3–4 µm	hyaline, septate, 74–78 × 3–4 µm	hyaline, septate, 60–100 × 2–3 µm	–	–
Asci	unitunicate with obtuse end, 8 ascospores/ascus, cylindrical, 58–68(94) × 8–10 µm	cylindrical, unitunicate with obtuse end, 8 ascospores/ascus, 68–81 × 8–10 µm	unitunicate, cylindrical, apedicillate, 8 ascospores/ascus, 63–73 × 8–10 µm	–	Absent
Ascospores	uniseriate, 1-celled, smooth-walled with granular content, 9(10)–12 × 5(6) µm, germ slit 8 × 5 µm, green to brown	uniseriate, 1-celled, smooth-walled with granular content, 9(10–)13 × (5–)6–7 (–8) µm, brown, longitudinal germ slit 7 × 6 µm	uniseriate, 1-celled, brown, smooth-walled, broadly ellipsoidal in top view and reniform from the side, dimensions (7.5–)8.5–10 (–11) × (5–)6–7.5(–8) × (3–)4–5 µm with granular content, germ slit	uniseriate, 1-celled, brown, granular contents, longitudinal germ slit 5.5–8 × 4–4.5 × 3–4 µm	Absent
Guttules	absent	absent	absent	present	Absent
Hyphae	–	hyaline, 2–3 µm wide	hyaline, 1–4 µm wide	–	hyaline, 1.5–3 µm wide
Conidia	hyaline, 1-celled, smooth walled, cylindrical to ovoid, (2–)3–6(–7) × 1–2 µm	hyaline, 1–celled, smooth walled, cylindrical to ovoid, sometimes allantoid (2–)3–4(–7) × 1–2 µm	hyaline, 1–celled, smooth-walled, mainly allantoid, sometimes cylindrical to ovoid (2.5–)3.5–6(–8) × 1–2(–3) µm	wider, not regularly allantoid	aggregated in heads, hyaline, 1–celled, smooth-walled, cylindrical with round ends or with one end slightly acute, occasionally biguttulate, 3.5–5.5 (–7) × 1.5–2 µm
Colonies on PDA	pale saffron, pale buff to white, flat, with sparse aerial mycelium	pale buff to white, flat, with sparse aerial mycelium	flat with sparse aerial mycelium, pale saffron, pale buff to white, 28 mm diam in 2 wk.		flat with felt-like aerial with fimbriate margin, ochraceous to luteous in middle
Chlamydospores	lacking	lacking	lacking	–	lacking

wall and without guttules, dimensions 9(10–)12 × 5(6) µm, with granular contents (Fig. 1l). Mature ascospores were broadly almond-shaped, ellipsoidal with a longitudinal germ slit 5–8 µm long (Fig. 1m).

Table 2. Basic morphological features of *Coniochaeta velutina* isolated from different hosts by several authors.

Causal agent	*Coniochaeta velutina*			
Author(s)	Basham et al. (1969)	Weber (2002)	Abdalla, Al-Rokibah (2003)	Asgari et al. (2007)
Host plant	*Acer saccharum*	*Picea abies*	*Phoenix dactylifera*	*Pistacia vera*
Plant part	stem	stem	leaves	twigs
Ascomata	–	dark brown, mature black, thick-walled (1 μm), smooth	globose-ovoid, black, gregarious perithecia	–
Setae	–	pointed setae 13–37 μm long, 3–4 μm wide	perithecia with setae 15–40 × 3–4 μm	–
Paraphyses	–	cylindrical, hyaline	–	–
Asci	–	8–spored with croziers, 55 × 6 μm	–	–
Ascospores	slighty discoidal, irregular –ovoid, ellipsoidal, 6–10 × 4–7 × 3–5 μm	ellipsoidal, brown, flattened with longitudinal germ slit 6–8 × 4–5 × 3.2–4 μm	dark brown, ovoid 7–9 × 5–7 μm with elongate germ slit	sometimes slightly, oval 6–10 × 4–7 × 3–5 μm
Guttules	–	present, 2 large guttules	–	present, 1–2 guttules
Hyphae	–	2–4(–5) μm wide, hyaline to olive, multiguttulate	–	–
Conidia	–	ellipsoidal to cylindrical hyaline, one-celled, biguttulate or with some small guttules (3–)3.5–6(–7) × (1–)1.2–2 μm	hyaline, ovoid, smooth, 1–celled 4–6 × 1.5–3 μm	–
Chlamydospores	–	absent	–	–

Causal organism was systematically isolated from leaf tissue showing the rusty to brown colour blight symptoms. Growth on PDA was slow. Colonies appeared white at first, than turned on pale saffron, pale buff to white. Conidia were produced abundant in culture media. Perithecia developed on PDA after about 8–10 weeks. Cultures of *Coniochaeta prunicola* do not turn dark as *Coniochaeta velutina* cultures (Weber 2002, Damm et al. 2010). This fact was also confirmed in our study with isolates of the fungus *C. prunicola* from peach trees. Slow-growing colonies on PDA in dark conditions and 24℃ formed aerial, pale saffron, pale buff later tanned mycelium (Fig. 1n). Vegetative hyphae were hyaline, 2–3 μm wide, lacking chlamydospores (Fig. 1o–q). Conidiophores formed directly on hyphae, mostly reduced to conidiogenous cells. Collarette are distinct, cylindrical, 2–3 μm long (Fig. 1r). Hyaline, one-celled, cylindrical or ovoid mainly allantoid conidia (2–)3–6(–7) × 1–2 μm (Fig. 1s) formed.

The key in Asgari et al. (2007) leads our results to *Coniochaeta velutina*, except that the ascospores of this species have guttules, and isolates of *Coniochaeta prunicola* produce larger ascospores than*Coniochaeta velutina*. These ascospore features correspond to those provided by Munk (1957), where isolates from *Prunus* sp. produced ascospores 6–8 × 4–6 × 3–4 μm in size and by description in Damm et al. (2010) and another authors. The main morphological differences of *C. velutina* isolated from different tree hosts are described in Table 2.

The other species (*Coniochaetidium* sp., *Ephemeroascus* sp. and *Poroconiochaeta* sp.) transferred into *Coniochaeta* by García et al. (2006) differed from *C. prunicola* by having ornamental ascospore walls, or by lacking *Lecytophthora* anamorphs. Most of the *Coniochaeta* species have different ascospore sizes: *Coniochaeta leucoplaca* (Berk. & Ravenel) 7–10 × 5–9 × 4–8 μm and *Coniolariella ershadii* (Zare, Asgari & W. Gams) Zare, Asgari & W. Gams (basionym *Coniochaeta ershadii* Zare, Asgari & W. Gams) 16 × 18 × 9.5–10 μm isolated from twigs of *Pistacia vera* L. (Asgari et al. 2007; Zare et al. 2010), *Coniolariella gamsii* (Asgari & Zare) Dania García, Stchigel & Guarro (basionym *Coniochaeta gamsii* Asgari & Zare) 16–19 × 6–11 μm isolated from leaves of *Hordeum vulgare* L. (Asgari & Zare 2006; Zare et al. 2010), *Coniochaeta ligniaria* (Grev.) Massee 9–20 × 8–15 × 4–8 μm (Mahoney & LaFavre (1981)and *Coniochaeta rhapalochaeta*

sp. nov. (Romero & Carmarán) 10–14 × 7.5–9 × 5–6 µm isolated from wood *Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. (Romero et al. 1999).

In anamorph stage of *C. velutina* described from various tree and shrub hosts in *Lecythophora* genus, sizes of conidia obtained from pure cultures varied; 3–6 × 2–4 µm (Taylor 1970), 2.5–3.5 × 1.5–2 µm (Udagawa & Horie 1982), 2–4 × 1–2.5 µm (Hutchinson & Reid 1988) and 3–8 µm long (Kirschner 1998). According to Damm et al. (2010) the anamorph of *Coniochaeta prunicola* is also similar to that of *Coniochaeta velutina*, but the collarettes in the latter are shorter, up to 1 µm long, and the conidia are wider and not regularly allantoid. This fact was also confirmed in our study.

According to Damm et al. (2010) *Coniochaeta* species were isolated from wood samples rarely showing necroses and were always found in combination with other fungi. According to the preliminary pathogenicity test, *C. prunicola* is pathogenic to apricot and *C. africana* to peach. In our experiments, the fungus *C. prunicola* was isolated from leaves samples of peach trees that showed necroses in combination with Hyphomycetes (*Alternaria alternata*, *Fusarium oxysporum*) and Coelomycetes (*Phomopsis* sp.) (Bernadovičová & Ivanová 2011).

The fungus *C. prunicola* were found in examined samples relatively uncommonly. Important finding is that *C. prunicola* was identified for the first time as a new pathogenic fungus associated with affected *Persica vulgaris* in Slovakia. Further studies are required for determination of pathogenicity and relevance of *Coniochaeta* infection in connection with peach trees damage.

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