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Anatomic-morphological characteristic of fungus *Coniochaeta prunicola* isolated from *Prunus cerasus* leaves

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Abstract

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Prunus cerasus L. is selected tree of genus *Prunus* susceptible to various pathogens, which caused discoloration, brown spots, blight symptoms and necroses, affecting their aesthetic value. The symptoms of infection observable from spring to autumn are increased, when the plants are in bloom – resulting in dieback and leaf drop. The damage is caused by fungus *Coniochaeta prunicola* Damm & Crous (Coniochaetales, Sordariomycetes, Ascomycota). The present work tries to specify – on the basis of light-microscopical morphological studies – the fungus *C. prunicola* that was isolated from *Prunus cerasus* symptomatic leaves from district Nitra and propose, for the first time, as a causative agent of sour cherry damage. The specific differences in spore size and anamorph morphology to the similar *C. velutina* could be confirmed. *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.

Keywords

anatomic-morphological characteristic*, Coniochaeta prunicola,* sour cherry

Introduction

Coniochaeta species had been found on many different substrates and hosts (on wood and bark, leaves and leaf litter of different trees, in dung of various animals, and in soil and water). This species was isolated from various parts of the genus *Prunus*, too. Decaying bark of *Prunus avium* L. in the Netherlands (CBS 178.75) enabled isolation the *Coniochaeta ligniaria* (Grev.) Massee, fruit trees (dry twigs of apricot, plum, pear, apple and cherry) in Moldavia isolation *Coniochaeta ambigua* (Sacc.) Cooke, *Coniochaeta calva* Tode, *Coniochaeta velutina* (Fuckel) Munk and *Coniochaeta ligniaria* (Grew.) Masse (Popushoi, 1971), necrotic wood samples of *Prunus armeniaca* and *Prunus salicina* in South Africa isolation *Coniochaeta velutina* (Fuckel) Munk and *Coniochaeta prunicola* Damm & Crous (Damm et al., 2010), leaves of *Prunus persica* in Slovakia isolation *Coniochaeta prunicola* Damm & Crous (Ivanová and Bernadovičová, 2012).

According to Mahoney and LaFavre (1981) *Coniochaeta* species are of low virulence on most hosts, usually appearing on dead tissue or as opportunistic invaders of previously infected, wounded or senescent tissue.

The genus *Coniochaeta* (Sacc.) Cooke belong to the family Coniochaetaceae. Their ascomata are perithecial, solitary, subglobose to pyriform, ostiolate with or without setae, ascospores are dark brown, discoid, nearly globose or ellipsoidal (Mahoney and LaFavre, 1981; Hanlin, 1990). Only 21 *Coniochaeta* species were included in the DNA phylogeny of the ascomycetous genus *Coniochaeta* (Asgari et al., 2007). *Coniochaeta* traditionally accommodates species with 4- to 8- or multi-spored asci. Their *Lecythophora* anamorphs are phialidic (Weber, 2002; Asgari and Zare, 2006) or polyblastic, nodulisporium like (Hawksworth, 1978; Asgari and Zare, 2006; Asgari et al., 2007). This genus represents the order Coniochaetales (class Sordariomycetes). Molecular studies have then demonstrated

the taxonomic relevance of anamorphs in the Xylariales (Sánchez-Ballesteros et al., 2000; Weber et al., 2002).

During an investigation on mycoflora of sour cherry trees growing in urbanized area the ascomycetous fungus *Coniochaeta prunicola* (Coniochaetaceae, Coniochaetales) that affects leaves of the host trees was noticed. This is the first record of this fungus as a pathogen of *Prunus cerasus* L. in Slovakia. The incidence of disease is sporadic, the infected trees showed relatively low damage.

The recently-noticed new disease of sour cherry trees becomes an especially relevant issue. The aim of our study was to isolate *Coniochaeta* species as a once in a factors involved in health state decline of *Prunus cerasus* and to present morphological description with distinctive features.

Material and methods

The issue was studied on samples of *Prunus cerasus* leaves showing blight symptoms. The samples were gathered from plants growing in private gardens of the town Nitra, during spring-autumn 2012. The samples of plant material were deposed at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plant Biology in Nitra.

For isolation and obtaining pure cultures we used classical phytopathological approaches. Leaf parts cut from the diseased plants were surface-sterilized in a sodium hypochlorite solution (1% available chlorine) for 20 minutes, rinsed twice or three times with sterile distilled water and placed in Petri dishes with a 3% potato-dextrose agar (PDA). Petri dishes were cultivated at 24 ± 1 °C and 45% air humidity in dark conditions in a versatile environmental test chamber MLR-351H (Sanyo). Pure fungal cultures were obtained by multiple purifications. The obtained isolates were transferred on 3% PDA medium to induce sporulation. The fungal structures were examined with a clinical microscope BX41 (Olympus) under a 400× and 1,000× magnification.

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

Results and discussion

Concerning all morphological characteristics and determined differences, the fungus under investigation in our study isolated from blighted leaves of sour cherry trees (Fig. 1a) was identified as *Coniochaeta prunicola*.

Coniochaeta prunicola Damm & Crous isolated from *Prunus cerasus* L. – anatomical-morphologically characteristics. Ascomata immersed or superficial on PDA medium developing after about 1 week were perithecial, solitary, subglobose to pyriform with a central ostiole, 188 (220) × 137 µm, neck 35–40 µm long (Fig. 1b). Peridium was pseudoparenchymatous, outer wall consists of dark brown angular cells (Fig. 1c), with setae. Setae were brown (or hyaline), straight, cylindrical, smooth-walled, 2–3 µm wide, up to 30–52 µm long (Fig. 1c, d). Prominent feature of the most *Coniochaeta* species are setae, but some species are described as lacking setae (Romero et al., 1999). Most of the described setae are dark brown to black rigid hairs, straight or bent, unbranched with a sharp apex. They may be scattered over the perithecial wall or concentrated in its upper portion (Mahoney and La Favre, 1981). According to Damm et al. (2010) subglobose to pyriform ascomata of the fungus *C. prunicola* isolated from branches of *Prunus armeniaca* and *P. salicina* formed pseudoparenchymatous peridium wide 20–25 µm with 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. Ascomata reached 200–250 µm in diameter, neck 50–60 µm long. Comparison of morphological characteristics of *Coniochaeta prunicola* isolated from different *Prunus* species and from examined material of *P. cerasus* are included in Table 1.

Unitunicate, fasciculate cylindric asci size 67 (92) × 5 (10) µm with truncate apex and small apical ring long 8 × 4 µm (Fig. 1e, f) form rosettes (Fig. 1e, h). Less numerous hyaline, septate paraphyses size 65 × 9 µm are formed between the asci (Fig. 1h). Each ascus contained eight ascospores, which were brown, onecelled, ellipsoidal, smoothwalled without ornamentation of the ascospore wall and with granular contents (Fig. 1h). Mature ascospores were broadly almond-shaped, ellipsoidal with a longitudinal germ slit 8–10 µm long (Fig. 1i). The key in Asgari et al. (2007) leads to *C. velutina*, except that the ascospores of that species have guttules and these isolates produce smaller ascospores compared to *C. prunicola* with larger ascospores (Fig. 1g). Ascospore shape is a valuable criterion for distinguishing species. Ascospores of *C. velutina* were ellipsoidal, brown, flattened with longitudinal germ slit and 2 large guttules, 6–8 × 4–5 × 3.2–4.0 µm size (Weber, 2002). Ascospores of *Coniochaeta ligniaria* (Grev.) Cooke are broadly spindle- or lemon-shaped, with tapering ends, size 14.5–16.0 × 7–8 × 6 µm. Colonies derived from ascospores became brownish and had sparse, thick walled chlamydospores with age (Holm and Ryman, 1977). Ascospores size in our experiments with *P. cerasus* isolates was 9 (10)–12 × 4 (5)–7 µm. Colonies

appeared white at first, than turned on pale buff to white, chlamydospores absent. These ascospore features are comparable to those provided by Munk (1957), where isolates from *Prunus* sp. produced ascospores 6–8 × 4–6 × 3–4 µm. Isolates from *Prunus laurocerasus* L. produced ascospores 9–10.5 (12.5) × 5 (7.5) µm in size (Ivanová and Bernadovičová, unpublished yet), from *Prunus persica* (L.) Batsch 9 (10)–12 × 5 (6) µm (Ivanová and Bernadovičová, 2012) or by description in Damm et al. (2010) isolates from *Prunus armeniaca* L. and *Prunus salicina* L. formed ascospores (7.5–) 8.5–10 (–11) × (5–) 6–7.5 (–8) × (3–) 4–5 µm in size.

The anamorph of *C. prunicola* is similar to that of *C. velutina*, but the collarette in the latter is shorter, up to 1 µm long and vegetative hyphas have 2–4 (–5) µm wide, are hyaline to olive, multiguttulate, chlamydospores absent (Weber, 2002; Damm et. al., 2010). Vegetative hyphae of *C. prunicola* isolated from *P. cerasus* were 3–4 µm wide, hyaline, without guttules and chlamydospores (Fig. 1j). Conidiophores formed directly on hyphae, mostly reduced to conidiogenous cells. Phialides were either short cylindrical or ampulliform (Fig. 1l). Collarettes were usually inconspicuous. Conidia obtained from pure culture of *C. prunicola* formed on hyphal coils (Fig. 1k). Conidia are hyaline, one-celled, cylindrical, mostly allantoid, 2–6 × 1–2 µm sizes (Fig. 1m). In anamorph stage of *Coniochaeta 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 and Horie, 1982), 2–4 × 1–2.5 µm (Hutchinson and Reid, 1988), (3–) 3.5–6 (–7) × (1–) 1.2–2 µm (Weber, 2002) and 3–8 µm long (Kirschner, 1998). Conidia of this species are mostly biguttulate or with some small guttules, but conidia of *C. prunicola* are without guttules (Damm et al., 2010; Ivanová and Bernadovičová, 2012). This fact was also confirmed in our study.

Similarly in anamorph stage of *Coniochaeta lignaria* (Grev.) Cooke conidia ellipsoidal to cylindrical, often somewhat curved, hyaline, one-celled, smooth-walled, mostly biguttulate, (3–) 3.5–6 (–8) × (1–)

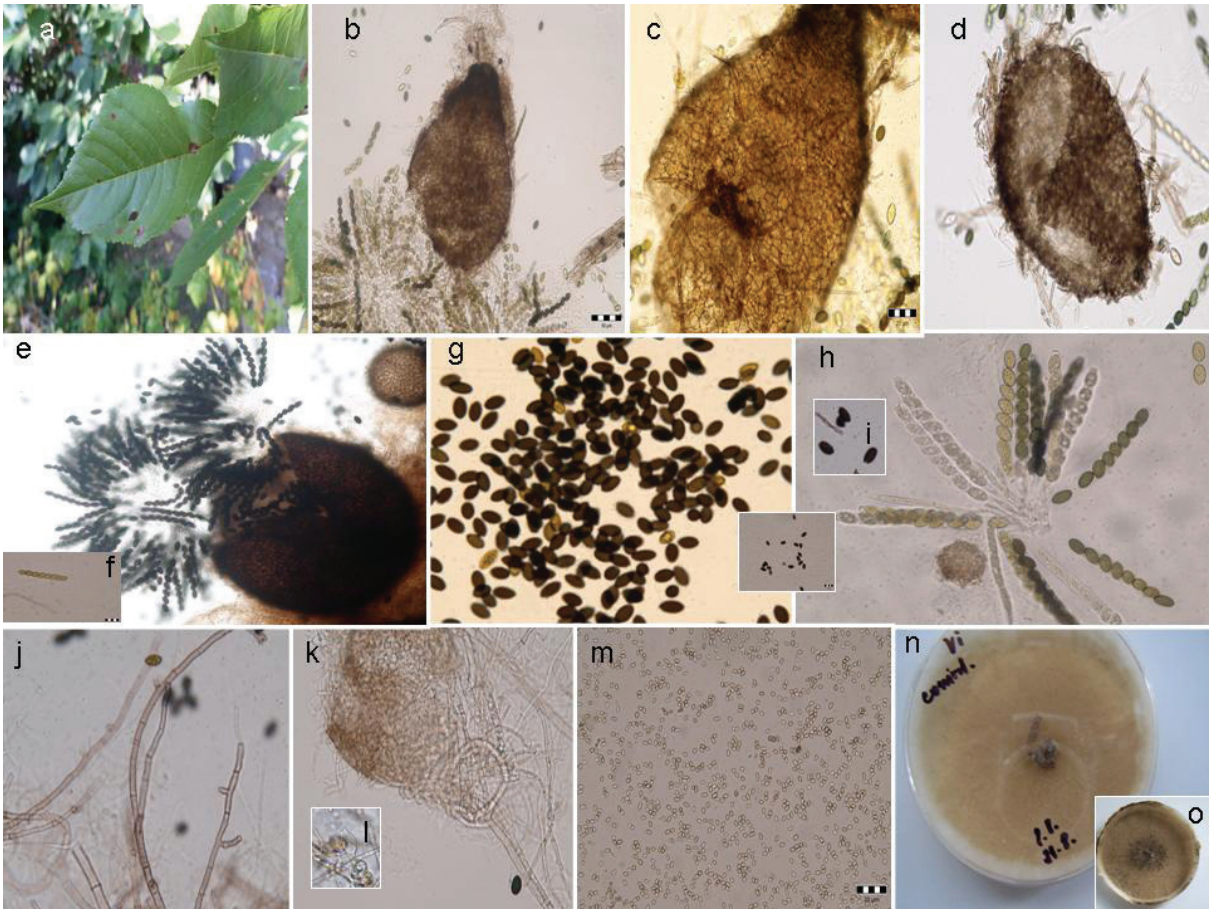

Fig. 1. *Coniochaeta prunicola* on *Prunus cerasus*. **a** affected leaves of *P. cerasus*, Teleomorph (**b–i**): **b** ascocarp with neck; **c–e** ascocarps in dehiscence; **c** peridium; **b–d** peridial setae; **f** 8-spored ascus, **g** ascospores, **e, h** rosettes of asci with paraphyses; **i** ascospore germ slit. Anamorph (**j–o**): **j** hyphas with collarettes, **k** hyphal coil, **l** ampulliform phialides, **m** conidia, **n** colony on PDA after 24 days; **o** colony on PDA after 1 week. Scale bars: c, d, f–m = 20 µm; b, e = 50 µm.

Table 1. Comparison of biometric characteristics and morphological features of fungus *Coniochaeta prunicola* on different species of genus *Prunus*

Authors	**Ours experiments**	**Ivanová, Bernadovičová 2012**	**Ivanová, Bernadovičová,** unpublished	**Damm et al. 2010**
Hosts / plant part	*Prunus cerasus* leaves	*Prunus persica* leaves	*Prunus laurocerasus* leaves, twigs	*P. armeniaca, P. salicina* wood
Causal agent	***C. prunicola***	***C. prunicola***	***C. prunicola***	***C. prunicola***
Ascomata	Perithecial, solitary, subglobose to pyriform, 188(220) × 137 µm, neck 35–40 µm	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
Setae	Hyaline or brown smooth walled setae, 2–3 × 30–52 µm	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
Paraphyses	Hyaline, septate, 65 × 9 µm	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 ascospres / ascus, apedicillate, 67(92) × 5(10) µm	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
Ascospores	Uniseriate, 1-celled, green to brown, smooth walled with granular content, 9(10)–12 × 4(5)–7 µm, longitudinal germ slit 8(10) × 5 µm	Uniseriate, 1-celled, smooth-walled with granular content, 9(10)–12 × 5(6) µm, longitudinal germ slit 8 × 5 µm, green to brown	Uniseriate, 1-celled, smooth-walled with granular content, brown, 9(10–)13 × (5–)6–7(–8) µm, 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
Guttules	Absent	Absent	Absent	Absent
Hyphae	Hyaline, 3–4 µm wide	–	Hyaline, 2–3 µm wide	Hyaline, 1–4 µm wide
Conidia	Hyaline, 1-celled, cylindrical to ovoid, 2–6 × 1–2 µm	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
Colonies on PDA	White, later pale buff to white, flat, with aerial sparse mycelium	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.
Chlamydosp.	Lacking	Lacking	Lacking	Lacking

1.5–2.5 µm, in the centre of the colonies conidia often larger, up to 7–8 (–11) × 4 µm (Weber, 2002).

Causal organism was systematically isolated from leaf tissue showing rusty to brown coloured blight symptoms and necroses in combination with fungus *Stigmina carpophila* (Lév.) M. B. Ellis (Ivanová and Bernadovičová, 2009) and *Blumeriella jaapii* (Rehm) Arx. (Ivanová and Bernadovičová, 2011). Colonies appeared white at first, than turned on pale buff to white (Fig. 1n). Conidia were produced abundant in culture media. Perithecia developed on PDA after about 1 week (Fig. 1o). Cultures of *Coniochaeta prunicola* do not turn dark as *Coniochaeta velutina* cultures (Weber, 2002; Damm et al., 2010) or do not turn more or less salmon-coloured as *Coniochaeta ligniaria* cultures (Weber, 2002). This fact was also confirmed in our study with isolates of fungus *C. prunicola* from peach trees (Ivanová and Bernadovičová, 2012), cherry laurel shrubs (Ivanová and Bernadovičová, unpublished yet) and with isolates from sour cherry in this study (Table 1).

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

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Anatomicko-morfologická charakteristika huby *Coniochaeta prunicola* izolovanej z listov *Prunus cerasus*

Súhrn

Prunus cerasus L. je drevina náchylná na ochorenie vyvolané škodlivými činiteľmi, spôsobujúcimi rôzne farebné zmeny a škvrnitosti listov, opad listov, usychanie a nekrózy konárov. Pri monitorovaní zdravotného stavu vybraných drevín v podmienkach mesta Nitry sme zaznamenali symptómy dobre viditeľné od jari do jesene, ktoré sa v čase kvitnutia zvyšovali. Na vzniku infekcie sa podieľa aj huba *Coniochaeta prunicola* Damm & Crous (Coniochaetales, Sordariomycetes, Ascomycota). Predkladaná práca špecifikuje príčinu poškodenia *Prunus cerasus* na základe mikroskopicko-morfologických štúdií huby *C. prunicola* izolovanej zo symptomatických listov danej dreviny. Potvrdzuje rozdiely vo veľkosti spór a v morfológii anamorfy vzhľadom k hube *Coniochaeta velutina*. *C. prunicola* je charakterizovaná tmavohnedými plodničkami pokrytými vláskami, jednovrstvovými cylindrickými vreckami vyrastajúcimi medzi málopočetnými parafýzami. Vrecká sú usporiadané do ružíc a obsahujú osem svetlohnedých, v dospelosti tmavohnedých, hladko-stenných elipsoidných, vo vnútri zrnitých vreckospór, ktoré klíčia pozdĺžnym klíčnym otvorom. Výskyt ochorenia je sporadický, často je spojený s výskytom huby *Blumeriella jaapii* (Rehm) Arx. (Ascomycetes) podieľajúcej sa na vzniku nápadnej škvrnitosti listov a huby *Stigmina carpophila* (Lév.) M. B. Ellis (Deuteromycetes) spôsobujúcej dierkovitosť alebo suchú škvrnitosť listov *Prunus cerasus.*

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