Needle disease on English yew caused by the parasitic fungus *Cryptocline taxicola* (All.) Petr. noticed in Nitra town

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Abstract

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This study reports the occurrence of the fungus *Cryptocline taxicola* (All.) Petr. on needles of *Taxus baccata* L. Symptoms of fungal infection, irregular necrotic spots and lesions, followed by turning brown of infected needles and whole shoots, were observed on *Taxus baccata* var. *fastigiata* (Lindl.) Loudon and *Taxus baccata* var. *fastigiata Robusta* in private gardens of the town of Nitra. Some important characteristics and distinctive morphological features were described. Black, subcircular fruiting bodies (acervuli) were observed on the upper, but more massed on the lower side of necrotic needles. Conidia were ellipsoidal to ovoid, smooth-walled, brownish to hyaline, nonseptate, grainy inside, rarely with two oil drops, average size 5–15 × 4–5 µm. The fungus formed on PDA aerial, pale brown to greyish colonies with olive-brown shade and hyaline, branched, septate mycelium. Average growth rate of mycelium in culture reached 3.02 mm day^{-1}. Experimental spraying with fungicide Dithane M45 showed to be effective. Because the English yew is affected by only a few serious fungal diseases, so the isolation and identification of *C. taxicola* – a fungal pathogen with sporadical incidence causing damage to needles of this woody plant was relatively uncommon. From this aspect the fungus *Cryptocline taxicola* seems to be important pathogen on needles of the English yew in Europa, including Slovakia.

Key words

Coelomycetes, Cryptocline damage, fungal disease, *Taxus baccata* L.

Introduction

Taxus is a genus of yews, small coniferous trees or shrubs in the yew family Taxaceae. *Taxus baccata* L. is a conifer native to western, central and southern Europe, northwest Africa, northern Iran and southwest Asia. This extraordinally long-lived and relatively slow growing tree known as common yew, European or English yew is the predominant yew species in all Europe. It is one of the oldest species in the world.

English yews are widely used in landscaping and ornamental horticulture, and thanks to their low site requirements and relatively high tolerance to harmful agents, they often belong to favourable evergreen tree species used especially for formal hedges and topiary in parks and gardens (Fig 1).

In contrast to other European tree species, English yew has a conspicuously low affinity for fungi. However, several authors reported infection of *Taxus* by several species of fungi (Brandenburger, 1985; Ellis and Ellis, 1997; Thomas and Polwart, 2003). From fungal disorders, parasitic fungi *Dotchichiza* sp. (syn. *Cytospora taxifolia* Cke. & Mass.), *Stagonospora* sp. (syn. *Hendersonia taxi* Hollós) and *Phyllosticta concentrica* Sacc. (syn. *Phyllosticta taxi* (Fr.) Wr. & Hoch.) are known commonly.

For the first time, *Cryptocline* fungus on yew needles was noticed in 1973 (Morgan and Jones, 1973). The fungus has been for long accounted as a weak pathogen but at present, strains with a higher degree of parasitism are occuring on various yew species and varieties. Towards the end of the 20th century, the disease occurrence was confirmed on Pacific yew (*Taxus brevifolia* Nuttall) in Canada (Vujanovic and St-Arnaud, 2001). In Europe, the disease was observed in 2001 on *Taxus baccata* var. *fastigiata* in Norway (Talgø et al., 2003), later in Germany on *Taxus baccata* L. (Wulf and Pehl, 2002). The first incidence in Slovakia on *Taxus baccata* was recently noticed in 2007 by Bukvayová (2007). In the Czech Republic, Šafránková (2008) recorded the disease on living and necrotic needles of *Taxus baccata* L. and its cultivars 'Adpressa', 'Fastigiata Aurea', 'Fastigiata Robusta', 'Nissens Praesident', 'Nissens Regent', also on *T. cuspidata* S. & Z and *Taxus × media* Rehd. 'Thayerae'.

Fig 1. *Taxus baccata* var. *fastigiata Robusta* often planted as a hedge in public greenery

Cryptocline taxicola (Allesch.) Petr. (Coelomycetes), teleomorph *Anthostomella taxi* Grove, current name *Anthostomella formosa* var. *taxi* (Grove) S.M. Francis (Xylariaceae, Xylariales) was described as *Gloeosporium taxicola* (All.) in 1896. In 1925, Petrak (1925) put the fungus – on the base of morphological features, into a new genus *Cryptocline* with characteristic subcuticular or intraepidermal development of acervuli. Later Von Arx (1957), by revision of genus *Gloeosporium,* classified the *Cryptocline taxicola* into the genus *Cryptocline.* Morgan-Jones (1973) categorized 14 species, including *Cryptocline taxicola* to the *Cryptocline* genus. Next revision of the genus *Cryptocline* was accomplished by Sutton (1980).

This study aims to describe the characteristic disease symptoms according to the previous diagnostic data and based on examination of cultural and morphological attributes, the distinctive morphological features of the fungus *Cryptocline taxicola* (All.) Petr. on *Taxus baccata* L. For this purpose, several important characteristics as cultural growth, conidial formation and size differences in microscopic structures were studied in pure hyphal cultures of *Cryptocline taxicola* isolated from symptomatic yew trees.

Material and methods

In the spring 2009, damaged twigs of yew (*Taxus baccata* var. *fastigiata* and *Taxus baccata* var. *fastigiata Robusta*) were delivered to the laboratory of the Institute of Forest Ecology of the Slovak Academy of Sciences in Nitra. The plant material had been taken from infected trees growing in private gardens of the town of Nitra – part Zobor. Visual characteristics of necrotic and chlorotic needles with fruiting bodies on the upper and lower surface were examined with a stereomicroscope SZ51 (Olympus). Investigation of fungal structures (conidia, conidiophores) immersed in water was performed with a clinical microscope BX41 (Olympus) under a 400× magnification.

The fungus *Cryptocline taxicola* was isolated from symptomatic needles and twigs with characteristic spots and lesions sampled during the growing season from the affected host trees of *Taxus baccata* var. *fastigiata*. The samples were surface-sterilized in 70% ethanol, then immersed for 15 minutes in sodium hypochlorite, rinsed in sterile distilled water (2–3 times) and dried carefully with filter paper. After the surface sterilization, the tissue samples were cut to small pieces (4–5 mm), placed on potato dextrose agar (PDA) and subsequently incubated in Petri dishes. Then followed cultivation in a versatile environmental test chamber MLR-351H (Sanyo) at 24 ± 1 °C temperature, 45% humidity and photoperiod 12/12 hours and isolation on potato-dextrose agar (PDA). Pure fungal cultures were obtained after multiple purification.

The collections from the town of Nitra (25 April, 8 May, 12 May and 15 May 2009, leg. H. Ivanová) were identified. The samples of material have been deposed at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plant Biology in Nitra.

The growth rate of the examined fungus was assessed on 10-day-old pure cultures grown on PDA in Petri dishes at 24 ± 1 °C and photoperiod 12/12 hours, based on the daily records of mycelium growth (mm day^{-1}; with precision of 0.5 mm) made during seven days. Agar columns with about 0.5 × 0.5 mm of the parent mycelium were used for inoculation of plates. Altogether 30 repetitions were made, and two dishes with fungal colonies were examined in each replication.

Results and discussion

Symptoms and morphology

In our observations, the first symptoms of damage caused by fungus *Cryptocline taxicola* (Allesch.) Petr. appeared on needles of new shoots as irregular necrotic lesions (Fig 2). They progressively increased in size, and finally affected the whole needles. According to Lawrence and Moltzan (2007), the symptoms include a browning of needles and the signs include black „pepper grains“ on the needles. Strong infection can lead to dying of whole shoots. Affected needles and whole shoots turn greeny up to brown, and they wither and fall off as the disease develops (Fig 3). Black circular or subcircular fruiting bodies (acervuli) are formed under epidermis on the both – upper and lower side of the needles. Acervuli reach 150–370 μm in diameter. According to Bukvayová (2007), the acervuli of the fungus observed with a stereomicroscope were circular to subcircular, black, surrounded by brown circles and 150–350 μm in diameter. Vujanovic and St-Arnaud (2001) observed circular to subcircular acervuli which were yellowish, surrounded by brown circles, subcuticular to intraepidermal, at first full covered, later having a fissure in the cuticule, and 150 to 350 μm wide. The fruiting bodies, sometimes more than 400 per a needle were observed on the upper surface. According to Talgø et al. (2003) acervuli are formed on the upper as well as lower side of the needles, and the epidermis breaks through. Acervuli are visible to the naked eye on green needles and shoots but a good stereomicroscope is needed to see them properly. The epidermis of the needle was first pushed upwards and then ruptured irregularly when the fruiting bodies became ripe and broke through.

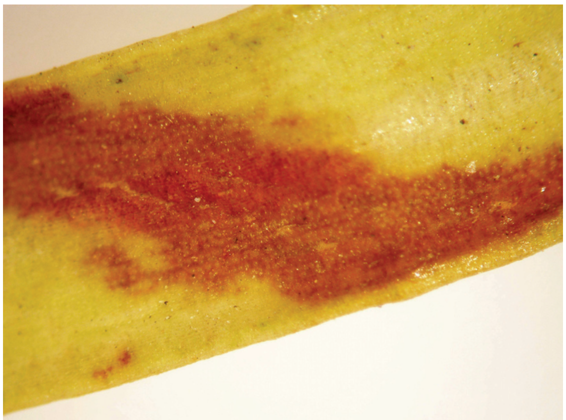

Fig 2. Detail of damaged needle of *Taxus baccata* var. *fastigiata Robusta* with irregular lesion

With an above normal precipitation, the epidermis arched and rupted, a creamy coloured spore (conidia) mass oozed out from the acervuli. During dry season, the needles manifested ocurrence of well visible rumpled and shrivelled, circular to oval or irregular fruiting bodies of the fungus (Fig 4a). When the infected material was incubated in saturated air at room temperature, a cream coloured spore mass of conidia oozed out from the acervuli (Fig 4b).

Fig 3. Necrotic needles and whole shoots of English yew affected by *Cryptocline taxicola* turn brown as the disease develops

Fig 4a. Mature acervuli of *Cryptocline* fungus on infected yew needles in dry conditions appear raised black „pepper grains“

Fig 4b. Initial formation of a cream coloured spore mass oozing out from the acervuli under sufficient humidity

According to Morgan-Jones (1973), the mature stroma of a fruiting body of *Cryptocline* fungus is subcuticular, intraepidermal, and the acervuli have on average 200–400 µm. Fruiting bodies, 15–25 µm in thickness, are relatively poorly-developed from thin-walled, pseudoparenchymatic (textura angularis), slightly brownish to hyaline cells, 5–7 µm in size, formed by the basic stroma. An acervulus towards to the centre can achieve a thickness about 40 µm. Cylindrical and hyaline conidiogenous cells are formed from the upper, compactly arranged, stromal cells. Conidiogenous cells are enteroblastic, phialidic, discrete, indeterminate, cylindrical, hyaline, smooth, with 1–2 percurrent proliferations (Petrak, 1924). Bifurcate and partite, smooth-walled fungal hyphas with subhyaline to tan appearance can be observed on cross-sections of the injured tissue of needles.

Despite the accounts by Morgan-Jones (1973) and Von Arx (1957), *Cryptocline* remains a very heterogeneous entity. The only common feature shared by the recently described species is the epidermal to subepidermal location of the conidiomata. According to the pressent knowledge, conidiogenous cells may be discrete or integrated on branched septate conidiophores. Conidiogenesis may be typically phialidic, percurrently phialidic or annellidic, conidia may be cylindrical, fusiform or doliiform and have either a small area of abscission or a relatively wide base scar. Separation of the taxa on the basis of conidial morphology and nature of the conidiogenous process would fragment the genus into several distinct groups.

Conidia are ellipsoid to oval, one-celled, hyaline to slightly pigmented, 5–15 × 4–5 µm, rarely with two oil drops (Fig 5). According to Petrak (1925) conidia are hyaline or brownish, thin walled, eguttulate or guttulate, smooth, cylindrical to doliiform or ellipsoid, with a broad, flat base. The fungus forms dark colonies with aerial, pale brown or olive to greyish mycelium on potato-dextrose agar (Fig 6a, b). The fungus grows relatively slowly, the average growth rate is 3.02 mm/day.

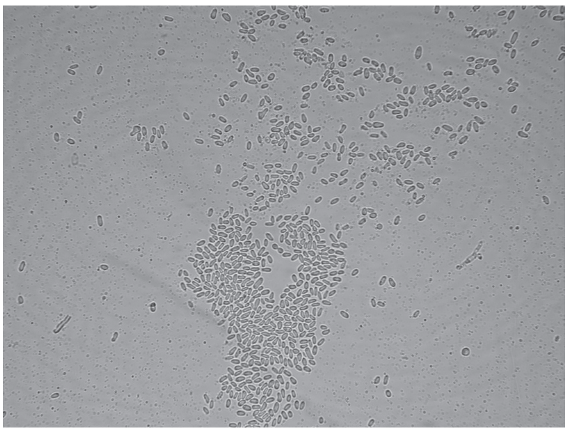

Fig 5. Ellipsoidal to ovoid and hyaline to slightly pigmented conidia (400x)

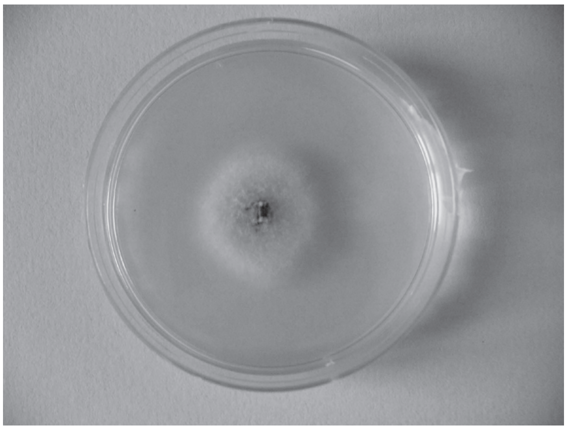

Fig 6a. Cultures of *Cryptocline taxicola* 5 days after inoculation on potato-dextrose agar

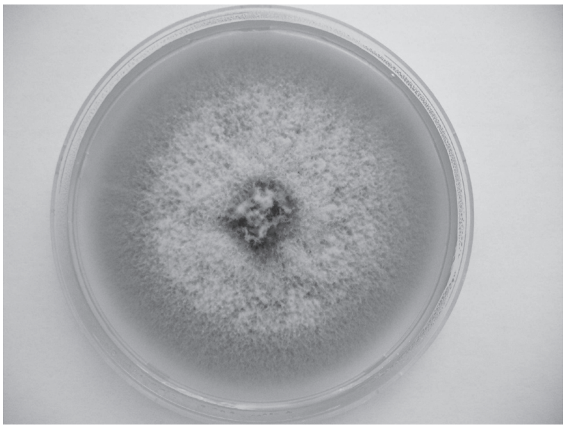

Fig 6b. Cultures of *Cryptocline taxicola* 9 days after inoculation on potato-dextrose agar

Our results are comparable with studies carried out in Canada (Vujanovic and St-Arnaud, 2001), Germany (Pehl and Wulf, 2002), Norway (Talgø et al., 2003) and in the Czech Republic (Šafránková, 2008), Table 1. In Slovakia, for the first time was the fungus recorded in 2007 (Bukvayová, 2007). An increasing occurence of this needle fungus with evident parasitic attributes on *Taxus baccata* – as a relatively resistant tree species has been affirmed by all the mentioned authors.

Table 1. Comparison of biometric characteristics and morphological features of *Cryptocline taxicola* on *Taxus* sp. reported by other authors and examined material from Slovakia

Author(s)	Acervuli	Conidia	Conidiophores	Mycelium
Vujanovic, St-Arnaud (2001)	On the upper surface of needles (circular to subcircular, yellowish, surrounded by brown circles, subcuticular to intraepidermal, at first covered, later exposed by the fissure of the cuticule, and 150–350 µm wide)	Ellipsoidal to oval, truncate at the base, obtuse at the apex, hyaline to slightly pigmented Size: 8–17 × 4–5 µm	Phialidic, cylindrical, hyaline Size: 10–20 × 2.5–4.5 µm	Septate, brownish on PDA
Pehl, Wulf (2002)	On the upper side, rarely at the bottom of needles	Elliptic to ovoid, smooth, thin-walled, hyaline, 1-celled, granular plasma inside, rarely with 1–2 oil drops Size: 12–18 × 5–8 µm	Hyaline, cylindrical Size: 25 × 3.5–5 µm	Wooly, aerial, olive-brown to grey colonies on MA Growth: 1.4 mm/day
Talgø et al. (2003)	On both – the upper and lower side of the needles	Ovoid, smooth Size: 11–18 × 5–8 µm	Absent	Dark colonies, aerial mycelium a greyish appearance on PDA
Bukvayová (2007)	On both the upper and lower side of the needles, black, surrounded by brown circles	Ellipsoidal to oval, thick-walled, truncate at the base, obtuse at the apex, hyaline to mildly pigmented Size: 15.6 × 6.85 µm	Hyaline, phialidic, cylindrical Size: 15 × 3.5 µm	Feltlike to wooly, dense, olive-brown to greyish brown, aerial, slow-growing mycelium
Šafránková (2008)	On both – the upper and lower surface of the needles, black, subepidermal, 160–370 × 90–145 µm, surrounded by brown circles	Ellipsoid to oval, pale brown to hyaline, with granular plasma inside, sporadically with two oil drops, flat at the base, rounded at the apex, thick-walled, without septa Size: 10–18 × 4.5–8.4 µm	Hyaline, cylindrical with phyalides Size: 12–18 × 2.8–3.4 µm	Dark colonies with greyish aerial mycelium on PDA
Examined material (2009)	Black, subcircular, on the upper surface, more massed on the lower side of necrotic needles	Ellipsoidal to ovoid, thick-walled, smooth-walled, brownish to hyaline, nonseptate, grainy inside, rarely with two oil drops Size: 5–15 × 4–5 µm	Absent	On PDA aerial, pale brown to greyish colonies with olive-brown shade, mycelium hyaline, branched, septate Growth: 3.02 mm/day

Control

Mechanical control consists in removing the infected needles as a source of new infection, possibly also in cutting off the damaged branches. According to Vujanovic and St-Arnaud (2001), the decreased disease occurrence was associated with sanitary measures that consisted of cutting off the symptomatic branches. However, favourable conditions during the growing seasons, eg high temperatures and excessive precipitation could be responsible for the increased incidence on the Pacific yew. It is evident that cutting measures have not stopped the fungal propagation and disease expression, and that the first are only temporary solutions. In our observations, favourable conditions (high temperatures and precipitation) during the growing season 2009 could be probably factors influencing the relatively increased incidence of *Cryptocline* needle disease also on English yew.

Lawrence and Moltzan (2007) state that more information is needed for better timing of chemical treatments. For now, the advice can be to apply in the spring – as needles emerge, treatment with a chlorothalonil-based fungicide. In chemical control, application of fungicides based on mancozeb – considerably decreasing the infectious pressure, is rather often used for elimination of the fungal spores. Considering the relatively sporadic incidence of the pathogen and short periods of investigation, many authors do not suppose that a chemical treatment were necessary. This approach accords with Šafránková (2008) because she suggests that, at present, there are no fungicides recognised as effective against the disease caused by *C. taxicola.* Therefore, spraying of fungicide Dithane M45 on sympto-matic yews growing in private gardens was applied at the owner's request. The positive effect of the fungicide treatment repeated three times at 14-day intervals is evident (Fig 7). However, if the fungus has already penetrated the tissue of the host tree, fungicides are ineffective. Therefore, preventive measures, in particular reduction of air moisture by adequate aeration of plan-tings are very important. It is equally important to use a noninfectious material in the new planting. According to Vujanovic and St-Arnaud (2001), inappropriate stand selection, unfavorable water conditions and a thin organic soil layer may predispose yew to infection by *Cryptocline* pathogen.

Within the needle diseases, *Cryptocline taxicola* was assessed to be a pathogen of low importance, but recent observations show that it possesses a large degree of potential parasitism (Wulf and Pehl, 2002).

To obtain more details about occurrence and important attributes of this fungal pathogen in Slovakia, the research requires to continue. Further investigations are needed in order to propose an adequate control strategy against the *Cryptocline* disease in our conditions.

Fig 7. *Taxus baccata* var. *fastigiata Robusta* after repeated fungicide treatment (Dithane M45)

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References

Arx, J.A. von 1957. *Revision der zu Gloeosporium gestellten Pilze* [Revision of fungi in Gloeosporium genus]. Amsterdam: Noord-Hollandsche Uitg. Mij. 153 p.

Brandenburger, W. 1985. *Parasitische Pilze an Gefäßpflanzen in Europa* [Parasitic fungi on vascular plants in Europe]. Stuttgart, New York: Gustav Fischer Verlag. 1248 p.

Bukvayová, N. 2007. Cryptocline taxicola (All.) Petr. – a new plant pathogen reported in Slovak republic. *Pl. Protec. Sci.*, 43: 122–124.

Ellis, M.B., Ellis, J.P. 1997. *Microfungi on Land Plants. An Identification Handbook*. Slough: Richmond Publishing. 868 p.

Lawrence, R., Moltzan, B. 2007. Cryptocline taxicola Discovered on Yew. Forest Pathologist's Notes. Missouri Forest Health Update, Missouri Department of Conservation [cit. 2009-05-07]. http://216.119.79.248/pdfs/Forest%20Health%20Update_2007-3.doc

Morgan-Jones, G. 1973. Genera coelomycetarum. VII. Cryptocline Petrak. *Can. J. Bot.*, 51: 309–325.

Pehl, L., Wulf, A. 2002. Nadelschäden an Taxus baccata L. durch Cryptocline taxicola (All.) Petr.

[Needle disease on Taxus baccata L. caused by Cryptocline taxicola (All.) Petr.]. *Nachr.-Bl. Dtsch. Pfl.-Schutzdienst*, 54: 266–268.

Petrak, F. 1925. Mykologische Notizen. VIII. [Mycological notes]. *Ann. mycol*., 23 (1/2): 24.

Thomas, P.A., Polwart, A. 2003. Taxus baccata L. Biological flora of the British Isles. No. 229. *J. Ecol*., 91: 489–524.

Sutton, B.C. 1980. *The Coelomycetes*. Key, Surrey: Commonwealth Agricultural Bureaux. 696 p.

Šafránková, I. 2008. Occurence of Cryptocline taxicola (All.) Petr. on needles of Taxus spp. – symptoms and morphological features. *Acta Univ. agric. silvic. Mendel. Brun.*, LVI (2): 199–202.

Talgø, V., Ørstad, K., Stensvand, A. 2003. Cryptocline taxicola. *Grønn kunnskap*, 7 (101D): 1–2.

Vujanovic, V., St-Arnaud, M. 2001. First report of Cryptocline taxicola infecting Pacific yew (Taxus brevifolia) in eastern North America. *Pl. Dis.*, 85: 922.

Wulf, A., Pehl, L. 2002. Needle disease on Taxus baccata caused by Cryptocline taxicola. In Uotila, A., Ahola, V. (eds). *Proceedings of the IUFRO working party 7.02.02 Shoot and foliage diseases, meeting in Hyytiälä, Finland, 17–22 June, 2001.* Helsinki, p. 198–201.

Choroba ihlíc tisa obyčajného spôsobená parazitickou hubou *Cryptocline taxicola* (All.) Petr. zaznamenaná v meste Nitra

Súhrn

Koncom jari a začiatkom leta vegetačného obdobia roku 2009 boli na ihliciach *Taxus baccata* var. *fastigiata* (Lindl.) Loudon a *Taxus baccata* var. *fastigiata Robusta* rastúcich v súkromných záhradách mesta Nitra zaznamenané charakteristické symptómy choroby spôsobenej hubou rodu *Cryptocline*. Z nekrotických a chlorotických ihlíc napadnutých stromov bola izolovaná huba *Cryptocline taxicola* (All.) Petr. identifikovaná ako pôvodca ochorenia ihlíc tisa. Bol potvrdený opakovaný výskyt tejto doteraz zriedka sa vyskytujúcej parazitickej huby tisa obyčajného, ktorá bola na Slovensku po prvýkrát zaznamenaná až v roku 2007. Práca popisuje na základe štúdia kulturálnych a morfologických vlastností skúmaného patogéna rozlišujúce morfologické znaky huby, jej anamorfného štádia (acervuly, konídie) a charakteristiku huby na živnom médiu (vzhľad kultúry, rýchlosť rastu mycélia). Čierne, takmer okrúhle plodničky (acervuly) huby boli prevažne sústredené na spodnej, menej na vrchnej strane nekrotických ihlíc. Huba vytvára elipsoidné až vajcovité, hrubostenné konídie s hladkou stenou, hnedasté až hyalinné, bez priehradok, so zrnitým vnútrom, zriedkavo aj s dvoma olejovými kvapkami, s priemernou veľkosťou 5–15 × 4–5 µm. Na zemiakovo-dextrózovom agare (PDA) formuje svetlohnedé až šedasté kolónie postupne s olivovohnedým nádychom a vzdušné, hyalinné, rozkonárené mycélium s priehradkami. Rýchlosť rastu mycélia huby na PDA je v priemere 3.02 mm deň$^{-1}$. Vzhľadom na to, že tis obyčajný je známy ako drevina napádaná iba niekoľkými hubovými patogénmi, izolácia a následná identifikácia huby *C. taxicola* ako sporadicky sa vyskytujúceho pôvodcu ochorenia bola pomerne neočakávaná. Z tohto hľadiska sa huba *C. taxicola* ukazuje ako významný patogén asimilačných orgánov *Taxus baccata* L. v Európe, vrátane Slovenska.

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