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FIRST REPORT

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First report of *Cryptosporiopsis tarraconensis* causing leaf lesion of *Corylus avellana* in Central Europe (Poland)

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Abstract

Key message As a result of our research, we determined that *Cryptosporiopsis tarraconensis*—as a new species for Central Europe—is the causative agent of leaf lesions in natural populations of hazel (*Corylus avellana*). Until now, this species had not been described in a natural population of *C. avellana* or out of the temperate climate. This is the fifth notification of this rare fungus in the world and the first from Central Europe and the natural population of the host.

Keywords Oak-hornbeam forest, Hazel, Nut production, Pathogenicity test, Hazelnut

Cryptosporiopsis tarraconensis Gené and Guarro is a rare fungal species (*Ascomycota, Helotiales, Dermateaceae*), occurring on hazel (*Corylus avellana* L.) buds, leaves, and twigs (Tagliavento et al. 2021). It can cause leaf desiccation and the dry rot of buds, leading to their abortion (Gené et al. 1990; Roohvarzi et al. 2013; Tagilavento et al. 2021). Hitherto *C. tarraconensis* has been found in the world only 4 times, each in temperate regions: in Spain (Gené et al. 1990), in Iran (Roohvarzi et al. 2013) in Italy (Tagilavento et al. 2021) and in Türkiye (Altin and Gulcu 2023), causing considerable damage to hazel orchards. Until now, this species had not been described in a natural population of *C. avellana* or out of the temperate climate. This is the fifth notification of this rare fungus in the world and the first from Central Europe and the natural population of the host.

The leaf lesions of *C. avellana* were observed in September 2021 in 15 locations in Wigry National Park (NE Poland), located in an oak-hornbeam forest (Table 1). On the infected leaves two types of lesions

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Table 1 An assessment of leaf lesion infection on hazel trees in 15 studied plots

Study area	WGS_N	WGS_E	Percentage of infected population of hazel [%]	Estimated percentage of infected hazel's leaves [%]
01A	54.10952	23.04164	25	10
01B	54.06696	23.01457	100	45
02A	54.10882	23.04376	5	1
02B	54.06875	23.01368	5	5
03A	54.04056	22.99401	100	15
03B	54.11032	23.04001	40	5
04A	54.06586	23.01985	1	1
04B	54.04016	22.99354	80	5
05A	54.06521	23.01721	6	2
05B	53.98456	23.11013	11	6
06A	54.06729	23.01378	5	5
07A	54.0665	23.01808	80	5
08A	53.98398	23.11023	50	15
09A	53.98349	23.10917	25	10
10A	54.06172	23.05709	15	15

were visible: one consisting of round, brown spots with a black point in the middle, and a second made of irregular, wet lesions, mostly occurring on the leave's edges (Appendix Figure 2). Depending on the location, the lesions were observed on a single tree or up to 100% of the trees at each location, and occurring from single leaves to 45% of a tree's leaves (Table 1).

Symptomatic tissues were disinfected for 30 s in 0.5% NaClO and rinsed in sterile water. Then, the leaves were cut into 2–5 mm fragments, placed on a PDA medium (Biocorp, Poland), and incubated at 21 ± 2 °C in darkness. After 10 days 810 fungal colonies were obtained, from which 134 were *C. tarraconensis* strains. The species identification of two main morphotypes: CtC1-21Ca and CtC7-21Ca, was classified, based on morphology, as *C. tarraconensis* (Gené et al. 1990; Sutton 1980). After 14 days of inoculation, a white-brown, velvet mycelium of *C. tarraconensis* was obtained, reaching 2,5–3,5 cm diam on the PDA medium, raising in the center with age (Appendix Figure 3). On the reverse of the colony, mostly dark yellow coloration with concentric brown rings was observed. After circa 30 days of incubation, ochre to dark brown globular fruiting bodies developed (Appendix Figure 4). Under a light microscope acervuli 115–250 µm were observed, exuding whitish conidial masses (Appendix Figure 4). The conidia were solitary, unicellular, hyaline, smooth, and cylindrical with evident basal scarring, in size (9) 12–16 (19) × (6) 7–8 (8,5) µm. All the macroscopic and microscopic morphological traits so far described suggest the presence of the fungus *C. tarraconensis*.

To prove the morphological identification, an analysis of the sequences of the ITS (internal transcribed spacer) region was conducted. Mycelia were grown on potato dextrose broth and freeze-dried prior to genomic DNA extraction using the CTAB method. The ITS sequences of *C. tarraconensis* isolates analyzed in these studies were deposited in NCBI GenBank with acc no. OM642119 and OM642128.

Due to the ambiguous result of the comparative analysis of our sequences with sequences available in GenBank NCBI, additional analysis was carried out. To confirm molecular identification and dispel doubts we made a phylogenetic analysis based on the ITS sequences of CtC1-21Ca and CtC7-21Ca, 8 sequences showing an identity match with them at the level over 99% and 6 chosen sequences of Helotiales and Pleosporales representatives (Appendix Table 2). Our sequences were 100% identical for two (EU707431, MT012300) of the six sequences of *C. terraconensis* deposited in GenBank originated from *C. avellana* and 99,82% similar with the next four (KF225578, EU707430, OM567738, OM567657) with a difference of one nucleotide (Fig. 1).

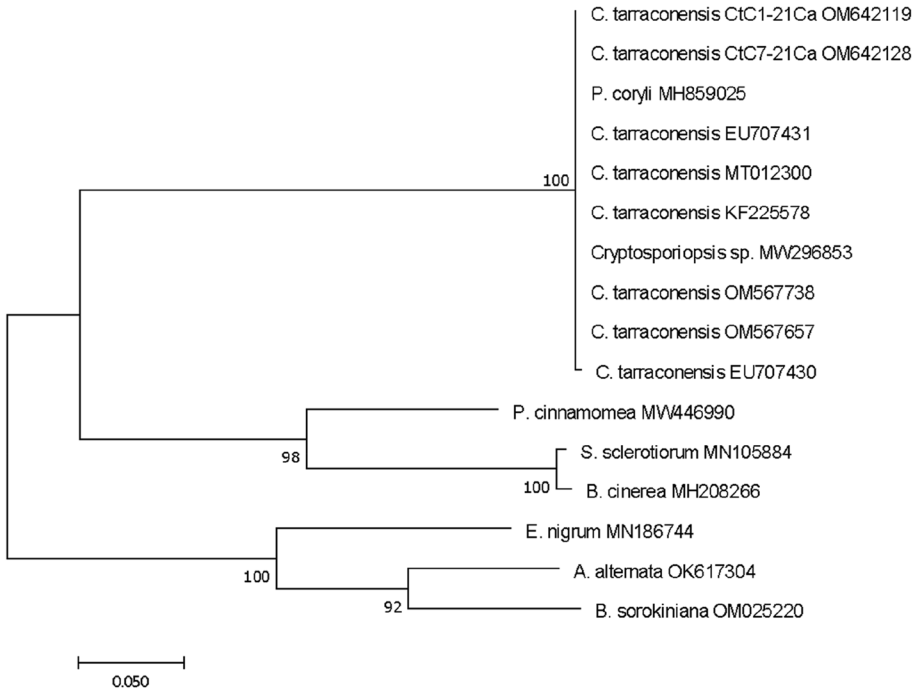

Fig. 1 Phylogenetic relationship between *C. tarraconensis* isolates CtC1-21Ca (OM642119) and CtC7-21Ca (OM642128) as analyzed in these studies, and 8 sequences showing an identity match with them at a level over 99% with 6 chosen sequences of Helotiales and Pleosporales representatives

Blastn analysis also revealed their identity in the first set with the singular GenBank isolate of *Piggotia coryli* (Roberge ex Desm.) Sutton (basionym-*Cheilaria coryli* Roberge ex Desm. (MH859025)). However, a match of our isolates to this species was ruled out. The phylogenetic tree (Fig. 1) shows a separate grouping of *Pezicula cinnamomea* (DC.) Sacc., *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* (Lib.) de Bary from the Helotiales order, which includes *C. tarraconensis*, and isolates from Pleosporales order, i.e., *Epicoccum nigrum* Link, *Alternaria alternata* (Fr.) Keissl. and *Bipolaris sorokiniana* Shoemaker, to which belongs *P. coryli*. Also, the level of identity between *C. tarraconensis* and the sequences of other species of both Pleosporales and Helotiales is below 93%, while the level of identity between *C. tarraconensis* is 100% or close to 100%. Therefore, this indicates that our isolates cannot belong to the species *P. coryli* and confirms the results of microscopic observations. The dendrogram was constructed in MEGA11 Toolbar (Tamura et al. 2021) using the Maximum Likelihood algorithm. The Kimura 2-parameter model was applied (Kimura 1980). Node support was calculated using 1000 bootstrap replicants.

The pathogenicity test was conducted on fresh, 1-month-old leaves of *C. avellana*. To inoculate pathogens on the leaves, two 10 µl of dilutions of *C. tarraconensis* isolates (C1 and C7) with a concentration of 1×10^6 conidia/ml each were prepared. After inoculation, the plants were kept under controlled conditions in a dew chamber (21 ± 2 °C at over 90% humidity) with controlled plants. After 7 days, the fungus was re-isolated from the inoculated plants. Morphologically identical to the original, C. *tarraconensis* isolate was reisolated from the infected tissues, thus fulfilling Koch's postulates (Appendix Figure 5).

Appendix

Fig. 2 Disease symptoms on *Corylus avellana* leaves were observed at Wigry National Park in 2021. **A** Regular, round, brown spots with a dark point in the middle of the spot. **B** Regular spots spread to the top of the leaf, creating long necrosis, limited by the leave's nerves, **C** and **D** wet, irregular lesions occurring on the borders of the leaves

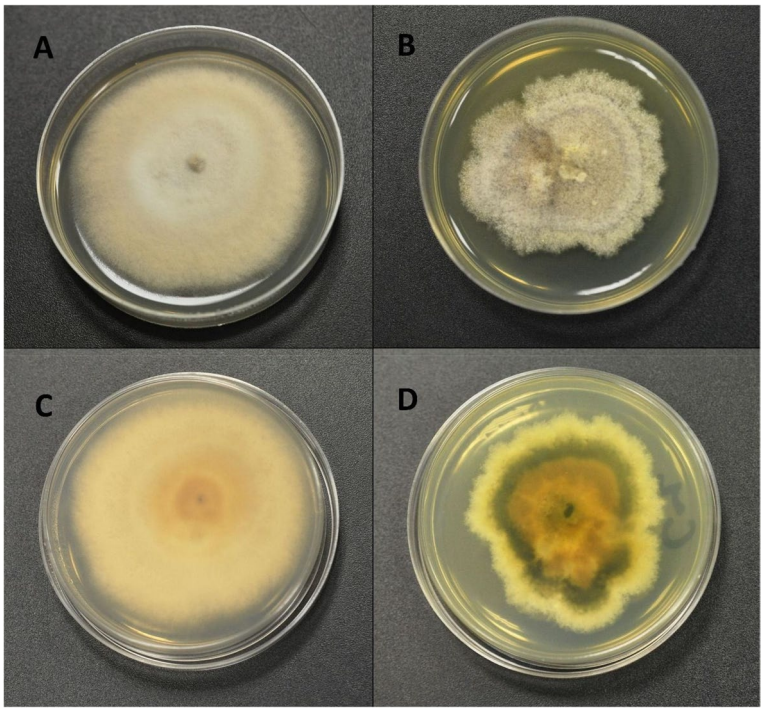

Fig. 3 Culture features of *Cryptosporiopsis tarraconensis* on PDA plates, 14 days after inoculation (**A**, **B** upper view of the plate and **C**, **D** reverse view)

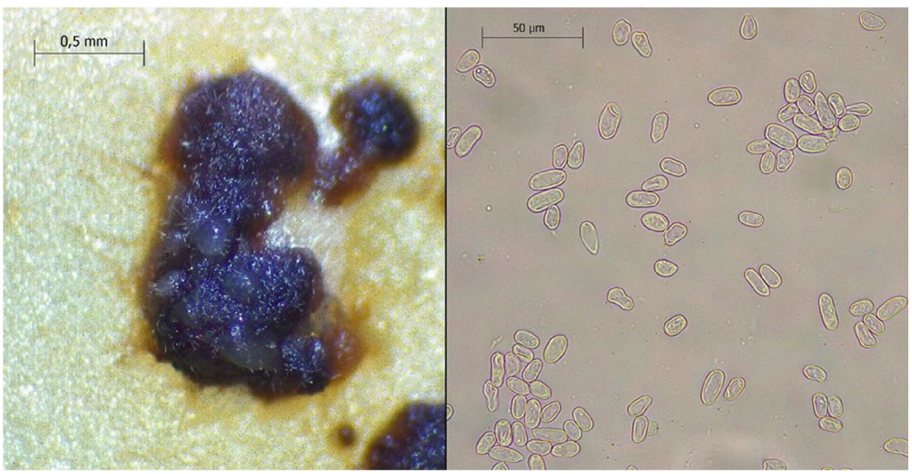

Fig. 4 Observation of acervuli and conidia of *Cryptosporiopsis tarraconensis*. An acervulus (scale bar = 0.5 mm) and conidia (scale bar = 50 µm) were recorded by optical microscope

Fig. 5 Symptoms of leaf lesions caused by *Cryptosporiopsis tarraconensis* on *Corylus avellana* after 7 days of reinoculation

Table 2 Details on isolates taken from GenBank NCBI for phylogenetic comparative analysis based on ITS sequences

Fungus	Accession number	Host	Percentage of identity with our isolates [%]	Class	Order	Family
Cryptosporiopsis tarraconensis Gené and Guarro	EU707431	*Corylus avellana* L	100	Leotiomycetes	Helotiales	Dermateaceae
Cryptosporiopsis tarraconensis Gené and Guarro	MT012300	*Corylus avellana* L	100	Leotiomycetes	Helotiales	Dermateaceae
Cryptosporiopsis tarraconensis Gené and Guarro	OM567738	*Corylus avellana* L	99.82	Leotiomycetes	Helotiales	Dermateaceae
Cryptosporiopsis tarraconensis Gené and Guarro	OM567657	*Corylus avellana* L	99.82	Leotiomycetes	Helotiales	Dermateaceae
Cryptosporiopsis tarraconensis Gené and Guarro	KF225578	*Corylus avellana* L	99.82	Leotiomycetes	Helotiales	Dermateaceae
Cryptosporiopsis tarraconensis Gené and Guarro	EU707430	*Corylus avellana* L	99.82	Leotiomycetes	Helotiales	Dermateaceae
Cryptosporiopsis sp.	MW296853	*Corylus avellana* L	99.81	Leotiomycetes	Helotiales	Dermateaceae
Piggotia coryli (Roberge ex Desm.) Sutton (basionym-*Cheilaria coryli* Roberge ex Desm	MH859025	unknown CBS 441.67	100	Dothideomycetes	Pleosporales	Didymellaceae
Epicoccum nigrum Link	MN186744	*Padus serotine* (Ehrh.) Borkh	93.48	Dothideomycetes	Pleosporales	Didymellaceae
Alternaria alternata (Fr.) Keissl	OK617304	*air, meadow*	91.30	Dothideomycetes	Pleosporales	Pleosporaceae
Bipolaris sorokiniana Shoemaker	OM025220	*wheat*	89.86	Dothideomycetes	Pleosporales	Pleosporaceae
Pezicula cinnamomea (DC.) Sacc	MW446990	*Fraxinus excelsior* L	86.87	Leotiomycetes	Helotiales	Dermateaceae
Botrytis cinerea Pers	MH208266	*Vicia faba* L	80.65	Leotiomycetes	Helotiales	Sclerotiniaceae
Sclerotinia sclerotiorum (Lib.) de Bary	MN105884	*Daucus carota* L	81.20	Leotiomycetes	Helotiales	Sclerotiniaceae

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Code availability
Not applicable.

Authors' contributions
Conceptualization: Katarzyna Patejuk; methodology: Katarzyna Patejuk, Anna Baturo-Cieśniewska; formal analysis and investigation: Katarzyna Patejuk, Anna Baturo-Cieśniewska, Agata Kaczmarek-Pieńczewska, Amelia Piegdoń, Wiesław Fałtynowicz, Pola Wasilewska, Magdalena Ogonowska; writing—original draft preparation: Katarzyna Patejuk, Anna Baturo-Cieśniewska; writing—review and editing: Katarzyna Patejuk, Agata Kaczmarek-Pieńczewska; funding acquisition: Wiesław Fałtynowicz; supervision: Katarzyna Patejuk. The authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare that they have no conflict of interest.

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