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DOI: 10.1111/epp.70069

ORIGINAL ARTICLE

The tree-parasitic fungus *Cryphonectria carpinicola* discovered on European chestnut and European hornbeam in Azerbaijan

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Funding information
Swiss Federal Office for the Environment (FOEN)

Abstract

The ascomycetous fungus *Cryphonectria carpinicola* is typically found in its asexual form on the European hornbeam, and its sexual state has only recently been discovered in deadwood of different *Carpinus* species in Japan. Recently, this fungus has been added to the EPPO Alert List due to its increasing incidence in the EPPO region and a possible East Asian origin. Although extensive field campaigns investigating chestnut blight in Europe have never detected this fungus on Fagaceae, a historical collection from a Japanese chestnut tree has demonstrated its ability to infect species in this family. This report expands the known distribution range of *C. carpinicola* to include Azerbaijan and provides the first evidence of its infection of *Castanea sativa* in the chestnut forests of the Greater Caucasus.

Le champignon parasite *Cryphonectria carpinicola* a été découvert sur le châtaignier européen et le charme européen en Azerbaïdjan

Le champignon ascomycète *Cryphonectria carpinicola* se trouve généralement sous sa forme asexuée sur le charme européen, et son état sexué n'a été découvert que récemment dans le bois mort de différentes espèces de *Carpinus* au Japon. Récemment, ce champignon a été ajouté à la liste d'alerte de l'OEPP en raison de son incidence croissante dans la région OEPP et de son origine probable en Asie de l'Est. Bien que les campagnes de terrain approfondies menées pour étudier le chancre du châtaignier en Europe n'aient jamais détecté ce champignon sur les Fagaceae, un échantillon prélevé sur un châtaignier japonais a démontré sa capacité à infecter des espèces de cette famille. Ce rapport élargit l'aire de répartition connue de *C. carpinicola* pour inclure l'Azerbaïdjan et fournit la première preuve de son infection du *Castanea sativa* dans les forêts de châtaigniers du Grand Caucase.

Обнаружение фитопатогенного гриба *Cryphonectria carpinicola* на каштане европейском и грабе европейском в Азербайджане

Аскомицет *Cryphonectria carpinicola* обычно встречается в бесполой форме на грабе европейском, или обыкновенном (*Carpinus betulus*). Половая стадия вида была обнаружена лишь недавно, в Японии, в мертвой древесине различных видов *Carpinus*. Недавно этот гриб был включен в Сигнальный перечень ЕОКЗР в связи с учащением его обнаружений в регионе ЕОКЗР и возможным восточноазиатским происхождением. Масштабные полевые исследования, связанные с изучением болезни каштана (*Castenea*) в Европе никогда не выявляли этот гриб на растениях семейства Fagaceae, однако образцы, взятые с японского каштана, указали на его способность поражать виды этого семейства. Данное сообщение расширяет

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известный ареал *C. carpinicola*, включая Азербайджан; впервые приводятся свидетельства о находке этого вида на *Castanea sativa* в каштановых лесах Большого Кавказа.

Since the early 2000s, repeated reports of declining hornbeam trees have emerged across Europe, spanning from Italy to the United Kingdom and from the Alps to the Balkans (Cornejo et al., 2021; Cornejo, Risteski, et al., 2023; Milenković et al., 2024; Nuskern et al., 2025; Papp et al., 2024). The trees are infected by one or both of two fungi and die within a few years if heavily attacked. One of these fungi produces large areas of necrotic bark with red, resin-like clumps on the trunks and has been identified as *Anthostoma decipiens* (Rocchi et al., 2010). The second fungus produces yellow-orange stromata on the bark and has been assigned to the newly described species *Cryphonectria carpinicola* (Cornejo et al., 2021).

Cryphonectria carpinicola was discovered in Japan in 2022, with its sexual form being described for the first time (Cornejo, Otani, et al., 2023). As only the asexual form has been observed in Europe to date, this finding suggests that East Asia may be the area of origin of *C. carpinicola*. Unlike in Europe, no symptomatic trees have been observed in Japan; however, stromata have been found saprotrophically on broken branches of *Carpinus* species on the forest floor. In the context of the increasing incidence of *C. carpinicola* in the EPPO region over the past two decades and given the possible East Asian origin of this fungus, *C. carpinicola* was included on the EPPO Alert List in 2024 (EPPO, 2024).

While *C. carpinicola* is clearly associated with infections in hornbeams in Europe and Japan, inoculation tests (Cornejo et al., 2021; Saracchi et al., 2015) and a strain isolated from *Castanea crenata* in Japan in 1998 (Liu et al., 2003; GenBank acc. OQ852902) have shown that this fungus can also infect Fagaceae species. However, despite extensive sampling of chestnut trees across Europe to study chestnut blight (causal agent, *Cryphonectria parasitica*), *C. carpinicola* has never been reported on European chestnut trees.

Our interest in *C. carpinicola* from Azerbaijan was initially inspired by a report of the fungus on hornbeam in the neighbouring country, Georgia (Cornejo et al., 2021), and its potential to infect Japanese chestnut. We therefore undertook two field trips: one to a mixed forest with chestnut trees in 2023 and another to a hornbeam forest in 2025, both of which were in the Greater Caucasus. Furthermore, we re-examined a strain that was collected during the 2010s field campaign to investigate the invasion dynamics of chestnut blight disease in chestnut forests. The strain M8194 was found to be morphologically distinct from the expected causal agent, *C. parasitica*, and was therefore cryopreserved in the WSL's fungus collection (Table 1).

The samples were collected in the districts of Zagatala, Gakh and Guba, which are all situated along the southern slopes of the Greater Caucasus mountain range in Azerbaijan (see Figure 1). Zagatala and Gakh are in the western part of the range, while Guba is in the eastern part. Chestnut trees are a common species in these mountain ranges, growing naturally in mixed forests alongside oak, beech and hornbeam, as well as in orchards and agricultural plantations. In the Chinarli orchards, chestnuts mainly grow alongside hazelnuts (Wall & Aghayeva, 2014).

Samples of stromata extracted from the tree bark were laid out on potato dextrose agar (PDA) (39 g/L; BD Difco) containing 0.1 mg/mL of the antibiotic streptomycin (Sigma-Aldrich) to prevent bacterial growth. Subsequently, the isolates were grown on PDA at 25°C and under a 14 h light, 10 h dark regime to document their cultural characteristics and to stimulate the production of asexual spores used for the long-term preservation. All isolates were stored frozen in 22% glycerol at −80°C in the Swiss Federal Research Institute WSL culture collection (Table 1).

To determine the species of isolates, DNA was extracted from mycelial cultures using the semi-automated KingFisher™ 96 Flex (Thermo Scientific™) using LGC reagents and Kingfisher 96/Flex (LGC Genomics

TABLE 1 Voucher information for the isolates of *Cryphonectria carpinicola* used in this study, including the GenBank accession number for their ITS sequences.

Tree host	Locality	Voucher	Collector (year)	Isolate ID[a]	GenBank
Castanea sativa	Chinarli village, Gakh district	Qakh 24.1	D. Aghayeva/J. Wall (2011)	M8194	PX998210
	Yukhary-Tala, Zagatala district	ZT75	D. Aghayeva (2023)	M10881	PX998211
Carpinus betulus		ZT-CB1	D. Aghayeva (2023)	M10882	PX998212
		ZT-CB2	D. Aghayeva (2023)	M10883	PX998213
	Galaduz village, Guba district	AZQ01	D. Aghayeva/C. Cornejo (2025)	M11673	PX998214
		AZQ04	D. Aghayeva/C. Cornejo (2025)	M11676	PX998215
		AZQ10	D. Aghayeva/C. Cornejo (2025)	M11682	PX998216

[a]M-number = culture collection of the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland.

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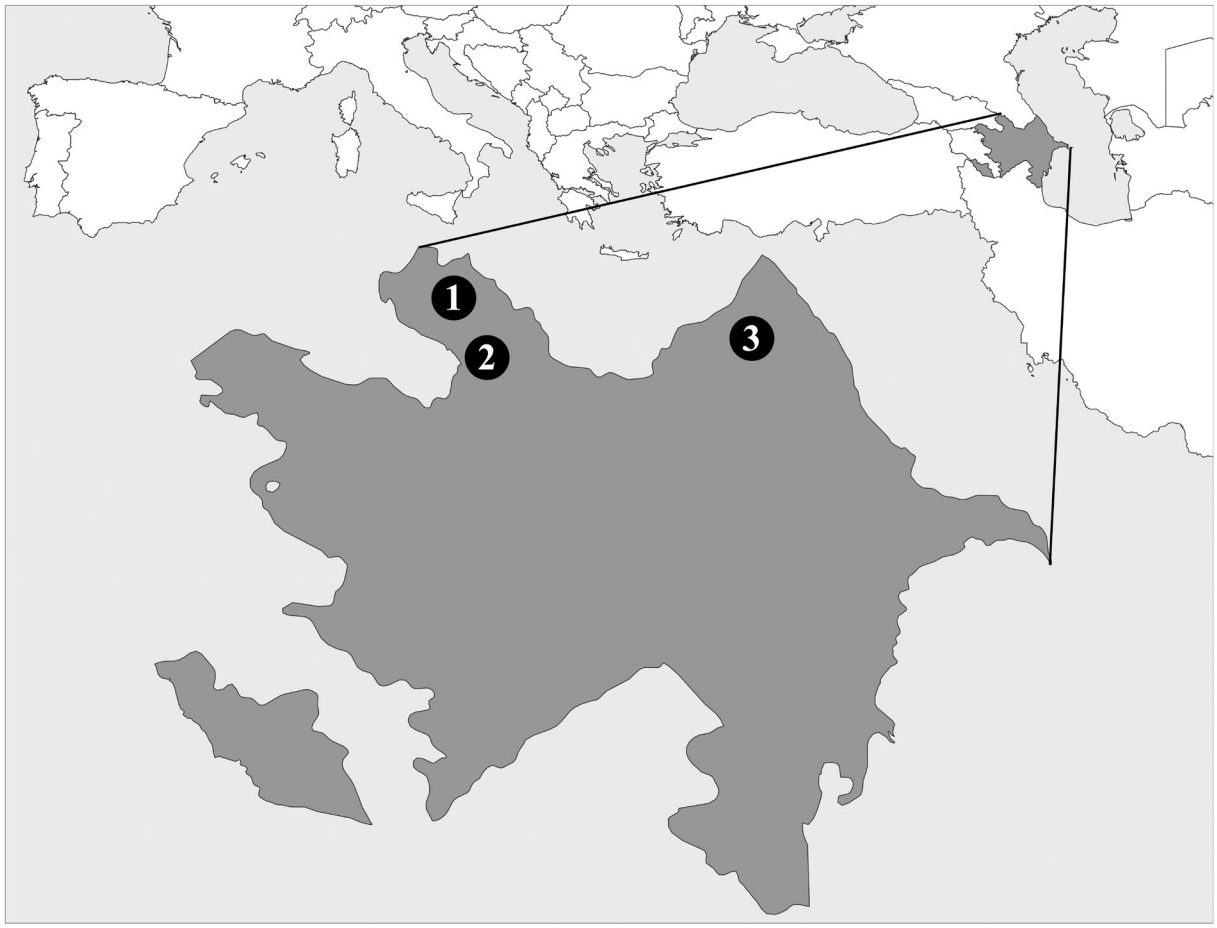


FIGURE 1 Partial map of Southern Europe and Western Asia, with the Republic of Azerbaijan enlarged. Numbers 1, 2 and 3 show where samples were collected in the districts of Zagatala, Gakh and Guba.

GmbH, Berlin, Germany), according to the manufacturer's instructions. The barcode internal transcribed spacers (ITS) of the nuclear rRNA gene cluster were amplified and sequenced using the primer pair ITS5-ITS4 according to published protocols (Cornejo et al., 2021; White et al., 1990). The resulting forward and reverse sequences were assembled using the software DNA Main Workbench v26 (CLC bio, Qiagen). The ITS sequences of several *Cryphonectria* species contained long stretches of single-nucleotide repeats, resulting in a rapid decline in Sanger read quality after the long poly(dA) and poly(dT) stretches. In such cases, reference sequences were used for assembling the forward and reverse reads. The ITS sequences were verified in GenBank (www.ncbi.nlm.nih.gov, accessed on 24-01-2026) using the nucleotide BLAST search.

Sequence alignments of closely related *Cryphonectria* species were directly downloaded from GenBank and used for a phylogenetic analysis with *C. parasitica* as the outgroup species. The phylogeny was reconstructed using the maximum likelihood criterion as applied in PhyML 3.0 (Guindon et al., 2010) on the ATGC platform (http://www.atgc-montpellier.fr, accessed on 24-01-2026), using the Smart Model Selection and the Akaike Information Criterion. The resulting tree was displayed in Tree-Graph 2 (Stoever & Mueller, 2010), including bootstrap percentages of 1000 pseudo-replicates. Samples from Azerbaijan were found to be part of a monophyletic group with other *C. carpinicola* samples from Europe and Japan, supported by 70% bootstrap (Figure 2).

The morphology and anatomy of the anamorph of *C. carpinicola* differ only slightly from those of the closely related species *C. radicalis*. Without a molecular analysis of their barcodes, it would be difficult to distinguish between the two species. While most *Cryphonectria* species occur on tree genera of the family Fagaceae, *C. carpinicola* was mainly found on *Carpinus* spp. of the family Betulaceae. It is noteworthy that historical records in the long-established BPI fungus collection include several herbarium specimens of *C. radicalis* (syn., *Endothia radicalis*) on various *Carpinus* species, which could potentially belong to the only recently described species *C. carpinicola*. For instance, there are records of this species on *Carpinus betulus* for Slovakia, on *Carpinus japonica* for Japan, on *Carpinus laxiflora* for the Korean peninsula, and on an unidentified *Carpinus* species for the USA. Specifically, the historical

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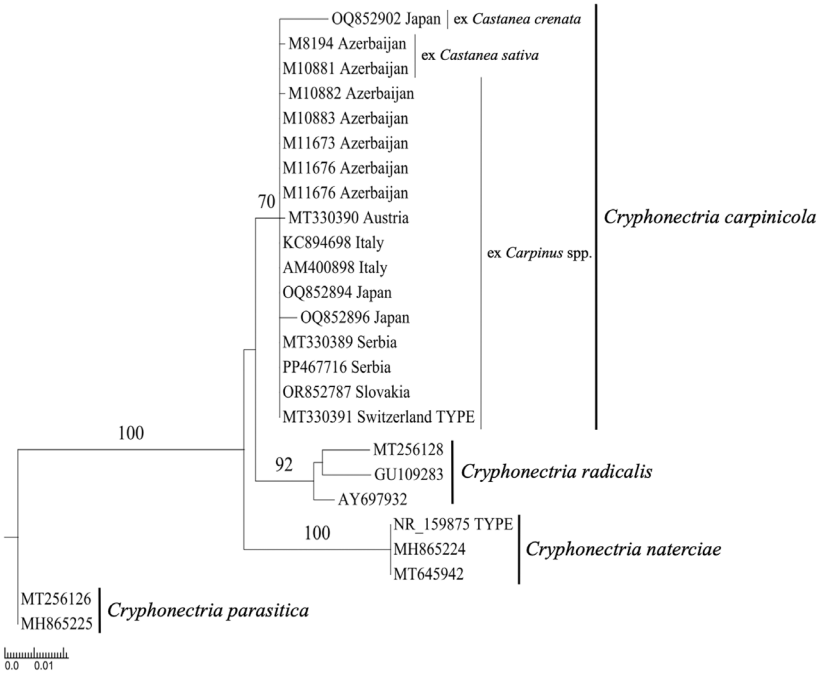


FIGURE 2 Phylogenetic analysis of *Cryphonectria* species. Most likely phylogeny of ITS sequences showing the relationship between closely related *Cryphonectria* species and among specimens of *Cryphonectria carpinicola* from Europe and Japan (PhyML v.3.0: 81 polymorphic out of 539 nucleotide sites, including gaps; GTR+G substitution model; 1000 bootstrap pseudo-replicates). Branches with Bootstrap support >70% are shown at nodes. ITS sequences of *C. parasitica* rooted this tree. Sequences extracted from GenBank are labelled with their accession numbers. The term 'TYPE' refers to the ITS sequence obtained from the holotype specimens of *C. carpinicola* and *C. naterciae*.



FIGURE 3 Typical orange stromata of *Cryphonectria carpinicola* on tree bark. (a) Dead water shoots at the base of a *Carpinus betulus* tree near Galaduz village in the Guba district. The white arrows indicate the orange stromata on the bark of the water shoot. (b) The bark specimen ZT75 was cut from the stem of a *Castanea sativa* tree in Yukhary-Tala, Zagatala district.

specimen BPI 797692, collected by the Russian botanist Michael Woronin (1838–1903) in Abkhazia from *Carpinus betulus*, could be a potential representative of *C. carpinicola* from the Greater Caucasus.

In Japan, *C. carpinicola* is saprotrophic and bark cankers have never been observed on *Carpinus* stems. Instead, orange *Cryphonectria* stromata have been found on fallen branches on the forest floor.

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A similar situation has been observed with *C. betulus* in Azerbaijan, where stromata were found on dead branches on the forest floor and on small water shoots that were dead but still attached to the tree (see Figure 3a). Interestingly, both strains of *C. carpinicola* that were found on European chestnut trees were found to be sporulating on their stems (see Figure 3b). Currently, it is not possible to determine the extent to which the trees are affected by this infection, since stromata were the only visible symptom. This finding corroborates the results of earlier inoculation experiments, which demonstrated that *C. carpinicola* can infect the bark tissue of *C. sativa* but does not produce significant lesions or cankers (Cornejo et al., 2021; Saracchi et al., 2015). To study the genetic diversity of *C. carpinicola* populations, our laboratory has developed *C. carpinicola*-specific, highly variable markers (simple sequence repeats, SSRs), which will enable the detailed study of regional genetic diversity and gene flow at different spatial scales between the European, Caucasian and Far Eastern regions. The study would also examine *C. carpinicola* specimens from *C. sativa* wherever possible. However, it remains to be seen whether new discoveries will be made regarding the rare occurrence of *C. carpinicola* on chestnut trees. We encourage other laboratories to contact us if they may be able to share any putative *Cryphonectria* specimens they have found on Fagaceae or Betulaceae trees to be examined for the presence of *C. carpinicola*.

Inoculations with *C. carpinicola* of chestnut and hornbeam seedlings produced small lesions on both trees and did not cause host mortality, suggesting that *C. carpinicola* is a secondary rather than a primary pathogen on its main host tree *C. betulus*. In Italy, *C. carpinicola* has been mainly reported on stressed hornbeam trees in urban environments often together with *A. decipiens* (Saracchi et al., 2010). Our field observations in Switzerland also confirm this. *Cryphonectria carpinicola* has never been observed as the sole pathogen of *C. betulus*, but mostly on trees that had already been damaged by *A. decipiens* or, less frequently, other fungi such as *Armillaria* spp. There have also been isolated findings on freshly felled hornbeams or individual dead branches in otherwise healthy tree crowns. In summary, *C. carpinicola* is probably not a primary pathogen but rather a secondary fungus or a weakness parasite. Which combination of environmental factors could additionally increase the pathogenic potential of *C. carpinicola* remains to be determined.

ACKNOWLEDGEMENTS

We would like to thank Daniel Rigling and Simone Prospero for their support throughout this study. Sampling in 2025 was supported by the SNSF SPIRIT project no. IZSTZ0_223681, TreeFunAZ: 'Tree-associated pathogenic fungi and oomycetes in Azerbaijan'. This work was partially funded by the Swiss Federal Office for the Environment (FOEN). Open access publishing facilitated by ETH-Bereich Forschungsanstalten, as part of the Wiley - ETH-Bereich Forschungsanstalten agreement via the Consortium Of Swiss Academic Libraries.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/, reference number PX998210-PX998216.

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How to cite this article: Cornejo, C., Aghayeva, D. & Beenken, L. (2026) The tree-parasitic fungus *Cryphonectria carpinicola* discovered on European chestnut and European hornbeam in Azerbaijan. *EPPO Bulletin*, 00, 1–6. Available from: https://doi.org/10.1111/epp.70069

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