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Short communication

Cryphonectria carpinicola discovered in Japan: first report of the sexual state on *Carpinus* tree

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ABSTRACT

Cryphonectria carpinicola is an ascomycetous fungus that has been regularly found in its asexual form on European hornbeam (*Carpinus betulus*) in Europe over the past two decades. Here we describe the discovery of *C. carpinicola* in Japan and report for the first time its sexual state on *Carpinus* species. No symptomatic trees were observed, but stromata were found saprotrophically on broken branches of *Carpinus* species on the forest floor. The sexual structures of *C. carpinicola* resembled that of other *Cryphonectria* species and strongly resembled those of the closely related species *C. radicalis*. A phylogenetic tree based on the internal transcribed spacer sequences showed monophyly for the Japanese and European isolates of *C. carpinicola*. Further studies on the distribution and host range of *C. carpinicola* in Japan and on the life history strategies of this fungus are needed.

Keywords: *Carpinus*, *Castanea*, Cryphonectriaceae, Fagaceae, saprotroph

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Scientists around the world have historically deposited biological material in curated herbaria, fungaria or culture collections, preserving an invaluable resource for future scientific study. Such preserved biological material includes the first documented living strain JS13 of a fungus that would not receive its species name until twenty-three years after its collection in 1998 (Liu, Linder-Basso, Hillman, Kaneko, & Milgroom, 2003).

The long history of naming began at the same time strain JS13 was collected, but on the other side of the globe, when Italian tree pathologists described declining hornbeams in the late nineties, reportedly infected by an unidentified *Endothiella* sp., obsolete name for anamorphs of the genus *Cryphonectria* according to the Melbourne Code (McNeill et al., 2012). From then on, reports of the decline of the European hornbeam (*Carpinus betulus*) followed continuously, first from various northern Italian cities, later from Austria, Germany, Switzerland, and from the easternmost limit of the European hornbeam's distribution in Iran (Cornejo, Hauser, Beenken, Cech, & Rigling, 2021). In addition to *Cryphonectria* sp., another fungus identified as *Anthostoma decipiens* was found to be involved in the decline phenomenon (Rocchi, Quaroni, Sardi, & Saracchi, 2010). The long naming history of the second fungus came to an end when it was described as the novel species *Cryphonectria carpinicola* (Cornejo et al., 2021).

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The strain JS13 was collected in 1998 from symptomatic Japanese chestnut (*Castanea crenata*) in the locality Jise of Kyoto Prefecture during an extensive sampling campaign to study the causal agent of the chestnut blight disease, *Cryphonectria parasitica*, and associated mycoviruses (Liu et al., 2003; Liu, Dynek, Hillman, & Milgroom, 2007). Bark samples with stromata and only a few samples without stromata but from symptomatic chestnut trees were collected. Isolations resulted in 472 exemplars of *C. parasitica* and 44 *C. nitschkei*, an obsolete synonym for *C. japonica* (Gryzenhout, Wingfield, & Wingfield, 2009). Even then, *C. japonica* was known to have a wide host range in Japan from Fagaceae, Pinaceae to Betulaceae (Kobayashi & Ito, 1956; Kobayashi, 1970). In addition, viral dsRNA has been detected in strain JS13 (Liu et al., 2007), which can alter the appearance of the mycelium. Therefore, it was assumed that this viral dsRNA was responsible for the peculiar colony morphology of JS13, whereas the possibility that it might be an undescribed fungal species was not considered at this time. Consequently, strain JS13 was later deposited in the American Type Culture Collection (ATCC) under the name *Cryphonectria nitschkei* and identification number MYA-4104 along with the notation 'contains novel virus'. However, in later studies on this virus, the fungal barcode of JS13 indicated that this exemplar did not belong to the species *C. japonica* but was closely related to *C. radicalis* (Shahi, Eusebio-Cope, Kondo, Hillman, & Suzuki, 2019). Only after its formal description could the species affiliation of isolate JS13 be definitively clarified as *C. carpinicola* twenty-three years after its collection (Sato et al., 2021).

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Thanks to the preserved strain JS13 our interest was aroused in whether *C. carpinicola* is native to Japan, and a scientific mission to Japan by the first author opened the opportunity to conduct a short field trip. The purpose of the present study was thus to verify (i) whether *C. carpinicola* is more common in Japan than this incidental finding in 1998, and (ii) whether it can also occur on *Carpinus* species according to reports from Europe (Cornejo et al., 2021; Cornejo, Risteski, Sotirovski, & Rigling, 2023; Crampton, Pérez-Sierra, & Denman, 2022).

In Japan five *Carpinus* species are known; *Carpinus japonica*, *C. tschonoskii*, *C. laxiflora*, and *C. cordata* are deciduous and arboreal trees, and *C. turczaninovii* is a deciduous shrub tree growing exclusively on limestone mountain ridges. *Carpinus japonica*, *C. tschonoskii*, and *C. laxiflora* are among the major components of deciduous broadleaf forests on the Island of Shikoku in southwestern Japan, and often found at sunny slopes. Therefore, three sites in the Island of Shikoku were selected for the 26–28 Oct 2022 field trip: the Ichinomata study forest (Shimanto, Kochi), Mount Ryuoh (Manno, Kagawa), and the Tengu Highland (Yusuhara, Kochi). Although Japanese chestnut is also common in Shikoku, chestnut trees were not considered for sampling because *C. parasitica* was assumed to be the predominant *Cryphonectria* species on these trees (Liu et al., 2003). Of the three sites visited, orange *Cryphonectria*-like stromata were not found on living trees at all, but only on fallen small branches on the ground in the Tengu Highland Forest (Fig. 1A).

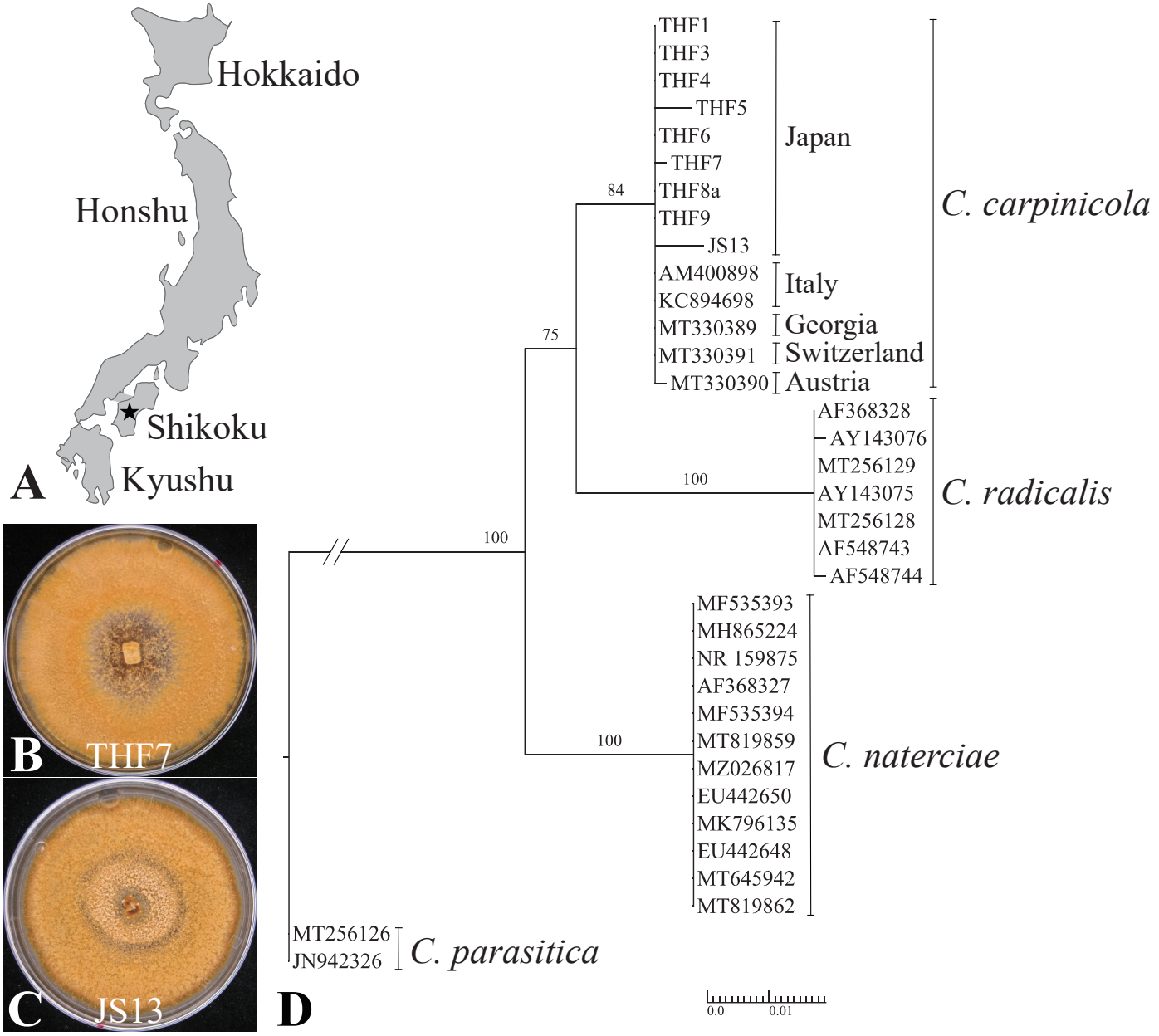

Fig. 1 – Collection site and phylogenetic analysis of *Cryphonectria* species. **A**: Map of the four main islands of Japan. Asterisk: Indicated is the Tengu Highland on the Island of Shikoku. **B–C**: Mycelium appearance of *C. carpinicola* grown 14 d under light/dark regime on PDA. **B:** Strain THF7 from *Carpinus* sp. **C**: Virus-free strain of JS13 from *Castanea crenata* that was obtained by single-conidium isolation from the originally virus-infected field isolate (Shahi et al., 2019). **C**: Most likely phylogeny of ITS sequences showing the relationship among Japanese and European specimens of *Cryphonectria carpinicola* (PhyML v.3.0: 65 polymorphic out of 521 nucleotide sites, including gaps; GTR+I substitution model; 1,000 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 labeled with their accession numbers.

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The collecting site in Tengu Highland encompasses the mountain ridges on the border between Kochi and the Ehime prefecture (1,350 m a.s.l.; 33° 28' 36''N, 133° 0' 28''E). At the Yusuhara Observatory, located 11 km southwest of the collection site and at 415 m a.s.l., the annual precipitation and the mean annual temperature are 2,729 mm and 13.4 °C, respectively (Japan Meteorological Agency, https://www.jma.go.jp/jma/indexe.html, accessed on 04-12-2022). Snowy weather is usually observed from Dec to Feb in the highland. Small twigs were collected from the ground along a trail leading from the Tenguso Hotel parking lot through a forest dominated by *Abies homolepis*, *Fagus crenata*, *Quercus crispula* var. *crispula*, *Carpinus japonica*, *C. tschonoskii*, *Stewartia monadelpha*, *Aesculus turbinata* and *Acer* spp. The forest floor is partially covered with dwarf bamboo (*Sasa nipponica*), and occasionally limestone rocks are found in the forest.

When stromata were found on fallen branches, the trees adjacent to the lying branches were visually inspected, but no disease symptoms were observed. Due to advanced autumn defoliation, only for the specimen THF5 the host tree species could be determined as *C. japonica*. Other hornbeam specimens were identified to a genus level. Therefore, the wood histology was checked using radial, longitudinal and transversal sections through the branches. All branches were determined to belong to *Carpinus* following Schweingruber (1990).

Cryphonectria-like stromata from eight branches were used for fungal isolation. Small stromata pieces 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 in order to document their cultural characteristics (Fig. 1B), 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 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 (White, Bruns, Lee, & Taylor, 1990; Cornejo et al., 2021). The resulting forward and reverse sequences were assembled using the software DNA Main Workbench v22 (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 28-12-2022) using the nucleotide BLAST search.

Sequence alignments of closely related *Cryphonectria* species (*C. radicalis* and *C. naterciae*) were directly downloaded from GenBank and used for a phylogenetic analysis with *C. parasitica* as outgroup species. Poly(dA) and poly(dT) regions produced ambiguous alignments and were thus excluded from further analyses by processing all datasets using Gblocks 0.91b (Castresana, 2000; Talavera & Castresana, 2007) on the Phylogeny.fr platform (Dereeper et al., 2008). 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 28-12-2022), using the Smart Model Selection and the Akaike Information Criterion. The resulting tree was displayed in TreeGraph 2 (Stoever & Mueller, 2010), including bootstraps percentages of 1,000 pseudo-replicates. Japanese samples were found to form a monophyletic group together with samples of *C. carpinicola* from Europe with 84% bootstrap support, including the ITS-barcode of the species holotype (GenBank accession, MT330391).

Additionally, the morphology of the stromata was investigated by hand sectioning of air-dried material and microscopic examination in tap water. Measurements and photos were taken with a Zeiss Discovery V8 stereo lens equipped with a Zeiss Axiocam 506 digital camera and a Zeiss Axio Scope A1 microscope equipped with a Axiocam 208 color digital camera and Zen 2.3 software (Carl Zeiss Microscopy GmbH, Germany). Asexual structures were found and documented on seven branches of *Carpinus* species, and ascostromata were discovered and studied in detail only on specimen THF7 of *Carpinus* sp. (Fig. 2).

Morphology

Cryphonectria carpinicola D. Rigling, T. Cech, Cornejo & L. Beenken, Fungal Biol. 125: 354, 2021.

MycoBank no.: MB 837752.

First description of sexual state occurring on *Carpinus* sp. Fig. 2 A–G: Ascostromata gregarious, pulvinate, semi-immerses in bark, pale orange, 0.2–0.8 × 0.4–1.4 mm and up to 0.8 mm high. Perithecia 2–20 per stroma, valsoid, embedded at base of stroma, black, +/- globous, 300–400 µm in diam.; necks 50–60 µm wide, black, emerging at stroma surface with ostioles imbedded in orange-brown papillae of stromatal tissue, 100–120 µm in diam. and up to 70 µm high. Asci 28–37 × 6–7(–10) µm (n = 10), fusiform, with ring-like apical ascus apparatus, 8-spored. Ascospores (6–)6.5–9(–11) × (2–)2.5–3(–3.5) µm (n = 40), 7.6 × 2.8 µm on average, length/wide ratio 2.2–5.6, 2.8 on average, colorless, hyalin, ellipsoidal to fusoid, ends round, with one median or sub-median septum, slightly constricted at septum, cell content hyalin, with small droplets. Remains of conidiomata in the same or separate stromata.

Substrate: On a dead twig, 1 cm in diam., of *Carpinus* sp. laying on the forest ground.

Specimen examined: JAPAN, Island of Shikoku, Tengu Highland, along the hiking trail starting from the Tengosou hotel, 33° 28' 38.40" N, 133° 0' 21.92" E, 1,350 m a.s.l. , 28 Oct 2022, C. Cornejo and T. Otani (THF7), ZT Myc 66431, MBK-0341550.

Conidiomata (Fig. 2 H–K) occurring on *Carpinus* spp. as described in Cornejo et al. (2021) but the stromata are smaller (0.2–0.9 × 0.5–1.5 mm) and with paler orange surface.

Substrate: On a dead twig, 0.5–2 cm diameter, of *Carpinus japonica* and *Carpinus* sp. laying on the ground.

Additional specimens examined containing only conidiomata: JAPAN, Island of Shikoku, Tengu Highland, along the hiking trail starting from the Tengosou hotel, 33° 28' 38.40" N, 133° 0' 21.92" E, 1,350 m a.s.l., *Carpinus japonica* (THF5), *Carpinus* sp. (THF1, THF3, THF4, THF6, THF8a, THF9), 28 Oct 2022, C. Cornejo and T. Otani.

Note: The sexual state of *C. carpinicola* described here for the first time strongly resembles those of other *Cryphonectria* species (Gryzenhout et al., 2009), with the closely related *C. radicalis* being the most similar. Its ascospores, which are (5.5–)6–7.5(–8.5) long and 2.5–3.5 µm wide (Gryzenhout et al., 2009), are only slightly shorter than those of *C. carpinicola*, while the ascospores of the other *Cryphonectria* species are clearly longer and broader (Gryzenhout et al., 2009). The stromata of *C. carpinicola* found on *Carpinus betulus* in Europe were with a size of 1–5(–10) × 0.5–1.5 mm (Cornejo et al., 2021) considerably larger than those from Japan. This difference could be related to the substrate. In Europe, *C. carpinicola* was found mainly on dead tree trunks and thicker

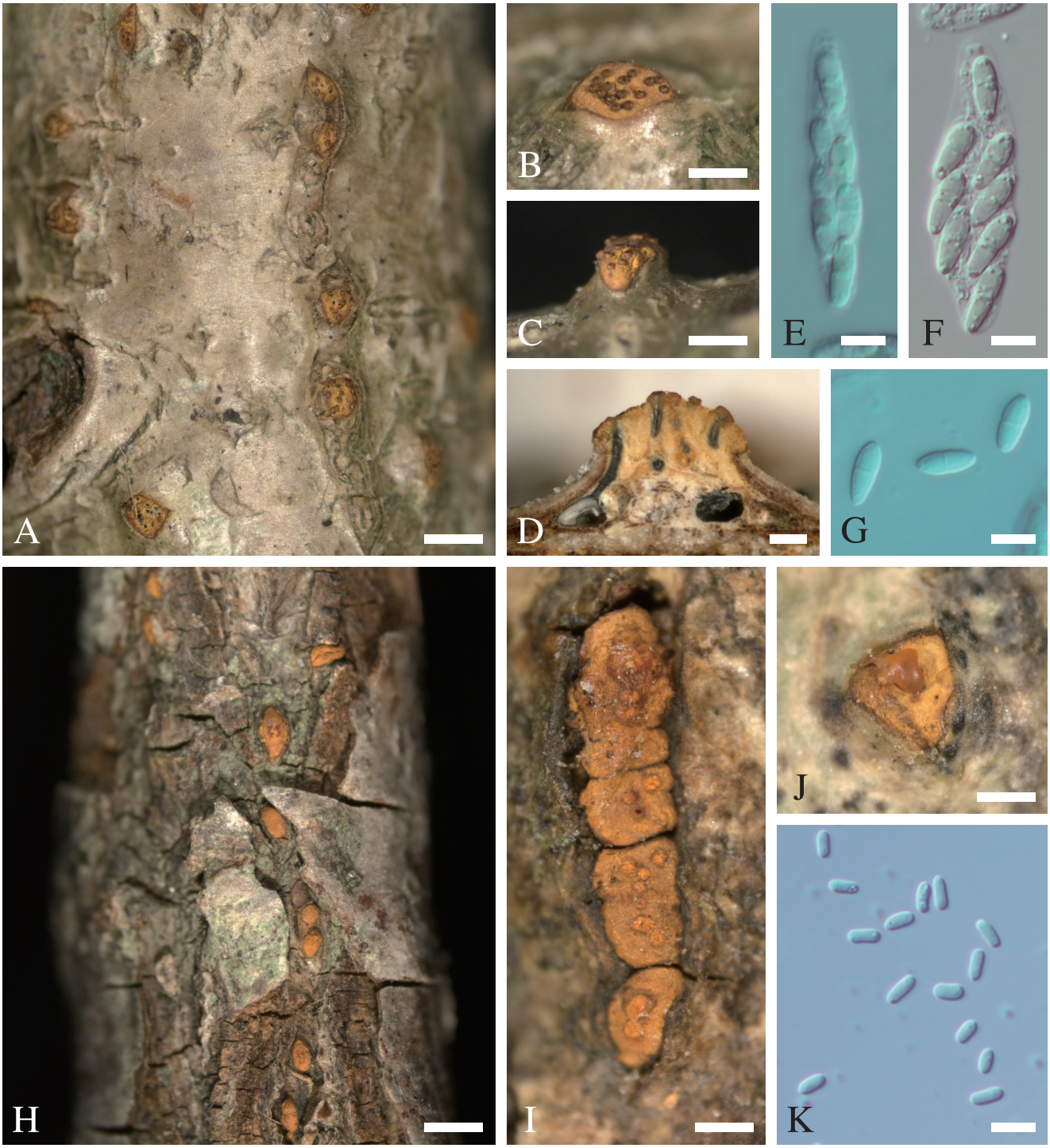

Fig. 2 – *Cryphonectria carpinicola*, **A–G**: Sexual stage on *Carpinus* sp. (THF7); **A–C**: Ascostromata immersed in bark, showing papillae of ostioles on the surface; **D**: Section of ascostroma with black perithecia and long necks; **E, F**: Asci, **G**: Ascospores. **H–K**: Asexual stage on *Carpinus* sp. (THF1). **H**: Orange conidial stromata on a twig; **I**: Several multilocular conidial stromata in a row; **J**: Conidial mass emerging out of a stroma; **K**: Conidia. Bars: A, H 1 mm; B, C, I, J 500 μm; D 200μm; E–G, K 5 μm.

branches, while the presented finds from Japan were from thinner twigs.

So far, this fungus is known only on *Carpinus betulus* in Europe and in the asexual conidial stage (Cornejo et al., 2021; field observations of the last author based on more than 40 collections from Switzerland, distribution atlas https://swissfungi.wsl.ch/en/index.html, accessed on 24-01-2023). The genus *Cryphonectria* appears to have a host preference for members of the Fagaceae, particularly the genera *Castanea* and *Quercus* (Gryzenhout et al., 2009). Despite the extensive sampling of chestnut trees in order to study the chestnut blight, *C. carpinicola* was never found on European chestnut, hence this fungus was considered an exception to this rule. However, our (Cornejo et al., 2021) and former inoculation tests by Saracchi, Sardi, Kunova, and Cortesi (2015), as well as voucher JS13 of *C. carpinicola* from *Castanea crenata*, confirm that this species has an affinity for Fagaceae, although it acts more as a weak parasite, as no

Table 1. Identification number of field samples of *Carpinus* sp. and isolates of *Cryphonectria carpinicola* examined in the present study.

Voucher	Tree host	Herbarium ID of the field sample[a]	Collection ID of the isolate[b]	GenBank accession no.
THF1	*Carpinus* sp.	MBK-0341545	M10783	OQ852894
THF3	*Carpinus* sp.	MBK-0341546	M10782	OQ852895
THF4	*Carpinus* sp.	MBK-0341547	M10781	OQ852896
THF5	*Carpinus japonica*	MBK-0341548	M10780	OQ852897
THF6	*Carpinus* sp.	MBK-0341549	M10779	OQ852898
THF7	*Carpinus* sp.	MBK-0341550	M10778	OQ852899
THF8a	*Carpinus* sp.	MBK-0341551	M10777	OQ852900
THF9	*Carpinus* sp.	MBK-0341552	M10776	OQ852901
JS13	*Castanea crenata*	n/a	ATCC:MYA 4104	OQ852902

[a] MBK = Herbarium of the Makino Botanical Garden, Kochi City, Island of Shikoku, Japan.
[b] M = Culture collection of the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland. ATCC = American Type Culture Collection, Manassas, Virginia, U.S.A.

bark cankers were observed.

For the present study, it was not possible to conduct a more intensive field excursion, but it is remarkable that the occurrence of *C. carpinicola* in Japan could be confirmed in this short time. Based on this positive result, we must assume that *C. carpinicola* is more abundant in Japanese deciduous forests than expected. Furthermore, the occurrence of the sexual state in Japan indicates a possible area of origin for this species. Considering that this fungus has been discovered in Central Europe only in the last twenty-five years, the question arises whether *C. carpinicola* has been introduced into Europe only recently or has been present here for a long time. Therefore, our laboratory is working on the development of highly variable *C. carpinicola* specific markers (Simple Sequence Repeats, SSR) that will allow detailed studies of regional genetic diversity and gene flow at different spatial scales. Future studies involving SSR markers and extensive sampling in deciduous forests will elucidate the genetic diversity of *C. carpinicola* and its host tree species in Japan.

Disclosures

The authors declare no conflicts of interest.

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References

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*(4), 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Cornejo, C., Hauser, A., Beenken, L., Cech, T., & Rigling, D. (2021). *Cryphonectria carpinicola* sp. nov. associated with hornbeam decline in Europe. *Fungal Biology*, *125*(5), 347–356. https://doi.org/10.1016/j.funbio.2020.11.012

Cornejo, C., Risteski, M., Sotirovski, K., & Rigling, D. (2023). Long-term preserved mycelium establishes the presence of *Cryphonectria carpinicola* in the Balkans and of *Cryphonectria radicalis* in Bulgaria. *European Journal of Plant Pathology*, *165*(2), 401–405. https://doi.org/10.1007/s10658-022-02613-8

Crampton, B., Pérez-Sierra, A., & Denman, S. (2022). Characterisation of Hornbeam Decline in England (UK). IUFRO Conference Division 7 – Forest Health Pathology and Entomology, Lisbon, Portugal.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, *36*, W465–W469. https://doi.org/10.1093/nar/gkn180

Gryzenhout, M., Wingfield, B. D., & Wingfield, M. J. (2009). *Taxonomy, phylogeny, and ecology of bark-inhabiting and tree-pathogenic fungi in the Cryphonectriaceae*. American Phytopathological Society (APS Press).

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, *59*(3), 307–321. https://doi.org/10.1093/sysbio/syq010

Kobayashi, T. (1970). Taxonomic studies of Japanese Diaporthaceae with special reference to their life-histories. *Bulletin of the Government Forestry Experiment Station 226*, 1–242.

Kobayashi, T., & Ito, K. (1956). Two new species of Endothia. *Japanese Journal of Phytopathology*, *21*(4), 151–152. https://doi.org/10.3186/jjphytopath.21.151

Liu, Y.-C., Linder-Basso, D., Hillman, B. I., Kaneko, S., & Milgroom, M. G. (2003). Evidence for interspecies transmission of viruses in natural populations of filamentous fungi in the genus *Cryphonectria*. *Molecular Ecology*, *12*(6), 1619–1628. https://doi.org/10.1046/j.1365-294X.2003.01847.x

Liu, Y.-C., Dynek, J. N., Hillman, B. I., & Milgroom, M. G. (2007). Diversity of viruses in *Cryphonectria parasitica* and *C. nitschkei* in Japan and China, and partial characterization of a new chrysovirus species. *Mycological Research*, *111*(4), 433–442. https://doi.org/10.1016/j.mycres.2006.12.006

McNeill, J., Barrie, F., Buck, W., Demoulin, V., Greuter, W., Hawksworth, D., Herendeen, P., Knapp, S., Marhold, K., & Prado, J. (2012). *International Code of Nomenclature for algae, fungi and plants*. Koeltz Scientific Books.

Rocchi, F., Quaroni, S., Sardi, P., & Saracchi, M. (2010). Studies on *Anthostoma decipiens* involved in *Carpinus betulus* decline. *Journal of Plant Pathology*, *92*(3), 637–644. https://www.jstor.org/stable/41998852

Saracchi, M., Sardi, P., Kunova, A., & Cortesi, P. (2015). Potential host range of *Anthostoma decipiens* and *Endothiella* sp., agents of hornbeam blight. *Journal of Plant Pathology*, *97*(1), 93–97. https://www.jstor.org/stable/24579135

Sato, Y., Shahi, S., Telengech, P., Hisano, S., Cornejo, C., Rigling, D., Kondo, H., & Suzuki, N. (2021). A new tetra-segmented splipalmivirus with divided RdRP domains from *Cryphonectria naterciae*, a fungus found on chestnut and cork oak trees in Europe. *Virus Research*, *307*, 198606. https://doi.org/10.1016/j.virusres.2021.198606

Schweingruber, F. H. (1990). *Anatomy of European woods*. Paul Haupt. Bern, Switzerland.

Shahi, S., Eusebio-Cope, A., Kondo, H., Hillman, B. I., & Suzuki, N. (2019). Investigation of Host Range of and Host Defense against a Mitochondrially Replicating Mitovirus. *Journal of Virology*, *93*(6), e01503-01518. https://doi.org/10.1128/JVI.01503-18

Stoever, B. C., & Mueller, K. F. (2010). TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics*, *11*(1), 7, Article 7. https://doi.org/10.1186/1471-2105-11-7

Talavera, G., & Castresana, J. (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology*, *56*(4), 564–577. https://doi.org/10.1080/10635150701472164

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications.* (pp. 315–322). Academic Press, Inc. https://doi.org/10.1016/B978-0-12-372180-8.50042-1