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SHORT COMMUNICATION

First report of *Cryptostroma corticale* on *Aesculus hippocastanum* causing sooty bark disease in Germany

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Abstract
Cryptostroma corticale is the causal agent of sooty bark disease, which was first described in the middle of the last century and has developed in recent years to a relevant threat for *Acer* spp. trees in Central Europe. Triggered by extreme heat and drought, this tree disease is becoming more and more important in the course of climate change. *Acer pseudoplatanus* is a particularly affected tree species, but the disease has also been observed on other *Acer* spp., and there is some indication that there are suitable hosts outside the *Acer* genus. In literature, *Aesculus hippocastanum* was mentioned twice to be a host, however, without any proof or details. With this study, we verify the assumption that *A. hippocastanum* is a host of *C. corticale* by morphological and phylogenetic analyses based on a case in Germany. Furthermore, we provide microscope pictures of microtome sections of the specimens, showing the spore production of *C. corticale* on *A. hippocastanum*.

Keywords Xylariales · Graphostromataceae · Sycamore maple · Buckeye · Microtome section

Introduction

The fungal species *Cryptostroma corticale* (Ellis & Everh.) P.H. Greg. & S. Waller (Xylariales, Ascomycota) is the causal agent of sooty bark disease, which was first described by Gregory and Waller (1951). This disease is triggered by extreme heat and drought and has led to significant damage of mainly *Acer pseudoplatanus* L. during the last decades in Europe (e.g. Koukol et al. 2015; Muller et al. 2023). Recently, the disease was also reported from Washington, USA (Washington State Department 2021). The disease occurs on trees of the genus *Acer* L., besides *Ac. pseudoplatanus* dominantly on *Ac. macrophyllum* Pursch in North America (Brooks et al. 2023). Furthermore single experiments and reports mention the fungus *C. corticale* on *Euonymus europaeus* L. (European spindle), *Populus tacamahaca* × *trichocarpa* (hybrid balsam poplar), *Salix viminalis* L. (osier), *Viburnum opulus* L., hickory, linden trees and *Aesculus hippocastanum* L. (buckeye) (Towey et al. 1932; Young 1978; Dickenson 1980; Washington State Department 2021). *A. hippocastanum*, addressed in this case as "horse chestnut", was mentioned shortly by Young (1978) as substrate for *C. corticale* without describing any detail, like locality or country. In a report on forest health from Washington, a number of tree species, including *A. hippocastanum*, are listed as having been positively tested by visible symptoms and molecular methods (without describing any details) for *C. corticale* (Washington State Department 2021). However, a comprehensively described study confirming *A. hippocastanum* as host of *C. corticale* has not been published so far. Here, we provide such evidence of *C. corticale* causing sooty bark disease on *A. hippocastanum*.

Material and methods

The infected *A. hippocastanum* tree, on which the following examinations are based, was discovered in December 2022 in the city of Trier in western Germany in a small group of *A. hippocastanum* trees planted in 1950 approx. 10–20 m apart. The group was located on a terrace on a

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southern-facing slope of around 20 degrees inclination, which was previously used as a gastronomic facility. The soil on the site is sandy and has been disturbed and heavily compacted through construction work, terracing and filling with various substrates including a top layer of gravel for its use as gastronomic facility. The tree had reached a height of 25 m in 2023, a tree crown diameter of 13 m, and at the height of one metre the perimeter of the stem was 2.22 m. In annual inspections, the tree had shown a loss of vitality since 2018 and in December 2022, although still alive, exhibited blackish spore production in bark fissures, loss of bark on some trunk areas up to a height of approx. 8 m, small buds untypical for vigorous trees, and death of crown parts. Therefore, the tree was felled on February 2023 because of concerns for traffic safety.

According to the German weather service, the location (Trier) has an average yearly temperature of 9.8 °C with 784.7 mm precipitation (calculation period 01/01/1981–31/12/2010). In 2022, a new weather extreme was measured with 39 hot days (maximal temperature at least 30 °C) per year (Deutscher Wetterdienst 2023).

On 19th December 2022, a specimen comprising bark and underlying wood tissue was taken at a height of 7 m from the symptomatic part of the trunk of the dying *A. hippocastanum* tree by cutting a horizontal wedge of bark and sapwood measuring approx. 13 cm (Fig. 1a). The fresh sample was processed for obtaining fungal material for culturing and histology.

Specimen examined: Germany, Trier, urban area, on dying tree of *A. hippocastanum*, 19.12.2022, leg. J. Esch, JKI-WSF-2023–001, ex-culture C 586.

For light microscopic examination and imaging, microtome sections of the specimen were produced, by cutting small cube-like parts that included wood and bark from the margin of the symptomatic area. This small part of the specimen was prepared with Modified Davidson's Fixative (Hartmann's Fixative) and subsequently embedded with help of the embedding system Technovit 7100 (Morphisto) based on 2-hydroxyethyl methacrylate. Pre-infiltration was done with 99% Isopropanol, twice evacuated for 5 min and then remained for 50 min in solution. According to the user instructions, infiltration was done with vacuum (2 × 5 min evacuated) and left for six hours at 6 °C. For mounting the embedded specimen with the Histobloc S, the kit Technovit 3040 (Morphisto) was used. Cutting was done by rotary microtome MICROM HM 350 with 10 µm widths and staining of the microtome sections was done with 0.1% Thionin for ten seconds and then rinsed out with water. The photographs were taken by the digital camera Axiocam 105 Colour R2, adapted to the Zeiss microscope Axio Imager.M2.

Conidia were taken from the specimen, mixed with sterile water and spread onto malt yeast pepton agar (MYP) for incubation at room temperature. After one day, germinated conidia were separated to gain single-spore isolates. Pure cultures were grown at room temperature with alternating light and dark for two weeks. Furthermore, pieces of discoloured sapwood, approx. 2 cm beneath the symptomatic cambial area, were cut out, surface sterilised 1 min. in 96% ethanol and placed on 2% malt extract agar (MEA) for cultivation. Resulting cultures were checked for contaminants and subcultured.

DNA-extraction was performed from both, single-spore isolates and isolates from sterilised wood, according to the protocol of Izumitsu et al. (2012) and polymerase chain reaction (PCR) was conducted with the primers ITS1F and ITS4 (Gardes and Bruns 1993; White et al. 1990) to amplify internal transcribed spacer 1, 2 and 5.8S gene. The PCR programme includes 240 s initial denaturation at 94 °C, followed by 35 cycles of 40 s at 94 °C, 40 s at 55 °C and 50 s at 72 °C, with a final 240 s extension at 72 °C. For purification of PCR products, the DNA Clean & Concentrator®-5 kit (Zymo Research, Irvine, California, USA) was used and the sequencing was implemented by Eurofins Genomics (Ebersberg, Germany). Resulting DNA sequences were deposited in NCBI GenBank (Benson et al. 2018).

To conduct the phylogenetic analysis, sequences of the closest relatives of *C. corticale* and outgroups shown in the phylogenies of Koukol et al. (2015) and Li et al. (2021) were used (Table 1). The sequences were edited manually by MEGA X (Kumar et al. 2018) and the alignment was constructed by MAFFT v.7 (https://mafft.cbrc.jp/alignment/server/, Katoh et al. 2019) using the L-INS-i algorithm with default settings. The phylogenetic tree (Maximum Likelihood analysis) was calculated by MEGA X using the following settings: 1000 bootstrap replications, Tamura-Nei substitution model (Tamura and Nei 1993), a partial deletion of gapped positions with 95% site coverage cut-off and otherwise default settings were used. The resulting phylogenetic tree was edited with Microsoft PowerPoint.

Results

Morphological analysis.

The morphological appearance of sporulation in the examined specimen is consistent with the description of *C. corticale* in Gregory and Waller (1951). Images of the specimen of *C. corticale* on *A. hippocastanum* are shown in Fig. 1. In *Ac. pseudoplatanus* infected with *C. corticale*, the superficial periderm is retained and sporulation takes place directly beneath the bark surface, whereas *C. corticale* on *A. hippocastanum* shows pronounced sporulation under peeling layers of corky bark (Fig. 1b). Cultures of *C. corticale* from *A. hippocastanum* were isolated from discoloured sapwood, which was observed below the cambial zone

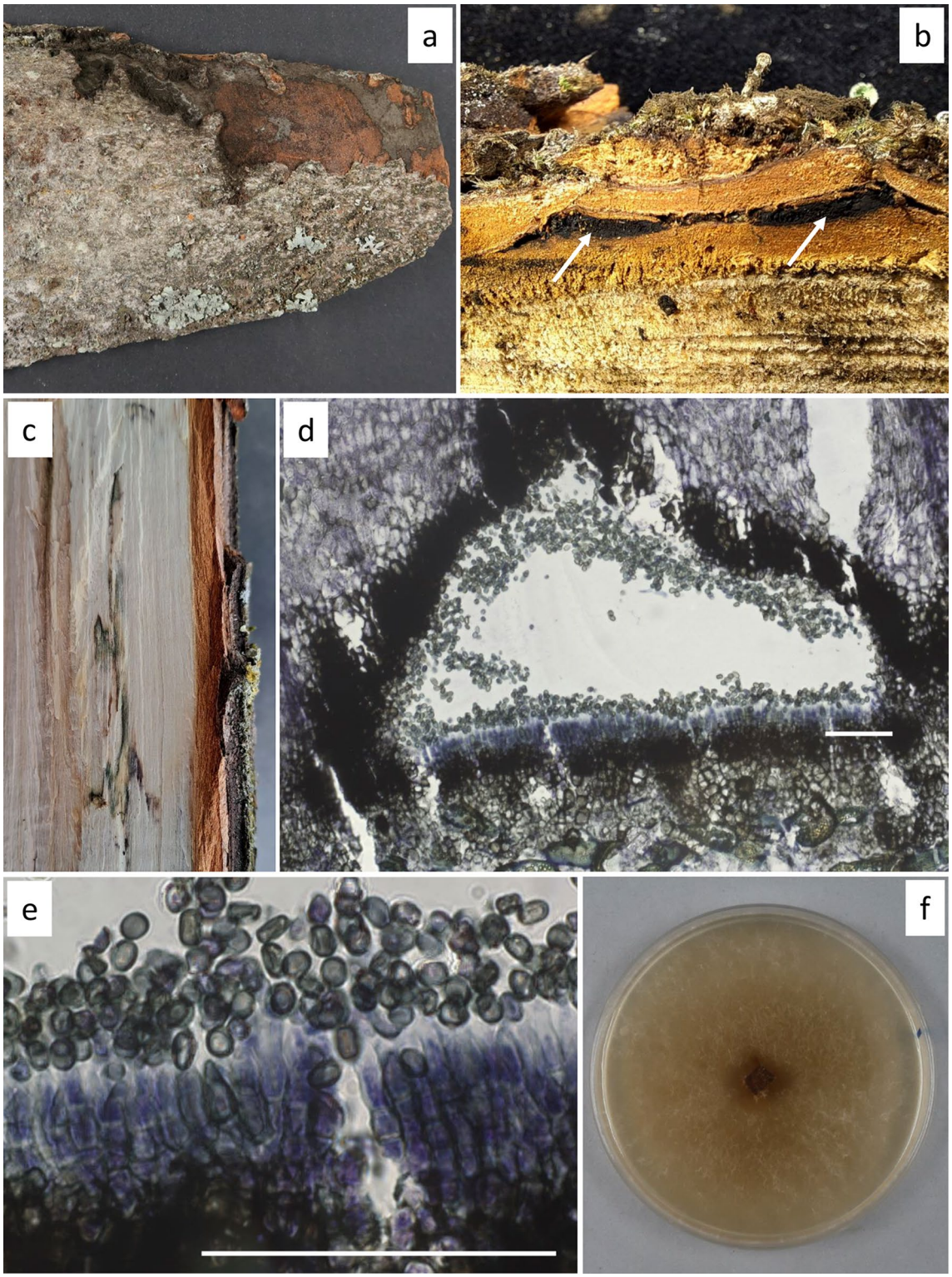

Fig. 1 *Cryptostroma corticale* on *Aesculus hippocastanum*: **a** specimen voucher JKI-WSF-2023–001; **b** cross-section of bark: sporulation (arrows) under peeling layers of corky bark; **c** radial section: discolouration of sapwood; **d** embedded microtome cross-section showing a cavity in the corky bark, surrounded by blackish pseudoparenchyma and created by masses of conidia that were produced by a layer of conidiophores at the proximal side of the cavity; **e** embedded microtome cross-section of corky bark showing conidiophores and produced conidia; **f** *C. corticale* MEA culture, 20 days old. Scale bars **d**–**e** 50 µm

Table 1 Details of DNA sequences used in the phylogenetic study. Newly generated sequences shown in bold

Species	Specimen voucher or isolate/strain	GenBank accession numbers	Host plant	Country	Reference
Jackrogersella cohaerens	CBS 114744	AY616688	*Fagus sylvatica*	Germany	Triebel et al. 2005
Biscogniauxia bartholomaei	ATCC 38992	AF201719	unknown	unknown	Pinto-Sherer 2001
Biscogniauxia granmoi	YMJ 135	JX507803	*Prunus padus* L.	Austria	Mirabolfathy et al. 2013
Biscogniauxia marginata	CBS 124505	KU684016	*Malus* sp. or *Pyrus* sp.	Germany	unpublished
Biscogniauxia nummularia	MUCL 51395	NR_153649	*Fagus sylvatica*	Germany	Wendt et al. 2018
Cryptostroma corticale	Acer7	HG934114	*Acer pseudoplatanus*	Czechia	Koukol et al. 2015
	JKI-GFF-2019–008	ON773408	*Acer pseudoplatanus*	Germany	Kespohl et al. 2022
	JKI-WSF-2023–001	**OR260270**	***Aesculus hippocastanum***	**Germany**	**this study**
Graphostroma guizhouense	GMBC0219	MW854659	dead wood	China	Li et al. 2021
Graphostroma platystomum	CPC:37,153	MT223799	*Lindera benzoin*	USA, New York	Crous et al. 2020
Hypoxylon pulicicidum	MUCL49879	JX183075	rotten wood	France, Martinique	Bills et al. 2012

(Fig. 1c). Similar reaction zones can be observed in infected *Ac. pseudoplatanus*.

Culturing of single conidia was as well successful and the morphology was similar to the cultures obtained from sterilised discoloured sapwood.

Phylogenetic analysis.

The ITS dataset consists of 869 positions, of which 431 positions were used in the phylogenetic study. The resulting phylogenetic tree shows a strong bootstrap support (99) for the five sequences of *C. corticale*, including the one isolated from *A. hippocastanum*. Phylogenetic analysis of the ITS region shows up to 100% identity between sequences of *C. corticale* isolated from *A. hippocastanum* and *Ac. pseudoplatanus* (Fig. 2).

Discussion

Both the morphology and the ITS sequence of the *A. hippocastanum* specimen show unambiguously that the organism can be assigned to the species *C. corticale*. Previous reports of *C. corticale* on *Aesculus* spp., which were not clearly documented, can now be supported with certainty. The fact that the species was isolated from the discoloured

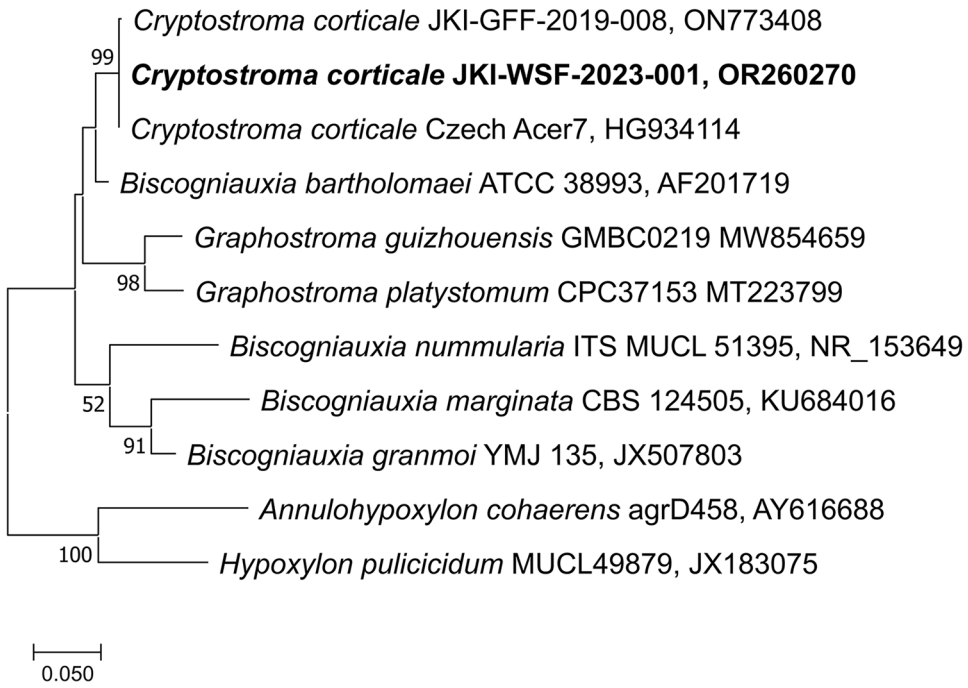

Fig. 2 Phylogeny based on ITS sequences, calculated with Maximum Likelihood method. Species names are followed by specimen or isolate numbers and accession numbers (GenBank, NCBI). All sequences of *Cryptostroma corticale* were isolated from *Acer pseudoplatanus*, despite the newly generated sequence, which is shown in bold; it was isolated from *Aesculus hippocastanum*. Only bootstrap values > 50 are shown. Scale bar indicates estimated number of substitutions per site

sapwood of *A. hippocastanum* further supports this evidence. Therefore, *A. hippocastanum* should be added to the host list of *C. corticale*.

An adjacent urban forest composed of predominantly *Acer* spp. is located approx. 25 m to the south of the here examined *A. hippocastanum* tree and has exhibited sooty bark disease (*C. corticale*) since 2017, which implies that some infection pressure was present at the site. The authors assume that *C. corticale* was involved in the tree´s loss of vitality as well as death of crown parts, cambium and bark, and thus can be regarded as a weak pathogen of *A. hippocastanum*. It can probably induce disease when the host is stressed by adverse site and climatic conditions, as is the case in *Acer* species, especially *Ac. pseudoplatanus* (Dickenson 1980; Gregory and Waller 1951; Muller et al. 2023).The affected *A. hippocastanum* tree was growing in an urban environment on compacted soil at a southern slope with enhanced exposition to solar radiation. Furthermore, in past years, it was infested by the horse chestnut leaf miner (*Cameraria ohridella* Deschka & Dimić). The leaf miner reduces annual growth and alters wood structure, but in general does not affect hydraulic efficiency to an extent that poses a lethal threat to older *A. hippocastanum* trees (Myśkow et al. 2021). Nonetheless, infestation by the leaf miner may have been a predisposing factor by reducing vitality of the here studied tree. A further important disease of urban *A. hippocastanum* is horse chestnut bleeding canker caused by the bacterium *Pseudomonas syringae* pv. *aesculi*, which can be lethal or lead to severe secondary damage by further pests and pathogens (Schmid et al. 2008; Webber et al. 2008). However, this disease was not observed in the examined tree or in its vicinity.

The fact that *C. corticale* was isolated from discoloured wood, as is also typical for sooty bark disease in *Acer* spp., can be seen as an indication of its pathogenic behaviour. However, further research, i.e. the testing of Koch's postulates, is needed to confirm the assumed pathogenic role of *C. corticale* in *A. hippocastanum.* Infection trials with controlled environmental stressors may further help understand this pathosystem and clarify the threat that *C. corticale* may pose to *A. hippocastanum* plantings under progressing climate change.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

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