

First detection of *Eremothecium coryli*, a plant pathogenic yeast transmitted by *Halyomorpha halys*, in the Czech Republic

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Abstract: We report the first detection of the plant pathogenic yeast *Eremothecium coryli* in the Czech Republic and identify the invasive brown marmorated stink bug, *Halyomorpha halys*, as a vector. *E. coryli* causes yeast spot diseases in crops such as soybeans, hazelnuts, and tomatoes, primarily in tropical and subtropical regions. The yeast was identified in the foregut of *H. halys* specimens collected in a cherry orchard near the hazelnut shrubs using PCR amplification and sequencing of the ITS and LSU gene regions. Phylogenetic analysis confirmed the identity of *E. coryli* and its association with *H. halys*, as discussed.

Keywords: ascomycetous yeast; stigmatomycosis; brown marmorated stink bug; yeast spot disease; invasive species; molecular detection; pathogen identification

Eremothecium coryli (Peglion) Kurtzman 1995 (syn. *Nematospora coryli*: Saccharomycetales: Saccharomycetaceae), an insect-transmitted ascomycetous yeast, is known as the causative agent of yeast spot diseases, often called stigmatomycosis (Kurtzman & Sybren de Hoog 2011). This yeast species infects various plants, including soybeans, hazelnuts, tomatoes, azuki beans, citrus fruits, and cotton in tropical and subtropical regions (Daugherty 1967; Kimura et al. 2008a, 2009; Hojjati et al. 2023). The genus comprises several related species (Kurtzman & Robnett 2003). These yeasts are always associated with insect damage and are transmitted by hemipteran vectors to host plants (Batra

1973; Burgess et al. 1983; Kimura et al. 2008b; Brust et al. 2018).

The yeast *E. coryli* is characterised by spindle-shaped ascospores, $72.4 \times 9.2 \mu\text{m}$ in diameter ($60\text{--}96 \times 8\text{--}12 \mu\text{m}$), with long, thin appendages, and is routinely isolated from diseased plant tissue on potato dextrose or yeast peptone agar medium (Miyao et al. 2000; Zečević et al. 2023; dos Reis et al. 2025). Although *E. coryli* is widely known as a pathogen of economically important crops, it has not been reported in the Czech Republic. In Europe, infections caused by *E. coryli* have been reported in hazelnuts in Bulgaria and Italy, and in soybeans in Serbia (Bobev et al. 2018; Scarpari et al. 2018;

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Zečević et al. 2023). Although we do not know the dispersal routes of *Eremothecium* spp. in detail, true bugs, such as *H. halys*, feeding on host plants may act as vectors for *E. coryli* (Brust & Rane 2024).

Hemipteran insects, such as stink bugs, are considered the most important vectors of plant pathogens, including viruses, bacteria, and fungi, due to their wide host range and feeding behaviour, which facilitate pathogen transmission (Mitchell 2004). The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), native to East Asia, is an invasive species that is currently established in North America and Europe (Rice et al. 2014). As a highly polyphagous species, BMSB is one of the most economically significant insect pests, causing direct damage through feeding and indirect damage as a vector of plant pathogens. It attacks numerous plant species, including fruit trees, vegetables, ornamental plants, and economically important crops (Leskey & Nielsen 2018). In Europe, BMSB was first detected in 2007 (Wermelinger et al. 2008) and was observed in the Czech Republic in 2018 (Kment & Březíková 2018).

This study originated from a survey of pathogens infecting invasive brown marmorated stink bug, *H. halys*, in the Czech Republic. The primary objective was to identify entomopathogens within the invasive BMSB. During this research, we detected the ascomycetous yeast, *E. coryli*. Although *E. coryli* is a known plant pathogen transmitted by hemipteran insects, there are no previous reports of its presence or disease caused by this pathogen in the Czech Republic. Therefore, the purpose of this work is to report the first molecular identification of *E. coryli* in the Czech Republic, detected directly from its insect vector, and analyse its relationship to other known species, raising questions about its cryptic presence in nature and the potential for future emergence as a plant disease pathogen.

MATERIAL AND METHODS

Adult living individuals of *H. halys* ($n = 4$) were collected in the vicinity of Valtrovice, South Moravia, Czech Republic, in September 2023 (48°49'21.592"N, 16°13'47.106"E). Pheromone traps with *H. halys* lures were distributed in an orchard on cherry trees. All captured living individuals were transported to the laboratory, immediately

placed in anhydrous ethanol, and stored at $-20\text{ }^{\circ}\text{C}$ until used for analysis.

Using sterilised blades, we inspected four adult specimens under a dissecting microscope. Before dissection, the legs and wings were excised. The digestive organs were extracted, and the whole gut was cut into three sections – foregut, midgut, and hindgut. Each part was transferred to a clean microcentrifuge tube containing lysis buffer (10 mM Tris-HCl, 10 mM EDTA, 10% SDS, pH = 8.0) and processed separately. DNA was extracted from each part of the alimentary canal using the phenol-chloroform method (Sambrook & Russell 2006). The isolated DNA was used as the template for the polymerase chain reaction (PCR).

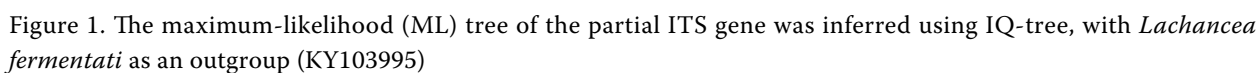
Both the partial large subunit (LSU) rDNA and the internal transcribed spacer (ITS) gene regions were amplified using the universal fungal primers LR0R/LR5 (annealing at $55\text{ }^{\circ}\text{C}$) and ITS1F/ITS4 (annealing at $60\text{ }^{\circ}\text{C}$) (Vilgalys & Hester 1990; White et al. 1990; Gardes & Bruns 1993). The PCR thermal cycling conditions were set as follows: 35 cycles of denaturation at $95\text{ }^{\circ}\text{C}$ for 30 s, annealing at the primer-specific temperature for 30 s, and elongation at $72\text{ }^{\circ}\text{C}$ for 120 s. To confirm amplification, PCR products were analysed using agarose gel electrophoresis. Successful amplicons were purified using ExoSAP-IT (Thermo Fisher Scientific, Czech Republic) according to the manufacturer's protocol. Sanger sequencing was performed by the company SEQme (Dobříš, Czech Republic).

The generated raw sequences were trimmed and manually revised using Geneious (version 8.1.9, <http://www.geneious.com/>). Then they were blasted on the NCBI website [<https://www.ncbi.nlm.nih.gov/>] (accessed February 25, 2025)] to ensure that the target amplicons were correctly amplified and to confirm the species identity of the samples. A single FASTA file was prepared from each of the two loci used to identify *E. coryli*. For each locus, a single file containing the obtained sequence and related sequences from a GenBank BLAST search was created. These combined datasets were then aligned with MAFFT (version 7) using default parameters and the 1 PAM/ $\kappa = 2$ scoring matrix for closely related sequences. The maximum-likelihood trees for each of the ITS and LSU partial sequences were constructed using IQ-tree (version 2.4.0) with best-fit substitution models (Minh et al. 2020). Subsequently, the trees were imported into FigTree (version 1.4.4, <http://tree.bio.ed.ac>

sequence data, the yeast was identified as *E. coryli* (Figure 1). This high sequence variability in the ITS locus (97–99%) indicates either significant intra-specific variation or the existence of a cryptic species complex. Further research is needed to confirm this hypothesis.

Similarly, the LSU gene region of the obtained sequence (PV495912) showed sequence identity from 99.44% (MH872931) to 99.77% (KY107675) with *E. coryli*, and less than 97.50% with other *Ermothecium* (Figure 2).

To our knowledge, this is the first report of *E. coryli* in the Czech Republic, though it was detected inside a vector insect. *E. coryli* is commonly isolated from lesions on bean seeds or tomato fruits, which have been damaged by true bugs (Pentatomidae) (Kimura et al. 2008a; Brust & Rane 2024). Although BMSB specimens were collected using pheromone traps placed in a cherry orchard, *E. coryli* has never been reported to infect cherry fruits or trees. In Europe, *E. coryli* has been reported to infect ha-



The bootstrap support values (1 000 replicates) and posterior probabilities of BI are shown above the branches. Branch lengths are proportional to distance. The isolate in bold type is the one obtained in this study

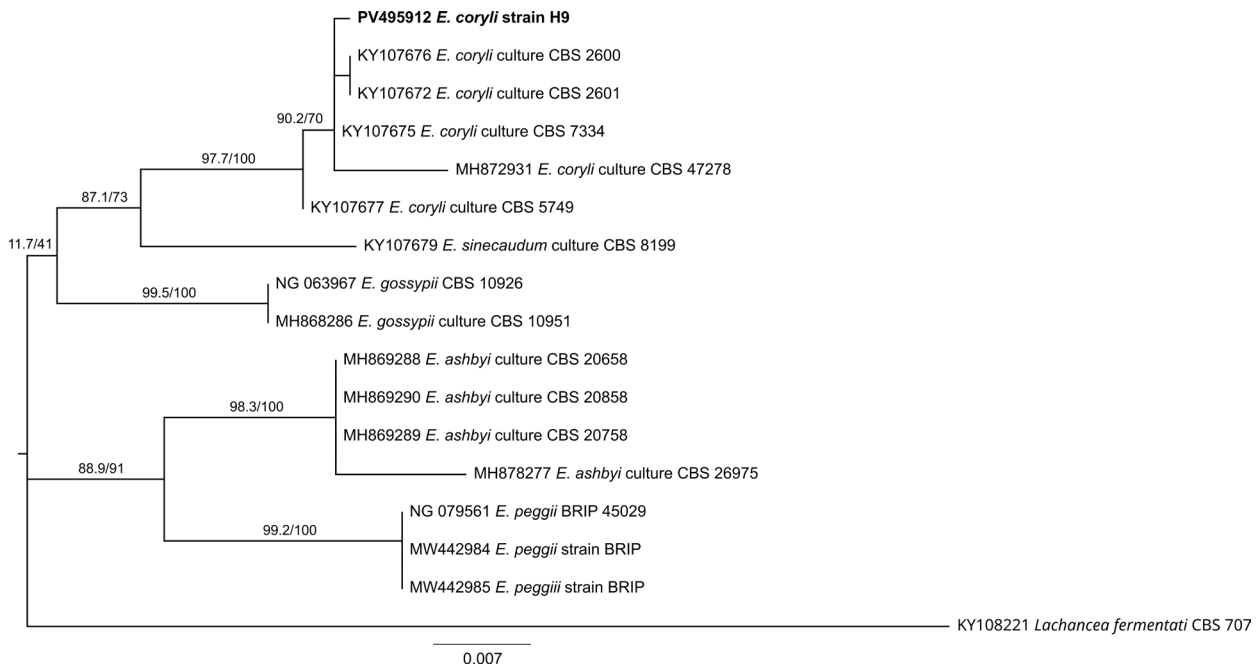


Figure 2. The maximum-likelihood (ML) tree of the partial LSU gene was inferred using IQ-tree, with *Lachancea fermentati* as the outgroup (KY108221)

The bootstrap support values (1 000 replicates) and posterior probabilities of BI are shown above the branches. Branch lengths are proportional to distance. The isolate in bold type is the one obtained in this study

zelnuts, with cases reported in Bulgaria and Italy (Bobev et al. 2018; Scarpari et al. 2018). Particularly, in this case, hazelnut shrubs and trees were growing near the cherry orchard. It is therefore likely that the *E. coryli* – infected specimen was attracted to the trap lure from the closely growing hazelnut shrubs.

The BMSB is one of the insect species that may transmit pathogenic bacteria and yeast, such as *E. coryli*, on the surface of the feeding stylets and may spread the fungal pathogen to new host plants (Rice et al. 2014; Brust & Rane 2024). Similar behaviour was also previously described in *Nezara viridula* L., in the transmission of yeast spot disease in soybean, and in the spread of *E. coryli* and *E. ashbyi* in azuki bean by *Riptortus pedestris* in Japan (Daugherty 1967; Kimura et al. 2009; Zečević et al. 2023).

Although mycelia of *E. coryli* have previously been found in stylet pouches and stylets, several reports have also detected *E. coryli* in the gut of various insect species. For example, the yeast of *Eremothecium* was found in the microbiome of termites and in a single specimen of unspecified Hemiptera (Handel 2017; Valentini et al. 2024). *E. coryli* was detected internally in *Lygus* bugs in the mustard

field in Canada (Burgess et al. 1983). It is widely accepted that yeasts of the genus *Eremothecium* are transmitted mechanically on the surfaces of feeding stylets; however, their repeated detection within internal organs suggests that an alternative route of transmission may exist.

One of the major reasons for the early and timely diagnosis of invasive fungi is the increased number of plant diseases, which cause both ecological and economic damage (Kenis et al. 2008). The growing number of non-native plant pathogens and their vectors increases the risk of chemical resistance, ecosystem disruption, and economic losses in fruit, vegetable, and crop production (Sosa-Gómez et al. 2019; Desper-Loustau et al. 2007; Scaccini et al. 2024). The growing demand for early and accurate diagnosis of fungal pathogens highlights the need to implement molecular methods as fast, reliable techniques for detecting non-native pathogens (Fang et al. 2023).

Stigmatomycosis is one of the important fungal diseases associated with Heteroptera (Mitchell 2004). The infection starts with direct feeding damage to seeds, fruits, or grains, and insect feeding lesions serve as entry points for the fungal pathogen. Seventeen pentatomid species, including *Nezara*

viridula and *H. halys*, are associated with this disease (Mitchell 2004; Brust & Rane 2024). The presence and recent population increase of both *N. viridula* and *H. halys*, the main vectors of *E. coryli*, in the Czech Republic, indicate that the introduction of this fungal pathogen is now more likely and could lead to severe yield losses and significant economic impacts on the production of fruits, vegetables, and soybeans. Further work is needed to obtain a pure culture of *E. coryli* to confirm our results using molecular, cultural, and morphological techniques.

CONCLUSION

We used PCR and Sanger sequencing of the LSU and ITS gene regions to detect and identify fungal plant pathogens. We identified *Eremothecium coryli* within its insect vector, *Halyomorpha halys* (BMSB), representing the first record of this pathogen in the Czech Republic. Given the lack of prior records, our results indicate that the invasion of *H. halys* likely facilitates its introduction.

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