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Symbiotic relationship between *Cerrena unicolor* and the horntail *Tremex fuscicornis* recorded in the Czech Republic

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From a specimen of *Acer saccharinus* collected in a Prague park, 38 females of *Tremex fuscicornis* (Hymenoptera, *Siricidae*) were reared and sixteen isolates of a symbiotic basidiomycete were isolated from their mycangia. All isolates shared morphology and RAPD patterns. The fungus was identified using rDNA (regions ITS1, 5.8S, ITS2, and D1D2 part of the 28S rDNA) as *Cerrena unicolor* (Basidiomycota: *Polyporales*). The identification is discussed with respect to related horntail taxa and former identification attempts.

Key words: *Tremex fuscicornis*, *Cerrena unicolor*, *Tremicinae*, insect–fungus symbiosis

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Ze vzorku napadeného dřeva odebraného z javoru *Acer saccharinum* rostoucího v pražském parku bylo vychováno 38 samic pilořitky *Tremex fuscicornis* (Hymenoptera, *Siricidae*). Z jejich mykangií bylo získáno 16 kultur symbiotické houby ze skupiny Basidiomycota, které se shodovaly morfologicky i v analýze RAPD. Houba byla určena na základě sekvence rDNA (oblasti ITS1, 5.8S, ITS2 a úsek D1D2 z 28S) jako *Cerrena unicolor* (Basidiomycota: *Polyporales*).

INTRODUCTION

Female wood wasps of the genus *Tremex* (horntail) carry symbiotic basidiomycetes in their mycangia (intersegmental pouches at the base of ovipositor) in the form of single cells and mycelial fragments (Francke-Grosmann 1939). During oviposition in the cambium layer, oidia are inoculated into the wood together with the egg and phytotoxic mucus.

The fungus then colonizes the wood; the larvae tunnel through the decaying wood, ingesting both wood and the hyphae of the fungus (Francke-Grosmann 1939, Eichhorn 1982). The symbiont is essential for further larval development; eggs of *Tremex columba* (L.) originating from females raised without the symbiotic fungus hatch, but larvae cannot develop beyond the first instar (Stillwell 1967).

In *T. columba*, a North American species, its symbiotic fungus was identified as *Cerrena unicolor* (Bull.) Murrill using the dikaryotization technique (Stillwell 1964, 1965). The same fungus was identified in mycangia of *Tremex longicollis* (Konow) collected in Japan (Tabata and Abe 1995). In this case, basidiocarps of *C. unicolor* were found near the emergence hole of the horntail and mating between single basidiospore mycelia of *C. unicolor* and single-arthrospore mycelia from the mycangia of the horntail showed that they were compatible.

The distribution of *Tremex fuscicornis* (Fabricius) is Eurasian. In Europe, especially in northern regions, *Tremex fuscicornis* is rather rare (Pisarski 1956, Eichhorn 1982, Taeger et al. 1998, Witmond 1999, Bergsten and Hedström 2004). *T. fuscicornis* was recently introduced to Chile probably with wooden crates from China infested with horntail larvae and pupae. Its spreading led to disastrous results (Baldini 2002) as there has been no natural predator of the invader.

In the Czech Republic, this horntail is also not often encountered, although its occurrence was recorded in the 1940's and 1950's (Gregor and Baťa 1940, Bouček and Pádr 1957). Records of rare specimens do not allow for basic bionomical studies – the only exception is the finding of *Ibalia jakowlewi* parasitizing *T. fuscicornis* (Pfeffer 1983). Recently, occasional findings were made in the course of faunistic searches using effective Malaise traps (Dr. Jan Macek, National Museum Prague, personal communication).

The first attempt at identification of a symbiont of *T. fuscicorni*s was made by Francke-Grosmann (1939). She isolated fungi from *T. fuscicornis* infested specimens of *Populus* sp. (poplar) and *Juglans regia* (walnut), collected in Germany. The "poplar fungus" was isolated from mycangia, whereas the "walnut fungus" was obtained only from galleries, as no females emerged from the wood specimen. The cultures differed in their mycelial morphology. An attempt to obtain basidiocarps on inoculated sterilized wood sticks in a wet chamber succeeded only with the "walnut fungus", which was then identified as *Polyporus imberbis* (now *Bjerkandera fumosa*) and later mentioned as a putative *T. fuscicornis* symbiont in the literature. The other fungus produced no fructification and therefore remained unidentified and unmentioned.

Palma et al. (2005) succeeded in producing basidiocarps of the symbiotic fungus from the invading *T. fuscicornis* on sterilized pieces of poplar wood. The cultivated fruit bodies of the symbiont were identified as those of *C. unicolor*.

Regarding the relationship between siricid species and their fungal symbionts it was shown that a specific species of *Sirex* or *Urocerus* always carried the same species of *Amylostereum*; however, one fungal species can be carried by several wasp species (Gaut 1970; Tabata and Abe 1997, 1999). On the other hand, xylariaceous symbionts of *Xiphydria* woodwasps belong to two albeit related genera and females of *Xiphydria longicollis* (even those emerging from the same tree) can carry either of the fungi (Šrůtka et al. 2007).

The data collected so far suggest that *T. fuscicornis* may share the *C. unicolor* symbiont with *T. columba* and *T. longicollis* outside Europe. The equivocal findings of Francke-Grosmann from Germany, however, raises questions about this hypothesis. The aim of this study was therefore to identify the symbiont in specimens of *T. fuscicornis* of European origin.

MATERIALS AND METHODS

Isolation and cultivation

Adults of *T. fuscicornis* have been reared (Šrůtka et al. 2007) from a part of a trunk of *Acer saccharinus* collected in Prague (50°6'2.40"N 14°23'42.53"E). From the incubated wood specimen (28 × 100 cm), thirty-eight females (Fig. 2) emerged but not a single male, probably due to the differences in the duration of male and female larval development. The insects were killed by an injection of ethylacetate into the thorax and dissected. The content of the mycangia was streaked with a sterile needle onto 2 % malt extract agar (MEA) (Difco, Detroit, USA) and the resulting 16 cultures maintained on MEA slants at 4 °C.

DNA analysis

DNA was isolated from young cultures grown on cellophane laid on MEA agar plates using an UltraClean Microbial DNA Isolation Kit (Mo-Bio Laboratories, California) according to the manufacturer's manual. Nuclear rDNA containing the internal transcribed spacers (ITS1 and ITS2), 5.8S rDNA, and the D1D2 region of 28S rDNA was amplified with primers ITS5 and NL4 (White et al. 1990) in a Mastercycler Gradient (Eppendorf, Germany) as follows: 1 cycle of 3 min at 95 °C, 30 s at 55 °C and 1 min at 72 °C, 30 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C and 1 cycle 30 s at 95 °C, 30 s at 55 °C and 10 min at 72 °C. The reaction mix consisted of PCR buffer (Finnzymes, Finland), deoxynucleotide mixture 0.2 mM, 2 pmol of each primer, 1 U of DynaZyme (Finnzymes, Finland) and 5–50 ng of DNA in 25 µl of total volume. Amplified fragments were purified and custom-sequenced in Macrogen (South Korea). The sequence was then deposited in GenBank under the accession No. EF577058 and compared to NCBI database sequences using BLAST (Altschul et al. 1990). RAPD patterns were obtained using primer 8F (5´-GCTCTGAGATTGTTCCGGCT) (Pažoutová and Frederickson 2005). RAPD patterns were obtained using primers 5R (TTTGTCCGGCTCAGAAAC), 8F (GCTCTGAGATTGTTCCGGCT), OPA-09 (GGGTAACGCC) and OPA-20 (GTTGCGATCC), both from Operon Technologies, CA. The reaction mixture contained in 20 µl: DynaZyme reaction buffer, 0.2 mM deoxynucleotides, 2 pmol of the primer, 1 U of DynaZyme, $MgCl_2$ in a total concentration of 1.75 mM, and 5–50 ng of DNA. The cycling was as follows: 33 cycles of 93 °C for 20 s, 38 °C for 1 min

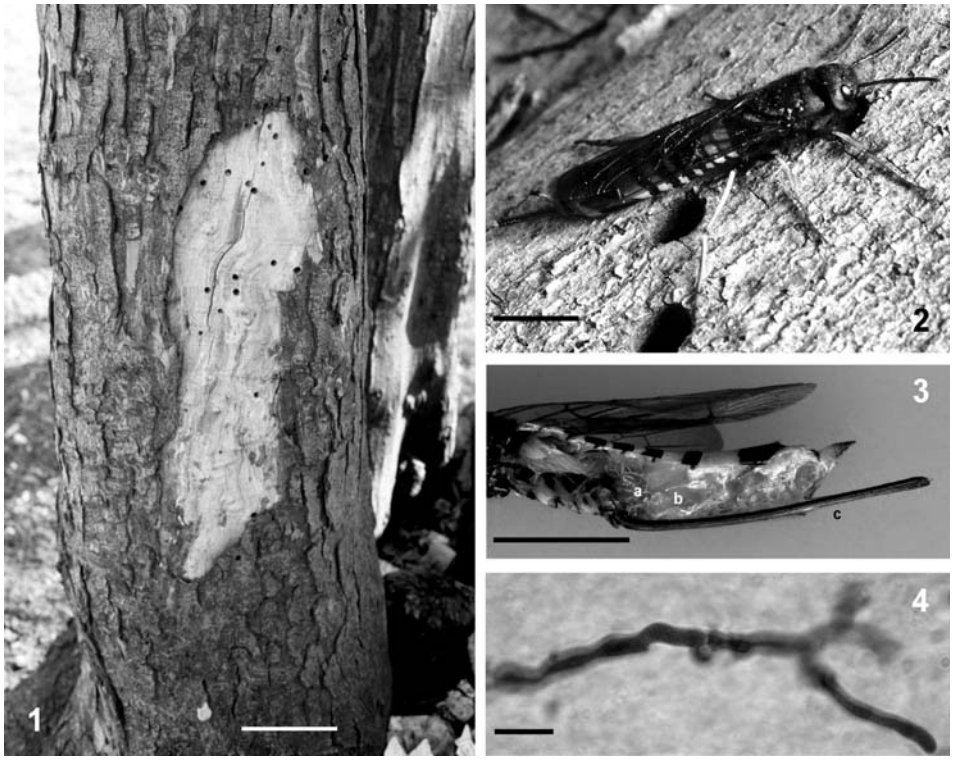

Fig. 1. Silver maple tree with emergence holes. Bar – 10 cm.
Fig. 2. Female of *Tremex fuscicornis* on silver maple. Bar – 1 cm.
Fig. 3. Dissected female of *Tremex fuscicornis*: a – mycangium, b – mucus reservoir, c – ovipositor. Bar – 1 cm.
Fig. 4. Oidium of *Cerrena unicolor* with a clamp. Bar – 10 µm.

72 °C for 20 s with an initial denaturation step at 93 °C for 3 min and the last elongation step at 72 °C for 6 min.

Microscopy

Samples were mounted in 1 % cotton blue in lactophenol and observed using a JENAVAL microscope (Zeiss, Germany) and a Nikon SMZ-1B Stereo Zoom Microscope. Photographs were taken using a Camedia C-5060 WZ camera (Olympus) and image-processing software QuickPHOTO Camera 2.2.

RESULTS AND DISCUSSION

The present study was initiated after discovering a heavily infested trunk of a silver maple tree (*Acer saccharinum*) in a busy urban park in Victory Place,

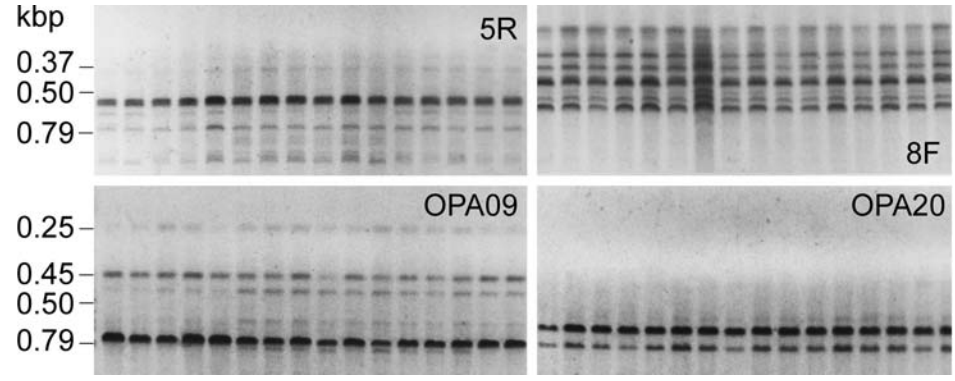

Fig. 5. RAPD patterns of 16 isolates obtained with four primers.

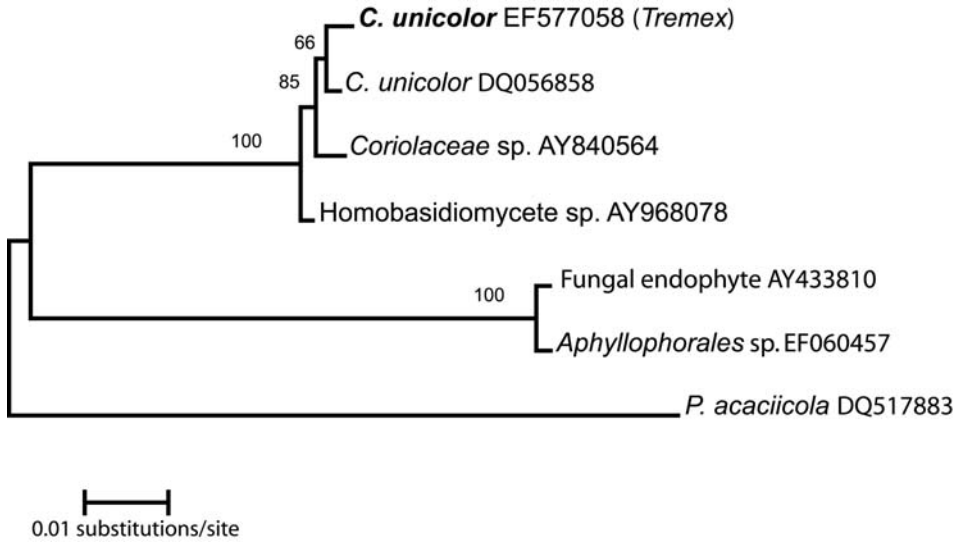

Fig. 6. Phylogram of *Cerrena unicolor* from *Tremex fuscicornis* and related fungi. The alignment consisted of 647 sites (54 parsimony-informative and 45 singletons). The phylogram was obtained using Kimura 2-parameter distance model (with pairwise deletion option and 1000x bootstrap) and the neighbour-joining tree constructing method as implemented in MEGA 3.1 software (Kumar et al. 2004). The bootstrap support values are given on the branches.

Prague (50°6'2.40"N, 14°23'42.53"E). The unusually high occurrence of the rather rare horntail in a city park might be explained by the absence of its natural predators – parasitic wasps, as the park is isolated from other green areas by surrounding high houses. Conspicuous and numerous emergence holes (3–7 mm in diameter) about 0.5-1 m from the trunk base were observed (Fig. 1, Fig. 2).

In the dissected female, paired mycangia were found directly below the subgenital plate (Fig. 3), immediately at the ovipositor entry. A reservoir contained clear colorless mucus. Inside the mycangia, mucus-suspended small hyphal fragments (oidia) bearing clamps were found (Fig. 4).

Pure cultures on MEA were all of identical appearance. The colony consisted of rich white cottony aerial mycelium. The reverse of the colonies was colourless; no pigments were produced even after 1 month of cultivation. During maximum growth the growth rate was 10-13 mm per day.

RAPD patterns of all isolates were identical with all four primers used (Fig. 5), confirming thus that the isolates belonged to the same fungal species. The identical patterns suggested that the isolates may even belong to a clonal population.

As the morphology was uninformative, rDNA sequence comparison was used for the identification of a *Tremex* symbiont. The 28S rDNA region (607 bp) was 98.3 % identical to *C. consors* AY515343 and 97.6 % identical to that of *Cerrena consors* AY515338 (Kim et al. 2005).

A database search of sequences of ITS1-5.8S-ITS2 region found the highest similarity to sequence of *Cerrena unicolor* (DQ056858) (Janusz and Rogalski, unpublished) with only a single base difference. The sequence originated from an isolate deposited at Botanical Institute II, University of Regensburg (Germany) as No. T143, and in Fungal Culture Collection of the Department of Biochemistry UMCS in Lublin (Poland) as No. 139 (G. Janusz, personal communication). Two unidentified white rot fungi (AY968078, AY840564) (Zhang et al. 2006) were also closely related. The phylogram (Fig. 6) has shown that these four fungi clustering together either all represent *C. unicolor*, or a complex of sister species.

In the distance tree generated by NCBI Blast from database sequences, the *C. unicolor* clade was further related to a clade containing several sequences of unidentified Aphyllophorales from *Theobroma* (here represented by EF060457) and of two endophytes from *Taxus mairei* (represented by AY433810, Wang and Wang, unpublished). A phytopathogenic fungus *Pseudolagarobasidium acaciicola* from wattle (Wood and Ginns 2006) was in the NCBI Blast distance tree on ancestral position to the above clades and was added as an outgroup. Database sequences of *Bjerkandera adusta* (DQ060097, AY319191) and *Bjerkandera fumosa* (AJ006673) were in the regions of ITS1 and ITS2 hardly alignable with that of the symbiotic fungus.

From the two fungi that Francke-Grosmann (1939) isolated, the colony morphology of the "poplar fungus" resembled that of our isolates of *C. unicolor*, especially in the relatively quick growth, lack of pigments in agar and rich white aerial mycelium formation. Francke-Grosmann noted a morel-like smell of the cultures, whereas we did not find any.

C. unicolor is confirmed by the above results and the observations of Palma et al. (2005) as a common symbiont of *T. fuscicornis* across its distribution area, shared also by *T. colomba*, and *T. longicollis*.

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