

## The nematophagous hyphomycete *Esteya vermicola* found in the Czech Republic

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During a study of surface mycoflora of the bark beetle *Scolytus intricatus* (Coleoptera: Scolytidae), the hyphomycetous microfungus *Esteya vermicola* was found associated with *Bursaphelenchus eremus* (Nematoda: Aphelenchoididae). *Esteya vermicola* and *Bursaphelenchus eremus* were recorded in the Czech Republic for the first time. The find of *E. vermicola* is considered to be the second one in the world. *Esteya vermicola* is a nematophagous species with two types of conidia, described in 1999 from the pinewood nematode *Bursaphelenchus xylophilus* in Taiwan. Our new records are from the surface of larvae and adult beetles of *Scolytus intricatus* and their galleries under bark of branches of three species of oak (*Quercus petraea*, *Q. polycarpa*, and *Q. robur*) on several localities in the Polabí and Křivoklátsko regions, Czech Republic. A description of morphological features and drawings of the fungus are given. Examined strains are maintained in the Culture Collection of Fungi (CCF), Faculty of Science, Charles University, Prague and Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno.

**Key words:** conidial fungi, *Esteya vermicola*, *Bursaphelenchus eremus*, *Scolytus intricatus*, nematodes, bark beetles

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Během studia povrchové mykoflóry bělokaza dubového (*Scolytus intricatus*, Coleoptera: Scolytidae) byl zjištěn výskyt mikroskopické vláknité houby *Esteya vermicola* ve spojení s háďátkem *Bursaphelenchus eremus* (Nematoda: Aphelenchoididae). *Esteya vermicola* i *Bursaphelenchus eremus* byli v České republice zaznamenáni poprvé. Nález *E. vermicola* je považován za druhý nález na světě. *E. vermicola* patří mezi nematofágní houby, tvoří dva typy konidií a byla poprvé popsána roku 1999 jako parazit háďátka *Bursaphelenchus xylophilus*, škůdce borovic na Taiwanu. Naše nálezy pocházejí z povrchu larev a dospělců bělokaza dubového (*S. intricatus*) a jejich chodbiček pod borkou větví tří druhů dubu (*Quercus petraea*, *Q. polycarpa*, *Q. robur*) na několika lokalitách v Polabí a na Křivoklátsku. V článku je uveden popis morfologických znaků, perokresba a mikrofotografie. Vybrané kmeny jsou uloženy ve Sběrce kultur hub (CCF) na katedře botaniky přírodovědecké fakulty UK v Praze a v České sbírce mikroorganismů (CCM) přírodovědecké fakulty MU v Brně.

## INTRODUCTION

*Esteya vermicola* J. Y. Liou, J. Y. Shih and Tzean is a nematophagous endoparasitic fungus recently described by Liou et al. (1999) from Taiwan. They found it attacking the pinewood nematode *Bursaphelenchus xylophilus* which causes the wilting disease of pine trees (e.g. *Pinus densiflora*, *P. thunbergii*, *P. luchuensis*) in eastern Asia. It is noteworthy that the life cycle of this nematode is associated with beetles. After Mamiya (1983), the dauerlarvae of the pinewood nematode enter tracheas of the just-pupated longhorn beetle *Monochamus alternatus*. Adults of the beetle fly from the dead trees to healthy ones for post-maturation feeding. On pine twigs, the nematodes leave the beetles and enter twig tissue through wounds caused by the beetles. Nematode feeding leads to the destruction of epithelial and parenchyma cells and infected trees die within 2-3 months. Liou et al. (1999) revealed the strong parasitic potential of *Esteya vermicola*. Infection by adhesive lunate conidia led to the killing of almost 100 % of the nematode population within 8-10 days.

There is wide range of relationships between bark beetles and nematodes, from simple phoresis to facultative or obligate parasitism. In the Czech Republic, the oak bark beetle *Scolytus intricatus* damages different species of oak (e.g. *Quercus robur*, *Q. petraea*, *Q. polycarpa*) and contribute to their dying. From 1997 to 1999, the surface mycoflora of *Scolytus intricatus* and its galleries was studied. The results of this study will be published elsewhere. During the study, the rare hyphomycete *Esteya vermicola* was found in connection with *Scolytus intricatus* (Coleoptera: Scolytidae) and its phoretic nematode *Bursaphelenchus cremus* Rühm (Nematoda: Aphelenchoididae). Both fungus and nematode were yet unknown from the Czech Republic. For the fungus it seems to be the first record in Europe. Therefore, a description of the fungus and notes on the significance of these species are given.

## MATERIAL AND METHODS

## Study sites

Oak branches (*Quercus petraea*, *Q. robur* and *Q. polycarpa*) infested with *Scolytus intricatus* were collected on four localities in central Bohemia, Czech Republic: Mlynářův luh and Kohoutov in the Křivoklátsko region, Libický luh and Bačov in the Polabí region. All localities are characterised by deciduous forest with prevailing oaks.

## Methods

The micromycete was isolated using two methods. (1) Larvae and mature beetles were excised from bark and separately washed with sterile water with

a Tween 80 in sonicator. Washed beetles, 1 ml of the suspension and detritus from galleries were inoculated each on a separate Petri dish with 1.5 % malt extract agar (MA1.5) and streptomycin (0.1 g/l). Most *Esteya* isolates were detected by this method. (2) Pieces of branches infested by *Scolytus* were superficially sterilised (96 % ethanol: 1 min., sodium hypochlorite: 3 min., 96 % ethanol: 30 s), divided into bark and wood, and inoculated onto Petri dishes with 2 % malt extract agar (MA2). After several days of incubation at 25 °C the fungi were isolated. *Esteya* strains were identified according to Liou et al. (1999). For identification, potato-glucose agar (PGA), potato-carrot agar (PCA), 2 % malt extract agar (MA2) and incubation in the dark at 25 °C were used. In addition, incubation at different conditions (room temperature: daylight/dark or UV light/dark) were tried. Representative strains were freeze-dried in skimmed milk and are deposited in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague and in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno.

Photographs were taken on Olympus BX-50 microscope with a DIC.

## RESULTS AND DISCUSSION

### Description of *Esteya vermicola* on the basis of our isolates

#### Representative strains:

- CCF 3115: ex dead female of *Scolytus intricatus* under bark of branch of *Quercus petraea*, Mlynářův luh, Křivoklátsko region, Czech Republic, isol. A. Kubátová as No. 116/97, VIII. 1997
- CCF 3116: ex male of *Scolytus intricatus* before emergence from *Quercus polycarpa*, Libický luh, Polabí region, Czech Republic, isol. A. Kubátová as No. 50/98, V. 1998
- CCF 3117: ex gallery of female of *Scolytus intricatus* before emergence from *Quercus polycarpa*, Libický luh, Polabí region, Czech Republic, isol. A. Kubátová as No. 51/98, V. 1998
- CCF 3118: ex gallery of larva of *Scolytus intricatus* before wintering in *Quercus robur*, Bačov, Polabí region, Czech Republic, isol. A. Kubátová as No. 218/98, XI. 1998
- CCF 3131: ex male of *Scolytus intricatus* before emergence from *Quercus petraea*, Kohoutov, Křivoklátsko region, Czech Republic, isol. A. Kubátová as No. 67/97, IV. 1997
- CCF 3132: ex larva of *Scolytus intricatus* after wintering in *Quercus polycarpa*, Libický luh, Polabí region, Czech Republic, isol. K. Prášil as No. 17A/98, V. 1998
- CCM 8247: ex male of *Scolytus intricatus* after emergence from *Quercus robur*, Bačov, Polabí region, Czech Republic, isol. D. Novotný as No. B12D12, VII. 1999
- CCM 8251: ex bark of twig of *Quercus robur* with gallery of *Scolytus intricatus*, Bačov, Polabí region, Czech Republic, isol. D. Novotný as No. B3K2, VII. 1999

#### Macroscopic features

PGA, 7 days, 25 °C, in the dark: colonies 24–30 mm diam., greyish green, reverse dark greyish to olive green. Colonies after 10 days 39–48 mm diam. and after 14 days 65–71 mm diam.

PCA, 7 days, 25 °C, in the dark: colonies 20–30 mm diam., grey, reverse dark grey. Colonies after 10 days 40–50 mm diam. and after 14 days 62–72 mm diam.

MA2, 7 days, 25 °C, in the dark: colonies 26–32 mm diam., dark olive green, reverse dark olive green. Colonies after 10 days 41–46 mm diam. and after 14 days 60–68 mm diam.

MA2, 7 days, 30 °C, in the dark: colonies 7–12 mm diam. Colonies after 10 days 10–21 mm diam. and after 14 days 18–36 mm diam.

Growth at 37 °C is nil.

### Microscopic features (Figs. 1 and 2)

Hyphae subhyaline, grey green to olive green, smooth to roughened, sometimes with slime sheath. Conidiophores, conidiogenous cells and conidia of two types, often growing on the same hypha.

Conidiogenous cells of the first type sessile, smooth to roughened, with olive green inflated base ca 3.1–5.4  $\mu\text{m}$  wide, abruptly tapering into a thin subhyaline neck of varying length, ca 1.5  $\mu\text{m}$  wide, often crooked, sometimes percurrent. Conidia one-celled, lunate, subhyaline, smooth-walled, 9.3–12.4  $\times$  3–3.2  $\mu\text{m}$ . They are formed very often on hyphae submerged in agar, too. Conidiophores of the second type loosely branched or simple, olive green, often roughened. conidiogenous cells about 30–40  $\mu\text{m}$  long, straight, mostly with swollen base (3–4.7  $\mu\text{m}$ ). Conidia cylindrical, one-celled, hyaline, smooth, 4.8–6.2  $\times$  1.3–1.5 (–2)  $\mu\text{m}$ .

The ability and mode of conidia germination were examined on MA2. Both types of conidia germinated the second day after inoculation of a water suspension. The lunate conidia formed one or more germ tubes in the centre of a concave side. The cylindrical conidia formed the germ tube at the end (Fig. 3).

Some details on a conidiogenesis are given by Liou et al. (1999): reportedly phialidic in the first type and phialidic enteroblastic in the second type. The unusual way of development, especially the forming of lunate conidia, needs further study using TEM.

The microscopic observations of our strains fit well with the description given by Liou et al. (1999). However, Liou et al. reported that on enriched media, cylindrical conidia were formed first. Our strains produced both types of conidia on nutrient media very early, mostly the third day of cultivation. Sporulation under different light conditions (daylight/dark, UV light/dark) seems to be nearly the same.

The teleomorph is not known and was not observed in this study. In strain CCF 3116, dark multicellular bodies of irregular shape were observed after 2 month of cultivation on malt extract agar (see Fig. 2 k).

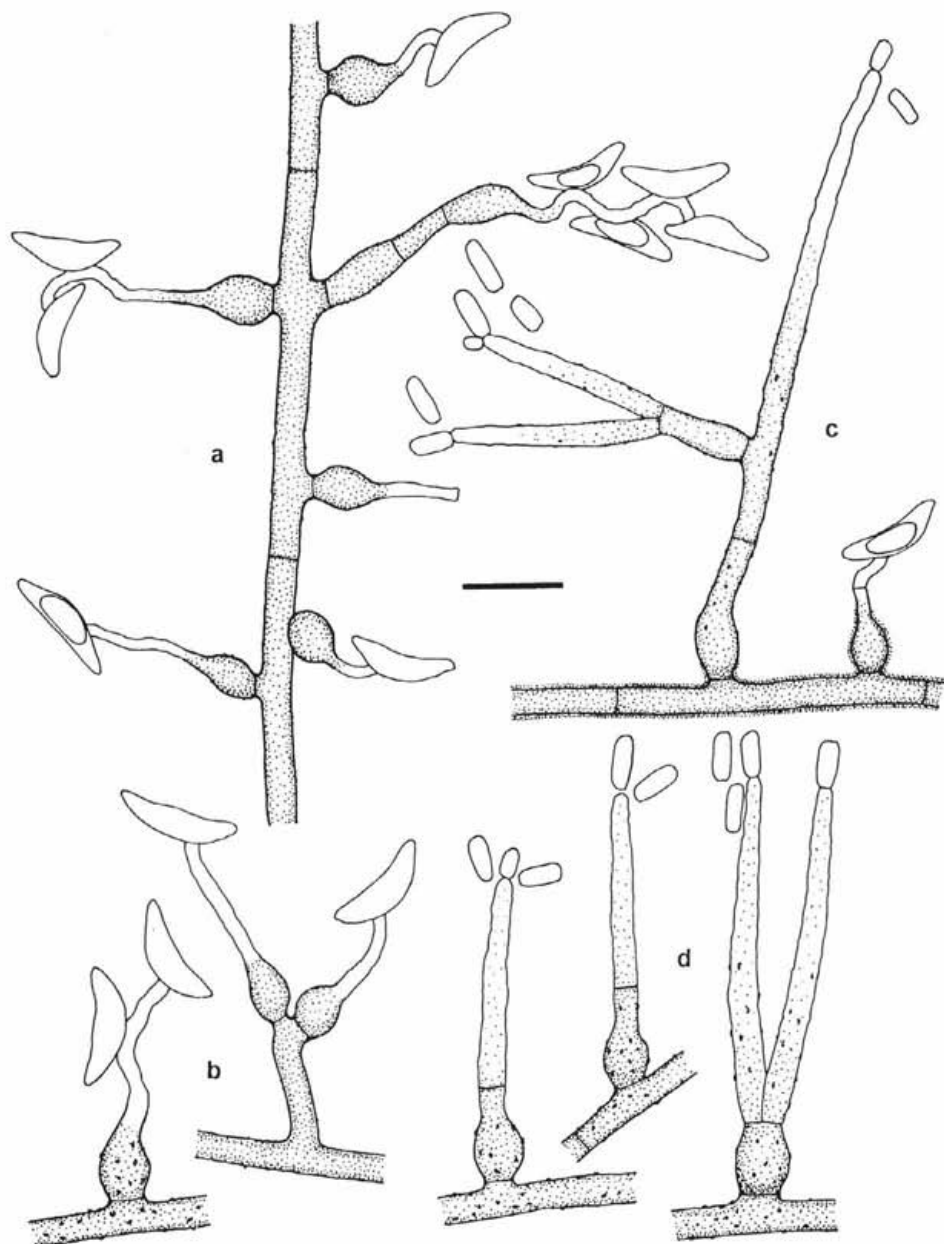
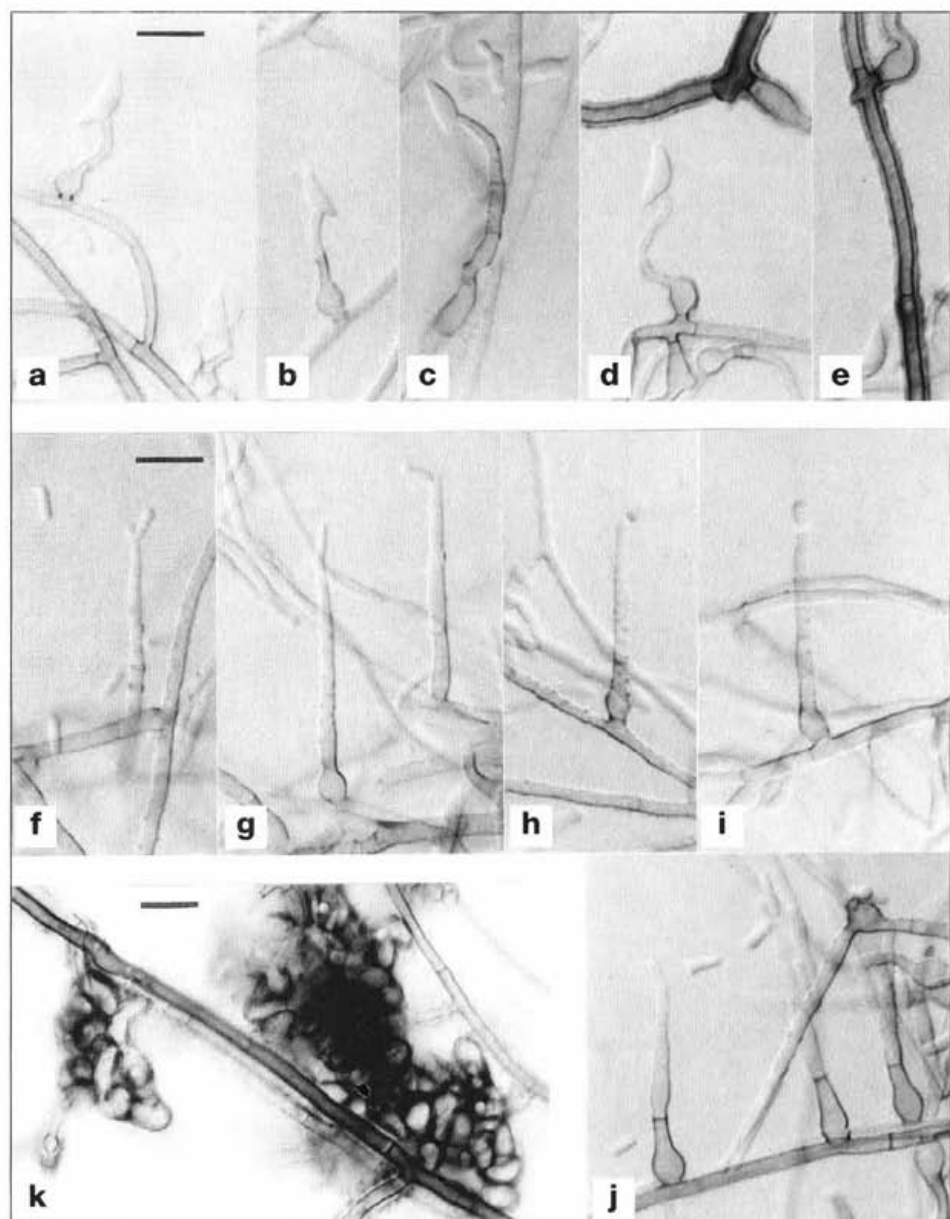


Fig. 1. *Esteya vermicola*. a, b: conidiophores with lunate conidia, c: hypha with two types of conidiophores and conidia, d: conidiophores with cylindrical conidia. Bar = 10  $\mu$ m.

A. Kubátová and K. Prášil del.



**Fig. 2.** *Esteya vermicola*. a - d: conidiogenous cells with lunate conidia, e: dark hypha with sheath, f - j: conidiogenous cells with cylindrical conidia, k: dark multicellular bodies. Bars = 10  $\mu$ m. Interference contrast.

Photo A. Kubátová

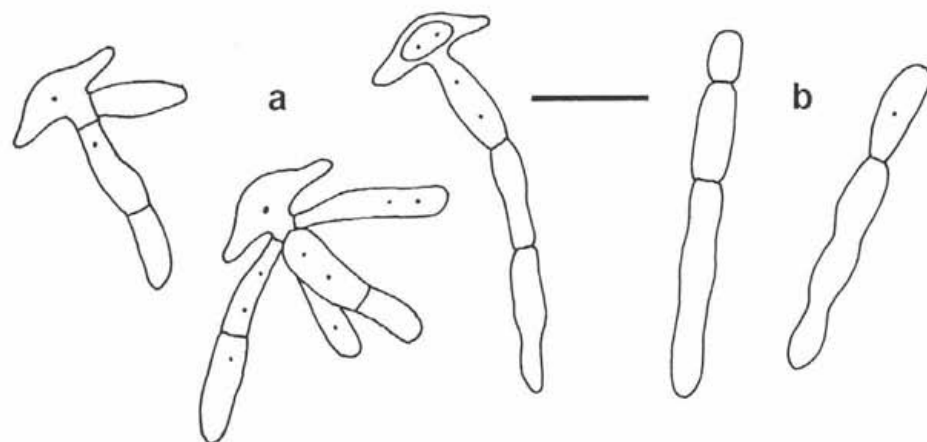


Fig. 3. *Esteya vermicola*, the strain CCF 3116. a: germinating lunate conidia, b: germinating cylindrical conidia. Bar = 10  $\mu$ m.

A. Kubátová del.

#### Occurrence and significance for forest pathology

Strains of *Esteya vermicola* were isolated from the surface of larvae and adult beetles of *Scolytus intricatus* and their galleries under bark of three species of oak (*Quercus petraea*, *Q. polycarpa*, and *Q. robur*) on four studied localities in the Polabí and Křivoklátsko regions, Czech Republic. The total number of finds reached 24 but not all records were isolated in pure culture. Most records of *Esteya vermicola* were from young beetles before emergence and their galleries on *Quercus polycarpa* at Libický luh, Polabí region. *Esteya* was observed on this locality associated with nine beetle bodies from a total of 25 beetles studied (see Table 1). It is obvious that *Esteya* was found in different stages of the life cycle of *Scolytus intricatus*, however predominantly on adults.

Interestingly, free-living nematodes – males, females, and larvae – were observed on several pieces of oak branches infested or non-infested with *Scolytus* and incubated in moist chambers as well as on some Petri dishes during isolation of micromycetes from *Scolytus intricatus*. The nematodes had a stylet, characteristic indistinct bursa and spicules with a prominent rostrum. It was identified as *Bursaphelenchus eremus* (Rühm 1956, Yin et al. 1988). The fourth larval stage and females of the nematode are known as a phoretic living under elytra or in intersegmental folds, while the facultative entomoparasitism of this nematode species was not proved. Young nematode larvae, males and egg laying females live in a frass of bark beetle galleries feeding on fungal hyphae. *B. eremus* may play a certain role as vector of micromycetes similarly as *Scolytus intricatus*. This is the first record of *Bursaphelenchus eremus* from the Czech Republic.



Table 1. List of records of *Esteya vermicola* associated with *Scolytus intricatus*

region	locality	species of oak	number of records of <i>Esteya vermicola</i> associated with <i>Scolytus intricatus</i>
Křivoklátsko	Kohoutov	<i>Quercus petraea</i>	• on 1 male before emergence (of 20 adults total)
	Mlynářův luh	<i>Q. petraea</i>	• on 1 male and 2 dead females (of 100 adults total) • on 1 larva (of 25 larvae total)
Polabí	Libický luh	<i>Q. polycarpa</i>	• on 6 males and 3 females before emergence and in their galleries (of 25 adults total)
	Bačov	<i>Q. robur</i>	• in galleries of 2 larvae before wintering (of 60 larvae total) • on 4 females in maturation feeding and on 1 female (of 30 adults total) • in 3 samples of bark from oak branch with beetle galleries (1 branch of 3 total)

Liou et al. (1999) recorded a strong killing potential of *Esteya vermicola* for the nematode *Bursaphelenchus xylophilus*. Its lunated conidia adhere to the nematode and penetrate the cuticle and muscle layer. Infection experiments with *Esteya vermicola* and *Bursaphelenchus eremus* were not carried out in our study. However, during incubation of a mixture of micromycetes from the surface of *Scolytus intricatus* on Petri dishes, coiled cadavers of nematodes overgrown by *Esteya vermicola* were observed.

In Fig. 4 a scheme is presented to demonstrate relations of the micromycete *E. vermicola* to nematode *Bursaphelenchus eremus* and bark beetle *Scolytus intricatus*, all organisms living in branches of *Quercus* spp.

Concluding, the study resulted in new data on the ecology of *Esteya vermicola*, a so far little known hyphomycete. It is evident that this fungus is not restricted to the subtropical climate of Taiwan, the nematode *Bursaphelenchus xylophilus*, the longhorn beetle *Monochamus alternatus* and *Pinus* spp., but is also living in the temperate climate of the Czech Republic, associated with the nematode *Bursaphelenchus eremus* and bark beetle *Scolytus intricatus* feeding on *Quercus* spp. trees. While nematode *Bursaphelenchus xylophilus* is the causal agent of pine wilt disease in eastern Asia (Mamiya 1983), *B. eremus* is probably not damaging oak tissues and feeding on microfungi in bark beetle galleries. The parasitic potential of *Esteya vermicola* for *Bursaphelenchus* nematodes is noteworthy and may be of value for plant pathologists. Therefore, virulence studies for other nematode genera should be carried out.



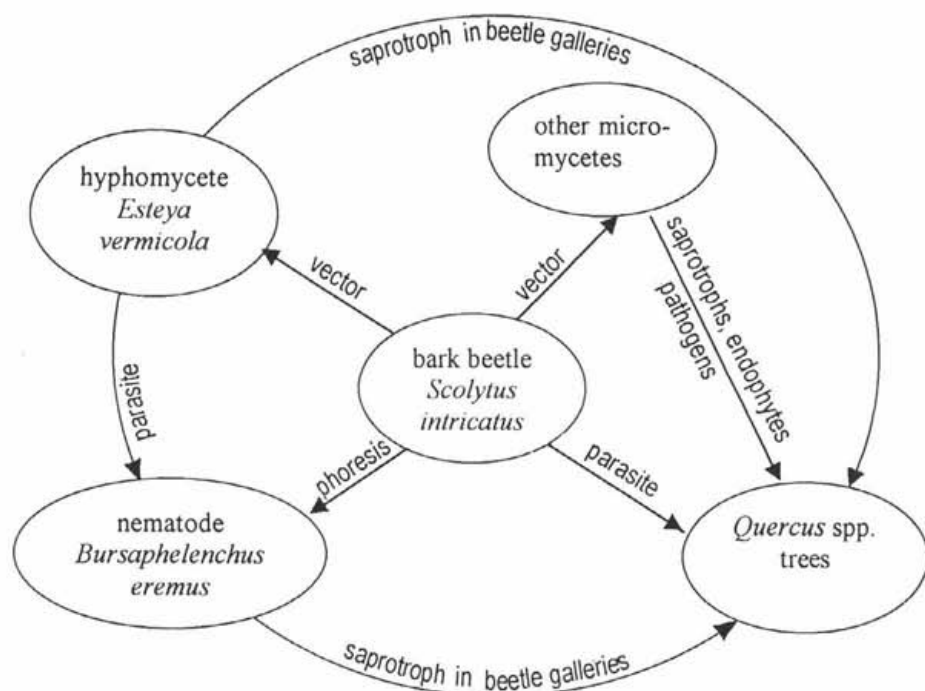


Fig. 4: Relations of the studied organisms: *Esteya vermicola*, *Bursaphelenchus eremus*, *Scolytus intricatus*, and *Quercus* spp.

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#### REFERENCES

- LIU J. Y., SHIH J. Y. and TZEAN S. S. (1999): *Esteya*, a new nematophagous genus from Taiwan, attacking the pinewood nematode (*Bursaphelenchus xylophilus*). – *Mycol. Res.* 103: 242–248.
- MAMIYA Y. (1983): Pathology of the pine wilt disease caused by *Bursaphelenchus xylophilus*. – *Ann. Rev. Phytopathol.* 21: 201–220.
- RÜHM W. (1956): Die Nematoden der Ipiden. – *Parasitol. Schriftenreihe* 6: 1–435.
- YIN K., FANG Y. and TARJAN C. (1988): A key to species in the genus *Bursaphelenchus* with a description of *Bursaphelenchus hunanensis* sp. n. (Nematoda: Aphelenchoididae) found in pine wood in Hunan province, China. – *Proc. Helminthol. Soc. Wash.* 55: 1–11.