

Tarichium hylobii* sp. nov., a pathogen of *Hylobius abietis

Siegfried Keller¹, Jaroslav Weiser² & Rudolf Wegensteiner³

¹ Rheinweg 14, CH-8264 Eschenz, Switzerland,

² Herálecká 964, Praha 4, Czech Republic,

³ University of Natural Resources and Applied Life Sciences,
Gregor-Mendel-Straße 33, A-1180 Vienna, Austria.

Keller S., Weiser J. & Wegensteiner R. (2008) *Tarichium hylobii* sp. nov., a pathogen of *Hylobius abietis*. *Sydowia* 61 (2): 249–254.

Tarichium hylobii sp. nov. (Zygomycetes, Entomophthorales) from larval *Hylobius abietis* L. collected in the Czech Republic is described. The resting spores are grey in mass and light brown in microscopic preparations. They are spherical to subspherical and have a diameter of 28.1–31.1 (24–36) µm including the irregularly undulating bulges and ridges of the endospore. Data are given to separate it from the similar species *T. cleoni*.

Key words: Entomopathogenic fungi, Entomophthorales, *Tarichium*, new species, morphology, Coleoptera, Curculionidae, *Hylobius abietis*.

The form-genus *Tarichium* (Entomophthoromycotina, Entomophthorales) (Hibett *et al.* 2007) comprises species known only from their resting spore stage and consists of 38 species (Bałazy 1993). Their biological and ecological life cycles are unknown. The criteria used to differentiate the species usually focus on host species and dimension, shape, colour, wall thickness, and wall and surface structures of the resting spores. Sometimes other structures such as rhizoids or hyphal bodies are present. We describe here a new species of *Tarichium* for which only the host, the large pine weevil *Hylobius abietis* L. (Coleoptera, Curculionidae), was known and resting spores were present. They resembled those of *T. cleoni* (Wize) Lakon (1915), pathogen of *Bothynoderes punctiventris* which belongs to the same host family but has distinct morphological features. Also, the ecology of their hosts is different. While *H. abietis* is a pest in forestry that attacks young conifers, *B. punctiventris* is an agricultural pest that attacks sugar beet. All these differences justify describing this species here as new.

Material and methods

Two infected larvae of *H. abietis* in closed pupal cells were collected at Brandys, 12 km northeast of Prague, Czech Republic, on Novem-

ber 15, 2006. The material was partly mounted in lactophenol-aniline blue (LPAB) and partly in lactophenol-aceto-orcein (LPAO) and examined under a stereomicroscope at 400x magnification. The material was compared with type material of *T. cleoni* from the collection of J. Weiser. The material was originally collected near Kiev, Ukraine. The material was mounted on two slides in Swann's medium (Romeis 1968). From each slide 25 spores were measured, having cared to include only spores with spherical appearance in the analysis. We investigated the outside diameter, which was defined by a sphere formed by the endings of the spines or the bulges respectively, the outside diameter of the endospores (only visible in *T. cleoni*) and the diameter of the inner wall of the endospores which corresponds roughly to the diameter of the cytoplasmic sphere. Several spores mounted in LPAO were ruptured by strong pressure on the cover slip with the aim to study the nuclei.

For the investigations of the fungus of *H. abietis* with the scanning electron microscope, air-dried spores were mounted on specimen stubs on carbon discs. The spores were coated in a sputter coater with gold-palladium using a voltage of 2 kV and a current intensity of 20 mA for 90 s under a vacuum of 10^{-2} to 10^{-3} mbar (Polaron E 5100, Hatfield, PA, USA). Electron micrographs were taken with a JEOL JSM 5200 scanning electron microscope (JEOL, Tokyo, Japan). Resting spores of *T. cleoni* were taken from a slide mounted in Swann's medium, cleaned and processed as above.

Results

***Tarichium hylobii* Keller, Weiser & Wegensteiner, sp. nov.** – Figs. 1a, 2a.
MycoBank: MB 515019

Sporae perdurantes sphaericae vel subsphaericae, 28.1–31.1 (24–36) μm episporio incluso. Sphaera cytoplasmatica 19.4–20.2 (16–25) μm diametro. Epispodium plurimis elevationibus vel cristis undulatis praeditum; sporae griseae in multitudine, singulae pallide brunneae. In larvis aut prepupis Hylobii abietii L. (hospitis typici) (Coleopterorum: Curculionidarum).

Holotypus: ZT Myc 1639. Czech Republic: Brandys. Coll. J. Weiser, November 15, 2006, det. S. Keller.

Description. – Mature resting spores measure 28.1–31.1 (24–36) μm, spherical to subspherical, grey in mass and light brown in microscopic preparations. In the light microscope the silhouette appears sinuous. The electron microscope shows irregularly undulating bulges and ridges. In the light microscope the separation between endospore and episporium was not clear (Fig. 1a). The diameter of the inner wall measures 19.4–20.2 (16–25) μm (Tab. 1).

Etymology. – Refers to the genus of the host.

Host and distribution. – Larvae or prepupae of *Hylobius abietis* L. (Coleoptera, Curculionidae) from closed pupal cells collected at Brandys, 12 km north-east of Prague, Czech Republic.

Distinguishing characters. – *T. hylobii* can easily be distinguished from other species by size and ornamentation of its spores and by the host species.

Remarks. – Developing resting spores of *T. hylobii*, which are probably azygospores, are smooth and form a white to pale gray powder. The hyphal remains form empty tubes measuring 10–15 × 25–35 µm. The surface structure develops when the spores mature. Under strong pressure some spores ruptured. With a few spores the episporium separated from the endosporium which had a smooth surface. No clear results were obtained in respect to the nuclei. In a few spores a single LPAO-stained structure was present while other spores contained 4–6 such structures. Most of them had an irregular shape. The few that appeared spherical had a diameter of 5–6 µm (n = 7) and are assumed to be undisturbed nuclei. Due to their internal appearance (Humber 1989), their size and their good stainability with LPAO we consider them as nuclei of a species of Entomophthoraceae.

T. cleoni (Wize) Lakon (1915) (Figs. 1b–c, 2b–c).

Resting spores 32.2–40.1 (28–47) µm, spherical to subspherical, red in mass and light brown in microscopic preparations, with regularly arranged 3.5–6.0 µm long spines. The endospores have a diameter of 21.2–23.6 (18–28) µm, the spore wall measures on average 3.5–4.0 µm. Endospores and episporium are tightly connected. In the light microscope the two walls appear clearly separated (Figs. 1a–b). The diameter of the inner wall measures 16.8–17.9 (14–21) µm (Tab. 1). Pathogen of *Bothynoderes (Cleonus) punctiventris* (Germar) (Coleoptera, Curculionidae).

The two species differ mainly by the size of the resting spores, by their ornamentation and by the colour of the resting spore mass. Weiser (1965) illustrated *T. cleoni* and compared it with eight other species of *Tarichium*.

Tab. 1. – Morphological characteristics of *Tarichium hylobii* and *T. cleonii*. Results of 25 measurements per slide.

Species, slide nr.	Diameter with episporium (µm)	Diameter without episporium (µm)	Diameter of cytoplasmic sphere (µm)
<i>T. hylobii</i> , 1	29.4 ± 1.69		19.8 ± 1.94
<i>T. hylobii</i> , 1	30.1 ± 2.22		20.2 ± 1.96
<i>T. hylobii</i> , 2	28.1 ± 1.49		19.4 ± 1.64
<i>T. cleoni</i> , 1	32.2 ± 3.35	21.2 ± 1.13	
<i>T. cleoni</i> , 1	40.1 ± 3.16		16.8 ± 1.71
<i>T. cleoni</i> , 2	36.7 ± 4.91	23.6 ± 2.86	
<i>T. cleoni</i> , 2	39.5 ± 2.85		17.9 ± 1.64

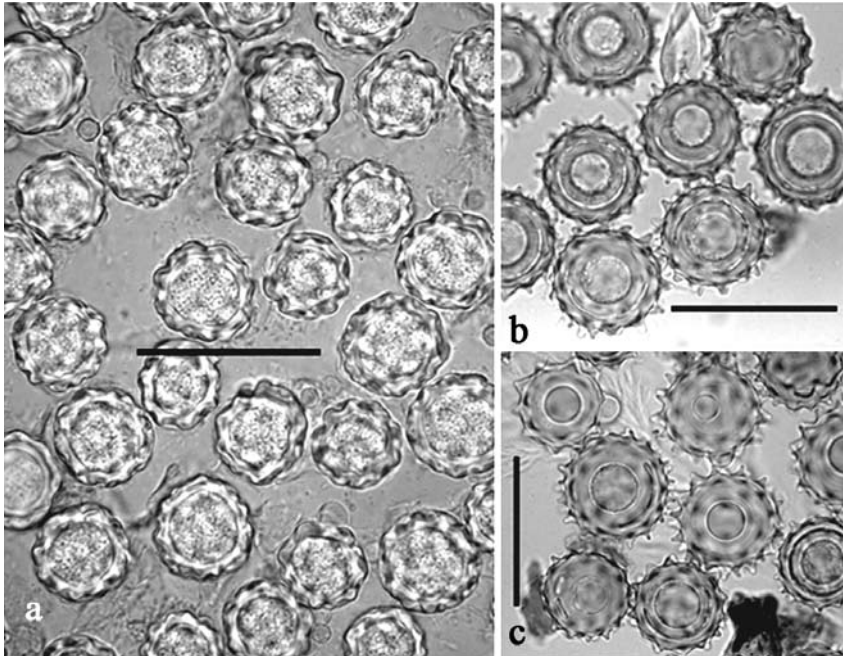


Fig. 1. – Light micrographs of resting spores of *Tarichium hylobii* (a) and *T. cleoni* (b, c). Bar = 50 μ m.

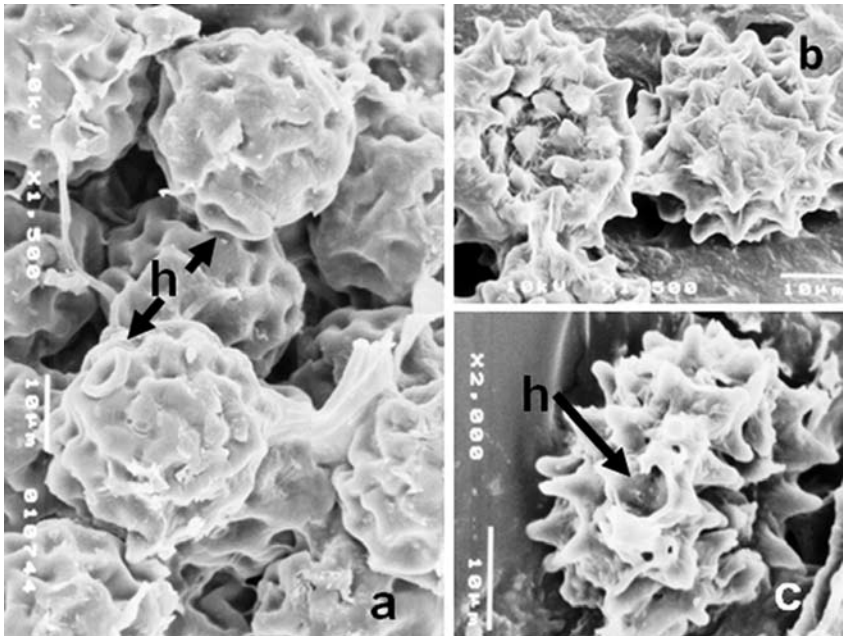


Fig. 2. – Scanning electronic micrographs of resting spores of *Tarichium hylobii* (a) and *T. cleoni* (b, c); h: hylus. Bar = 10 μ m.

Discussion

The diameter of the resting spores of *T. hylobii* are not very variable. Their size range between 28.1 and 30.1 μm (average: 29.2 μm). Those of *T. cleoni*, on the other hand, are rather variable (range: 32.2 – 40.1 μm ; average 37.1 μm), even within the same slide (Tab. 1). The diameter of the inner wall, which is identical with the diameter of the cytoplasmic sphere, is larger in *T. hylobii* as compared to *T. cleoni*. The difference between the diameter of the episporium and of the inner wall corresponds to the thickness of the walls of both endosporium and the episporium including the outgrowths. *T. hylobii* has clearly thinner walls than *T. cleoni*.

In the light microscope the resting spores of *T. hylobii* did not show a separation between endosporium and episporium (Fig. 1a) in contrast to *T. cleoni* which seemed to have a smooth endospore wall with a thick episporium (Fig. 1b–c). The prominent spines seem to be a structure of the episporium. In both species endosporium and episporium are strongly connected. In fact, even strong pressure on the cover slip could not break away the episporium. Only with *T. hylobii* the episporium exceptionally separated from the endosporium showing the smooth surface of the latter.

In the scanning electronic microscope (SEM), the differences in the surface structures are clearly visible. While *T. cleoni* has marked, flattened spines, the surface of *T. hylobii* is covered with rounded bulges or ridges. The hylum of the latter species appears as a ring-like protruding structure (Fig. 2a) while the hylum of *T. cleoni* appears as rounded hole (Fig. 2c). Using a light microscope as well as in SEM, the two species can be separated unequivocally.

Seventeen species of Entomophthorales known in their conidial form (some of them also produce resting spores) and four species known only in their resting spore form (form-genus *Tarichium*) are known to attack Coleoptera (Bałazy 1993, Keller 2007a). One of the four species of *Tarichium*, *T. rhagonycharum*, was recently transferred to the genus *Zoophthora* due to typical characteristics like compound rhizoids and hyphae-like hyphal bodies (Keller 2007b). A comparison of all resting spores from species attacking Coleoptera showed that the resting spores of *T. hylobii* clearly differ from those of either species which justifies describing it as a new species.

Acknowledgments

The authors are greatly indebted to Dr. O. Petrini for correcting the Latin diagnosis.

References

- Bałazy S. (1993) *Flora of Poland. Fungi (Mycota)*, vol. 24, Entomophthorales. Polish Academy of Science, 356 pp.

- Hibett D.S. *et al.* (2007) A higher-level phylogenetic classification of the Fungi. *Mycological Research* **111**: 509–547.
- Humber R.A. (1989) Synopsis of a revised classification of the Entomophthorales (Zygomycotina). *Mycotaxon* **34**: 441–460.
- Keller S. (2007a) Type host-pathogen lists. In: *Arthropod-pathogenic Entomophthorales: biology, ecology, identification* (ed. Keller S.), COST office Luxemburg, ISBN 978-92-898-0037-2: 135–146.
- Keller S. (2007b) Arthropod-pathogenic Entomophthorales from Switzerland. III. First additions. *Sydowia* **59**: 75–113.
- Lakon G. (1915) Zur Systematik der Entomophthorengattung *Tarichium*. *Zeitschrift Pflanzenkrankheiten* **25**: 257–272.
- Romeis B. (1968) *Mikroskopische Technik*. Oldenburg Verlag, München.
- Weiser J. (1965) Notes on two new species of the genus *Tarichium* Cohn (Entomophthoraceae). *Česká Mykologie* **19**: 201–204.

Manuscript accepted 14 July 2009; Corresponding Editor: R. Pöder

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Sydowia](#)

Jahr/Year: 2009

Band/Volume: [61](#)

Autor(en)/Author(s): Keller Siegfried, Weiser J., Wegensteiner Rudolf

Artikel/Article: [Tarichium hylobii sp. nov., a pathogen of Hylobius abietis. 249-254](#)