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# New records of Entomophthoraceae (Fungi, Zygomycetes) from aquatic insects

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Collections of diseased midges (Diptera, Nematocera), caddisflies (Trichoptera) and stoneflies (Plecoptera) were examined and two species of Entomophthoraceae identified. The fungus from caddisflies and stoneflies was identified as a member of the *Zoophthora radicans* complex and that from the midges as *Erynia gracilis* (Thaxter) Humber. This is the first record of this species since its original description from the eastern USA in 1888.

Keywords: Entomopathogenic fungi, Entomophthoraceae, new records, Diptera, Trichoptera, Plecoptera.

## INTRODUCTION

The fungus family Entomophthoraceae comprises nearly 200 species which attack exclusively insects and phalangiids. Many species prefer humid habitats and it is not astonishing that insects infected with Entomophthoraceae are frequent in the vicinity of water, and epizootics on midges and caddisflies (Trichoptera) are common (Keller 1985). In this paper we report on species of Trichoptera and Plecoptera as new hosts of the *Zoophthora radicans* species complex and on a species that had never been recorded since the original description in 1888.

## MATERIAL AND METHODS

Three collections were examined (Tab. 1). Collection 1 includes three different species of the plecopteran family Nemouridae and the material originates from Switzerland and from Austria. The infected adult Plecoptera were fixed to the underside of leaves of elder (*Alnus glutinosa* Gärtn.), hazelnut (*Corylus avellana* L.) and other deciduous trees and bushes up to about two meters above the water level of small rivers, and to the wall of a house bordering the lake Lunz (Austria). Collection 2 comprises five species of adult Trichoptera belonging to three different families (Tab. 1). They were fixed to the underside of leaves of lime (*Tilia* sp.), hazelnut, elder and other deciduous trees and bushes up to about four meters above the water level, bordering the river Rhine. Collection 3 (Tab. 1) includes about 20 specimens of small midges fixed a few centimetres above the water level to a small branch of a tree lying in a small river.

The infected insects or parts of them were teased on microscopic slides and the fungus stained with lactophenol-aniline-blue (0.1 % aniline) or with lactophenol-aceto-orcein (0.25 % orcein). All counts and measurements were based on 25 objects per individual host, if not otherwise stated, and defined as a series.

| Collection Number | Host species (family) | Location, collection date, collector |
|---|---|---|
| 1a | *Amphinemoura sulcicollis* (Nemouridae) | Switzerland: Fischingen TG, river Murg, 4.–18.8.2004, S. Keller |
| 1b | *A. sulcicollis* (Nemouridae) | Austria: Lunz am See, NÖ, 7.7.2004, H. Malicky-Ruzicka |
| 1c | *Nemoura marginata*<br>*Nemoura* sp.<br>*Amphinemoura* sp.<br>(Nemouridae) | Austria: Schreierbach, NÖ, 5.–10.8.2004, P. Zwick |
| 2 | *Mystacides azurea* (L.) (Leptoceridae)<br>*Hydroptila forcipata* (Eaton),<br>*H. sparsa* Curtis<br>(Hydroptilidae)<br>*Cheumatopsyche lepida* (Pictet),<br>*Hydropsyche contubernalis* McLachlan<br>(Hydropsychidae) | Switzerland: along the river Rhine between Rheinau and Eglisau, ZH, 10.6.–10.10.2001, S. Keller |
| 3 | Unidentified small midges (Diptera, Nematocera) | Switzerland: Fischingen TG, along the river Murg,18.8.2004, S. Keller |

Tab. 1: The examined collections of Entomophthoraceae

## RESULTS

### *Zoophthora* cf. *radicans* from Plecoptera

The infected stoneflies collected in 2004 were fixed to the substrate with compound pseudorhizomorphs typical for the genus *Zoophthora*. No sign of fungal infection was visible on the host surface. The cadavers contained only resting spores. They were spherical, hyaline and had an average diameter of 25.8–28.6 µm, the extreme values ranged from 22–31 µm. They matched the dimensions given for material collected in June 1981 from the same host family (Keller 1985). All important fungal structures were present in that material and allowed to identify the fungus as *Zoophthora* cf. *radicans*. The primary conidia measured 19.8–21.6 x 6.9–7.2 µm with a length/diameter-ratio (L/D) of 2.79–2.96. The capilliconidia measured 20.1–21.3 x 5.1 µm, L/D = 3.94–4.18. The resting spores were spherical, hyaline and smooth and measured on average 25.5–27.2 µm with extremes ranging from 20–33 µm.

### *Zoophthora* cf. *radicans* from Trichoptera

The infected caddisflies were fixed to leaves with compound pseudorhizomorphs typical for the genus *Zoophthora*. Infected specimen had their wings slightly spread, sometimes white or greyish mycelial bands were visible along the intersegmental membranes of head and thorax. The primary conidia measured on average 15.7–20.9 x 6.0–7.1 µm with extremes of 15–23 x 5–8 µm, L/D = 2.07–3.23. The capilliconidia measured on average 18.9–21.4 x 4.8–5.1 µm, L/D = 3.74–4.17, and

were produced on a capillary tube with an average length of 48–66 µm. The resting spores were spherical, hyaline and smooth and had an average diameter of 25.1–25.9 µm with extreme values of 23–28 µm.

*Erynia gracilis* (Thaxter) Humber (1989)

The hosts were unidentified midges about 3 mm long. The cadavers were grey with a distinct violet touch and fixed some centimeters above the water level to a branch of about 2 cm diameter, which stuck in the water of a small pre-alpine river.

The rhizoids are monohyphal with a diameter of 14.9–16.8 (8–34) µm (2 series, n = 25), distal portion gradually enlarging or branched, the endings are bulbous, sucker-like or cork-screw-like. The subspherical to elongate hyphal bodies measure on average 30.8–32.3 x 25.0–28.0 µm (extremes: 23–45 x 19–36 µm), L/D = 1.15–1.22 (4 series) and contain 8.3 (6–12) nuclei (1 series, n = 18). The conidiophores are branched and terminally enlarged to 7.3–9.0 (7–11) µm (2 series, n = 15 and 25). The primary conidia measure on average 30.1–39.0 x 7.6–8.6 µm (24–46 x 6–10 µm), L/D = 3.50–5.10 (4 series, n = 25) and are elongate, curved to slightly sickle-shaped with the largest diameter in the basal half. The secondary conidia are like the primary ones or nearly spherical. The tapering cystidia are rare and resting spores were absent.

## DISCUSSION

Adult Plecoptera are known as hosts of two other species of Entomophthorales: *Erynia plecopteri* (Descals & Webster 1984) and *Entomophthora rivularis* Keller *et al.* in Keller (2002). The presence of *Z.* cf. *radicans* on this host order has only been reported by Keller (1985). There are good reasons to assume that the fungus reported here is identical with the previous finding.

Adult Trichoptera are well known as host of *Erynia rhizopspora* (Thaxter) Humber (1989) which regularly causes epizootics. *Z.* cf. *radicans* on this host order has never been reported. The dimensions of the primary conidia of this fungus from Trichoptera showed a wide variation. Especially conidia from Hydroptilidae were distinctly smaller than conidia from the other host families. The question arises if different fungus species of the *Z.* cf. *radicans* group might be involved.

*Z. radicans* sensu lato is known to have an extremely wide host range which is unusual for species of Entomophthoraceae. On the other hand the fungus seems to be very specific within a host taxon. Therefore, Turian (1957) proposed to create subspecies using the host taxon as subspecies epithet. Later on, Bałazy (1993) described the fungi from a defined host taxon as new species. He also gave some minor morphological differences which, however, do not allow to separate the species unequivocally. Probably, the two fungi from Plecoptera and Trichoptera identified as *Z.* cf. *radicans* should as well be described as own species. However, further studies are needed which should include not only morphological but also genetic characteristics.

*Z. radicans* sensu stricto was originally described from larvae of *Pieris brassicae* L. (Lepidoptera) (Brefeld 1870). The morphological data in the original description are limited to the dimension of the primary conidia (17.6 x 5.4 µm) and do not allow a thorough comparison with similar species. According to Bałazy the conidia are larger; unfortunately he did not mention the origin of his material. A

redescription of *Z. radicans* from the type host would substantially contribute to clarifying the status of the species involved in this complex.

The finding of *E. gracilis* is the first record of this species since the original description from the eastern USA and rejects doubts about a possible synonymy with *E. conica* (Bałazy, 1993). The conidia vary widely but match the dimensions given in the original description. However, the dimensions given by Thaxter (1888) do not correspond with his drawings which show distinctly more slender conidia. The material examined contained only very few secondary conidia, but both types were present. The observation that cystidia are rare agrees with the original description. In contrast to Thaxter's observation the mycelial mass on the insect was not white but dark grey with a distinct violet touch, which distinguished the infected midges from those attacked by *E. conica* found on the same branch. However, the colour of the mycelial mass may be influenced by the host species.

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