

## New hyphomycete species from streams in the Šumava National Park (Bohemian Forest, Czech Republic)

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Three new species, *Enantioptera bialata*, *Tricellula ornata* and *Tricladium obesum*, are described on the basis of pure cultures derived from conidia isolated from stream foam. All occur in clean, softwater streams in a temperate climate.

**Key words:** mitosporic fungi, new taxa, *Enantioptera*, *Tricellula*, *Tricladium*.

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Na bázi čistých kultur izolovaných z pěny na potocích jsou popsány tři nové druhy hyfomycetů: *Enantioptera bialata*, *Tricellula ornata* a *Tricladium obesum*. Všechny se vyskytují v mírném pásmu v čistých potocích s měkkou vodou.

### INTRODUCTION

Biodiversity studies of freshwater hyphomycetes in the temperate zone usually reveal at least several unknown spores, very probably belonging to undescribed fungi. In spite of the fact that in waters on the European continent the freshwater mycobiota is relatively well known, authors publishing lists of taxa collected in foam usually report and illustrate tens of undescribed species (e.g. Descals et al. 1995, Descals 1998, Voglmayr 1996, Marvanová and Gulis 2000, Marvanová 2001). The following formal descriptions presented here should diminish the number of some “unknowns” reported several times by various authors. These new species were isolated from running waters in the Šumava National Park.

### MATERIALS AND METHODS

Foam samples were collected into glass jars. Inoculations were performed in the field or the same day after return to the laboratory. The foam was smeared on thin layers of 0.1 % malt agar (MA, Difco), on object slides. After incubation at c. 10 °C for 1–2 days, the agar layers were scanned under low power of a compound

microscope. Germinating conidia were cut out with a flamed needle together with a piece of agar and transferred to Petri dishes with 2% MA. All isolates were monocolonial. Descriptions of colonies are based on pure cultures on 2% MA incubated at 15 °C. Sporulation was obtained by submerging pieces of agar cultures into standing sterile distilled water in Petri dishes, incubated at 15–18 °C in diffuse daylight.

## RESULTS AND DISCUSSION

***Enantioptera bialata* Marvanová sp. nov.**

Fig. 1.

Etym.: *bialatus* (L.) = double-winged.

Fungi anamorphosi, hyphomycetosi. Teleomorphosis ignota.

Coloniae ad agarum maltosum albae, restrictae, mycelium aerium gossypinum, margine abrupto, parte reversa pallide brunnea. Sporulatio subaquatica. Conidiophora micronematosa. Cellulae conidiogenae breves, terminales vel intercalares, cum elongationibus sympodialibus. Conidia singularia vel usque ad tres per cellulam conidiogenam, tetra radiata. Axis cylindricus, rectus vel paulo curvatus, 18–25 × 1–2 μm, apice subulatus, basi truncatus, uniseptatus, rami typice duo, laterales, mediani, suboppositi vel paralleli, subulati, basi non constricti, 7–11 × 1–1.5 μm. Dehiscencia conidiorum schizolytica.

Anamorphic fungi, hyphomycetes, teleomorph unknown.

Colonies on MA 2% white, restricted, reaching 12 mm diam. after 19 days at 12 °C, aerial mycelium low, cottony, margin abrupt, reverse beige. Sporulation after submergence, under water. Conidiophores micronematous. Conidiogenous cells short, terminal or intercalary with a lateral peg, sympodial, elongations few. Conidia single or in groups of up to 3 per conidiogenous cell, tetra radiate. Axis cylindrical, straight, arcuate or slightly bent at branch insertion, 18–25 × 1–2 μm, with a single septum between the branches, subulate above the branches, base truncate or rounded. Branches typically two, lateral, median, synchronous, subopposite, in one plane or divergent, sometimes unilateral and then either parallel or appearing crossed when seen in preparations, subulate, 7–11 × 1–1.5 μm, insertion unstricted. Conidial secession schizolytic.

Holotype: PRM 901637 (preparation from the culture CCM F-23899), Czech Republic, South Bohemia, Šumava National Park, foam on the left branch of the stream Křemelná, near the road from the perished settlement Zhůří to Stará Huť, above the confluence with the right branch, c. 850 m alt., 19–10–1998 coll. and isol. L. Marvanová.

Detached conidia of this species were relatively frequently encountered in foam on streams of the catchment areas of the upper Vltava and upper Otava rivers (Marvanová 2001).

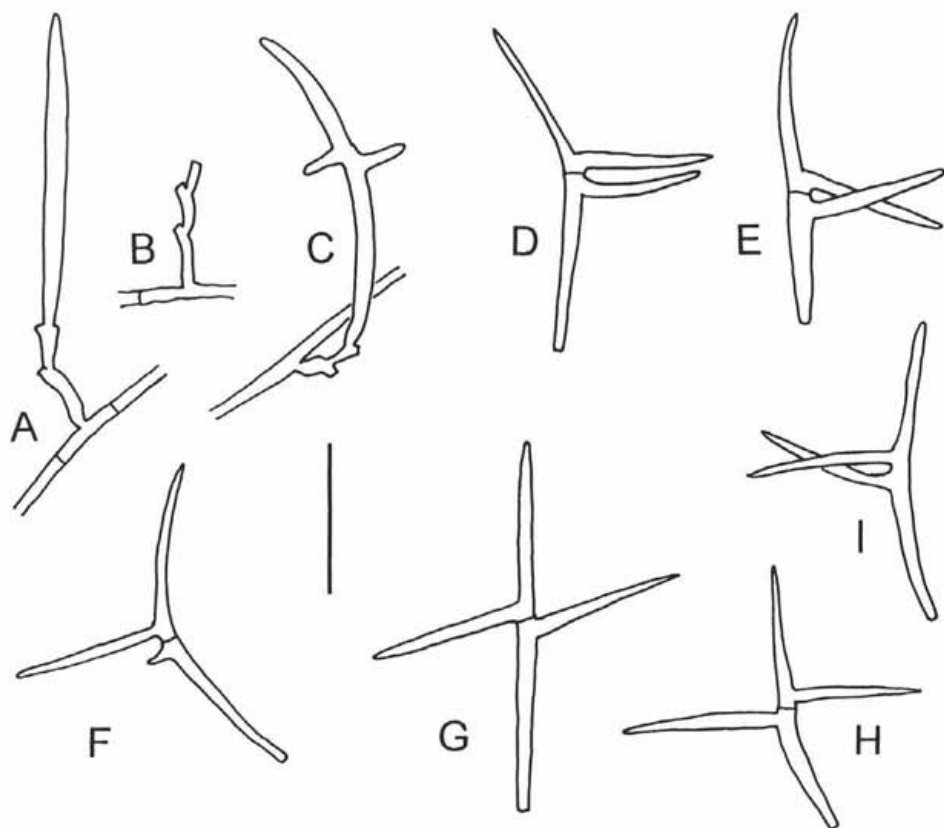


Fig. 1. *Enantioptera bialata* CCM F-23899. A. Conidial primordium. B. Spent conidiophore. C. Young conidium developing two lateral branches. D-I. Detached conidia. Some appear with unilateral branching (D-F, I), the rest has subopposite arms. Scale bar = 10  $\mu\text{m}$ .

Conidia of *Enantioptera bialata* are similar to those of *E. tetraalata* Descals, the type and so far the only species of the genus. The latter differs by typically two parallel laterals on both sides of the conidial axis which is longer: 30–38  $\mu\text{m}$  given in the protologue (by Descals and Webster 1983), but in their Fig. 2S according to the scale bar it reaches 47  $\mu\text{m}$ . The generic character of *Enantioptera*, i.e. two opposite pairs of laterals perpendicularly attached to the conidial axis is very unique among hyphomycetes. Adding a species with just a single pair of laterals seems inappropriate at first sight. However, *E. tetraalata* itself is very variable, often producing conidia with one pair of laterals missing.

Conidia strongly resembling those of *E. bialata* were depicted by Regelsberger et al. (1987: Fig. 3C<sub>1</sub>-C<sub>6</sub>, as 'close to *Taeniospora*') and by Marvanová and Gulis (2000: Fig. 3H, with longer axis and branches, as unknown) from the Ysper

stream in Austria. The fungus isolated in Canada (Marvanová and Bärlocher 1988: Fig. 6A-L) in pure culture and considered a monocyron of *Taeniospora descalsii* Marvanová et Stalpers was very probably *E. bialata*. The white colony with low cottony mycelium supports this opinion. Unfortunately the Canadian culture is no more viable and therefore no comparison of colony character of both isolates is possible at the moment.

*Enantioptera bialata* conidia are somewhat similar to those of *Arborispora paupera* Marvanová et Bärlocher, but here the conidial branches are sequential, branch bases are obconic and hence the insertion is very narrow (Marvanová and Bärlocher 1989). *Descalsia cruciata* A. Roldán et Honrubia has sequential conidial branches which are subopposite, never unilateral.

***Tricellula ornata* Marvanová sp. nov.**

Fig. 2.

Etym.: ornatus (L.) = decorated.

Fungi anamorphosi, hyphomycetosi. Teleomorphosis ignota.

Coloniae ad agarum maltosum albidae, restrictae, pars reversa isabellina. Hyphae in substrato 1-7  $\mu\text{m}$  latae. Sporulatio subaquatica. Conidiophori laterales, curti, ramosissimi, ad septa constricti. Cellulae conidiogenae polyblasticae, acrogenae vel pleurogenae, ellipsoideae, 5-9  $\times$  2-3  $\mu\text{m}$ . Conidia stauroformia, aggregata, acro- vel pleurogena, sequentia. Ramus primus ellipsoideus, ramum apicalem duoque ramos laterales gerens. Ramus apicalis sagittiformis, rami laterales sequentes, retrorsi, inaequilaterales, lobati; rami toti per isthmus angustos cum axe connecti. Conidia tota 11-17  $\mu\text{m}$  in diam. Dehiscencia schizolytica.

Anamorphic fungi, hyphomycetes. Teleomorph unknown.

Colonies on MA 2 % off white, restricted, reaching 6 mm diam. after 16 days at 15 °C, aerial mycelium elevated in the centre, finely funiculose, margin low, exudate copious, clear, reverse isabelline. Substrate mycelium with hyphae 1-7  $\mu\text{m}$  wide, some cells inflated. After submergence the colony gains a pale bluish-green tinge. Sporulation underwater, copious. Conidiophores lateral, short, profusely branched, strongly constricted at septa. Conidiogenous cells polyblastic, acrogenous and pleurogenous, in groups, ellipsoidal, 5-9  $\times$  2-3  $\mu\text{m}$ . Conidia stauroform, in groups, acrogenous or pleurogenous, closely sequential. First formed arm ellipsoidal, bearing one apical and two lateral arms, the apical one sagittate, with more or less wavy side walls, the lateral arms sequential, wing-shaped, retrorse, usually appearing lobed due to various irregularly disposed projections. All arms connected by narrow isthmi. Conidial span 11-17  $\mu\text{m}$ , secession schizolytic.

Holotype: PRM 901638 (preparation from the culture CCM F-15299), Czech Republic, South Bohemia, Šumava National Park, foam on the stream Mlýnský

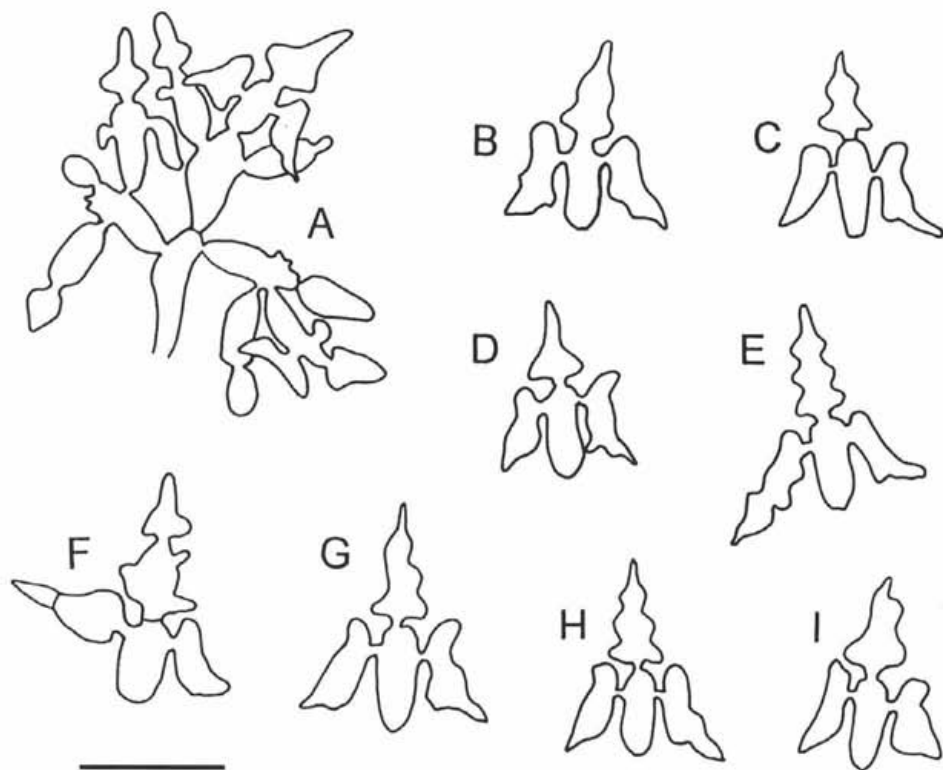


Fig. 2. *Tricellula ornata* CCM F- 15299. A. Conidiophore with conidiogenous cells and conidia in various stages of development. B-I. Detached conidia. Scale bar = 10  $\mu$ m.

potok, near the bridge on the road from Prášily to Srní, near the perished settlement Velký Bor, c. 900 m alt., 19-10-1998 coll. and isol. L. Marvanová.

Besides the type locality, conidia of this species were seen only in a single foam sample from a stream in the upper Vltava river catchment area (Marvanová 2001).

This fungus has all features of a member of the genus *Tricellula*, but the colony lacks the orange pigment which is typical for the other species of this genus. Morphologically it differs from all other members of the genus by the lobed conidial arms. In permanent preparations with lactofuchsin as the mountant, the conidiogenous cells tend to swell considerably and the wavy outline of conidial elements becomes much less pronounced.

Roldán and Puig (1992: Figs. 3G and 5C,D) and Descals (1987: Fig. 5G,H; 1997: Fig. 19) illustrated very similar conidia of a *Tricellula* sp. with less lobed arms from Spain which may be conspecific. However, they did not see the conidiogenesis and the colony of Descals' fungus was described as pale orange. Matsushima (1971)

classified a fungus with very similar but larger and more symmetrical conidia tentatively as *Isthmotricladia* sp. His isolate differs from ours by the conidiogenesis: conidia arise singly on micronematous conidiophores. The accommodation in *Isthmotricladia* seems not appropriate, because the latter is characterised by conidia with a fascicle of antrorse branches on a stalk.

***Tricladium obesum* Marvanová sp. nov.**

Fig. 3.

Etym.: *obesus* (L.) = fat.

Fungi anamorphosi, hyphomycetosi. Teleomorphosis ignota.

Coloniae ad agarum maltosum atrae, medium celeriter crescentes, mycelium aerium copiosum, funiculosum, pars reversa nigra. Mycelium in substrato brunneum, hyphis glabris, sed nonnullis verrucosis. Sporulatio sub- vel supraaquatica.

Status macroconidialis: Conidiophora micronematosa, cellulae conidiogenae mono- vel polyblasticae, terminales vel laterales, cum conidiophoris conidiisque integratae, elongationes sympodiales vel prolificantes. Conidia singularia vel duo per cellulam conidiogenam, ramificata, ex uno axi et (1-)2 ramis consistentia. Elementa conidialia septata vel continua, cellulis inflatis, apicibus obtusis vel subulatis. Axis saepe subcurvatus, 40-63(-90)  $\times$  5-10  $\mu$ m, basi truncatus vel mamillatus. Rami alternati, sequentes, raro oppositi, recti vel subcurvati; ramus proximus 5.5-40  $\times$  4-6.5  $\mu$ m, ramus distalis 5-16  $\times$  3.5-5  $\mu$ m, basi subconstricti. Dehiscencia conidiorum schizolytica.

Status microconidialis: hyphomycetosus. Conidiophora semimacronematosa, terminalia, recta, ca. 2  $\mu$ m lata. Cellulae conidiogenae acrogenae, aggregatae vel singulares, phialidicae, 9-17  $\times$  2-3.5  $\mu$ m, cum collare cupulato. Conidia aggregata, ellipsoidea, glabra, 3-5  $\times$  1.5-2  $\mu$ m.

Anamorphic fungi, hyphomycetes. Teleomorph unknown, probably ascomycetous.

Colonies on MA 2 % dark grey, reaching 20 mm diam. after 20 days at 15 °C, aerial mycelium hairy to funiculose, reverse blackish. Substrate mycelium brown, with thicker walls, some hyphae finely warty. Sporulation after submergence, under and above water.

Macroconidial state: conidiophores micronematous, conidiogenous cells mono- or polyblastic, terminal or lateral, integrated with conidiophores as well as with the conidia, elongations sympodial or percurrent. Conidia single or rarely two per conidiogenous cell, branched, with one axis and (1-)2 laterals. In pure culture sporulating in standing distilled water, the distal branch is often absent. Conidial elements septate or continuous, cells often inflated, apices rounded to subulate. Axis usually slightly bent, 40-63(-90)  $\times$  5-10  $\mu$ m, base flat or with a teat or short conical extension. Branches single or alternate when two, appearing in acropetal

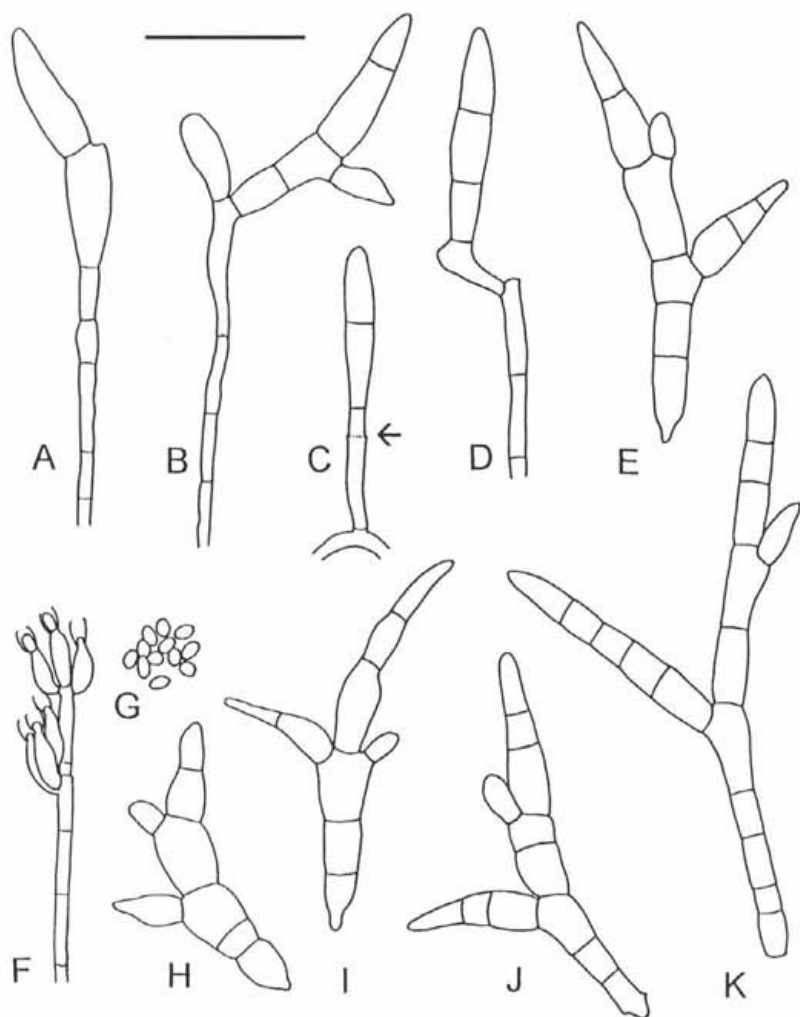


Fig. 3. *Tricladium obesum*. A-E, H-K. Macroconidial state. A. Young conidium developing on a terminal conidiophore. B. Polyblastic conidiogenous cell with two conidia in various stages of development. C. Short lateral conidiophore with percurrent elongation (arrow). D. Conidiophore with sympodial proliferation. E, H-K. Detached conidia. F, G. Microconidial state. F. Conidiophore with phialides. Note the deep collarettes. G. Group of microconidia. (A, C, D-F, K from CCM F-14598; B, H, J from CCM F-14098; I from CCM F-13798). Scale bar = 25  $\mu$ m.

sequence, or rarely opposite, straight or slightly pendulous, the proximal one  $5.5-40 \times 4-6.5 \mu$ m, the distal one  $5-16 \times 3.5-5 \mu$ m, base not or only slightly constricted. Conidial secession schizolytic.



Microconidial state: hyphomycetous. Conidiophores terminal, integrated with the supporting hypha, straight, stalk c. 2  $\mu\text{m}$  wide. Conidiogenous cells acrogenous, grouped, or scattered singly down the conidiophore stalk, discrete, phialidic, sometimes with a secondary septum, 9–17  $\times$  2–3.5  $\mu\text{m}$ , collarette cup-shaped, deep. Conidia in groups, ellipsoidal, glabrous, 3–5  $\times$  1.5–2  $\mu\text{m}$ .

Holotype: PRM 901639 (preparation from the culture CCM F-14598), Czech Republic, South Bohemia, Šumava National Park, foam on the right branch of the stream Křemelná in the vicinity of the perished settlement Zhůří, near a broken wooden bridge, c. 900 m alt., 19–10–1998 coll. and isol. L. Marvanová.

Other cultures examined: CCM F-13798, CCM F-13998, CCM F-14098, Czech Republic, South Bohemia, Šumava National Park, foam on the left branch of the stream Křemelná, near the road from the perished settlement Zhůří to Stará Huť, above the confluence with the right branch, c. 850 m alt., 20–10–1998 coll. and isol. L. Marvanová.

In foam samples, conidia of this species were more frequently seen in streams of the upper Otava river catchment area (Marvanová 2001).

Conidia of undoubtedly this species were reported and illustrated several times, often from soft waters on acidic bedrock: e.g. Messner and Stüve (1986: Fig. 2A, as *Tricladium* sp.); Regelsberger et al. (1987: Fig. 3B<sub>1</sub>, B<sub>2</sub>, as 'the form might belong to *Tricladium*'); Roldán et al. (1987: Figs. 4P-S, as *Tricladium* sp., 'similar to *T. angulatum* Ingold, but more fat'), locality not specified; Voglmayr (1996: Fig. 13d, as unknown); Descals (1998: Fig. 4B, D, E, as ?*Tricladium* sp.), water chemistry unspecified.

The genus *Tricladium* is heterogeneous. It comprises mitosporic fungi and two ascomycete anamorphs with relatively simple conidia: axis and typically two alternate primary branches. This is a primitive branching pattern, which might be derived from a branching hyphal end. As concerns the conidial morphology, our fungus is similar to *T. angulatum* as suggested by Roldán et al. (1987), but the latter has pale colonies and conidia with narrower elements.

There is a fungus with strikingly similar conidia described as an unnamed anamorph of the bitunicate loculoascomycete *Perisporiopsis lophirae* (Deighton) Arx (Müller and von Arx 1962, *Perisporiopsidaceae*; Sivanesan 1984, *Parodiaceae*). At present, *Perisporiopsis* is classified among Dothideomycetes incertae sedis, in *Parodiopsidaceae* (Eriksson 2004). The conidiogenesis of the *Perisporiopsis* anamorph involves percurrently proliferating conidiogenous cells and repeated formation of a funnel-like collarette, left behind by each seceding conidium (cf. Moreau and Moreau 1959: Fig. 1i), similar to that seen by Descals and Webster (1982) in *Culicidospora aquatica* R. H. Petersen. Besides this, the authors report sympodial proliferation of the conidiogenous cell (Moreau and Moreau 1959: Fig. 1e-h), also accompanied by a funnel-like collarette appearing after secession. A holoblastic microconidial state with amerosporous small conidia borne on



denticles is also illustrated (Moreau and Moreau 1959: Fig. 1s,t). Macroconidial anamorphs similar to that of *Perisporiopsis lophirae* are reported also for two other members of *Parodiaceae* by Sivanesan (1984). Ecologically, *Perisporiopsis* spp. belong to leaf parasites on forest trees in tropical and subtropical climates in South and Central America and in tropical Africa.

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