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Anguillospora mediocris sp. nov. from streams in Hungary

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Anguillospora mediocris sp. nov. is described from the Morgó stream system in Hungary. It produces relatively short, falcate or sigmoid conidia on percurrent conidiogenous cells. A pycnidial microconidial synanamorph was observed in pure culture. The natural substrate is leaves, mainly of alder (Alnus glutinosa). The fungus seems to prefer hard waters. Conidia in nature, even when abundant in stream, occur only sporadically in foam.

Key words: aquatic hyphomycetes, taxonomy, ecology.

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V článku je popsán druh Anguillospora mediocris sp. nov z vodních toků v Maďarsku. Je charakterizován tvorbou srpovitých nebo sigmoidních konidií na krátkých nevětvených konidioforech. V čistých kulturách bylo pozorováno také mikrokonidiové stadium vytvářející pyknidy. V přírodě se tento druh vyskytuje na ponořených listech, zejména olšových (Alnus glutinosa). Dosavadní nálezy jsou převážně z tvrdých vod. Konidie se jen sporadicky najdou ve vzorcích pěny a to i v případech, kdy jsou ve vodním toku hojné.

INTRODUCTION

Stream water filtration and identification of detached conidia on filters is a common method used in ecological and biodiversity studies of aquatic hyphomycetes. Whereas identification is relatively safe in the case of stauroconidia, it often fails when applied to scolecoconidia, not only because few scolecoconidia have diagnostic characters distinct enough, but also because of the great variation in size, especially in length, which causes frequent overlap and uncertainty in identification unless conidiogenesis is seen.

Abundant scolecoform conidia of undoubtedly the same species were repeatedly encountered in the Morgó stream system since the first analyses of the stream water with membrane filtration in 1996. The conidia have been referred to as *Filosporella* sp. 1 in Gönczöl et al. (1999) and Gönczöl and Révay (1999a), and *Filosporella* sp. in Gönczöl and Révay (1999b). After several attempts the first author succeeded in isolating the fungus into pure culture. It appears that it fits relatively well into the genus *Anguillospora* and it is described here as a new species.

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MATERIAL AND METHODS

Decaying leaves of Alnus glutinosa (L.) Gärtn. were collected from the Nacsagrom stream in the Börzsöny Mountains, NE Hungary, in November 2000. Leaves were placed into Petri dishes with distilled water and incubated for several days at 10 °C. Some of the incubation water was then poured onto a plate with 0.1 % malt agar (MA), germinating conidia were located with the aid of a New England Finder, and with a sterile needle transferred to 2% MA. Subcultures were made on 3% MA. Pieces of 15–60 day old colonies from 3% MA were submerged in standing or aerated sterile distilled water and incubated at 12 °C. Aeration had negative effect (no conidia detected), whereas in standing water a weak to abundant production of conidia took place after 5–7 days.

DESCRIPTION

Anguillospora mediocris Gönczöl et Marvanová, sp. nov. (Figs. 1, 2)

Etym. mediocris (Lat.) = medium, not large, size of the conidia in comparison with other species of the genus.

Coloniae in agaro maltoso moderate crescentes, griseae vel atrogriseae, margine lobatae. Mycelium in substrato crustosum, e cellulis plus-minusve isodiametricis seu breviter elongatis, 7.5–15 μ m in diametro, cum parietibus tenuibus, atris, compositum. Pars reversa nigra. Conidiophora singularia vel aggregata, brevia, non ramificata, 10–60 × 2–3 μ m. Cellulae conidiogenae incorporatae, percurrenter vel raro sympodialiter prolificantes. Conidia scolecoformia, septata, falcata vel raro sigmoidea rectaque, attenuata in parte distali, 62–104 × 3.3–4.8 μ m, apex subulatus, basis truncata vel cum extensione brevi. Dehiscentia conidiorum schizolytica.

Status microconidialis (andromorphosis ?) coelomycetosus, in cultura agarosa. Pycnidia inter mycelium immersa, globosa, brunnea, 50–175 μ m in diametro, parietibus tenuibus, e cellulis isodiametricis compositis. Conidiophora cellulae conidiogenaeque non visae. Conidia hyalina, ellipsoidea vel irregulariter elongata, 1.5–4 × 1.5–1.8 μ m.

Holotypus: BP-95701, praeparatum e cultura monoconidiali JG 2000–5 e folio putrido *Alni glutinosae* isolata, in rivulo Nacsagrom-patak pr.pag. Szokolya, montibus Börzsöny-hegység, Hungaria, 14 Nov. 2000., J. Gönczöl.

The monoconidial primocultures on 2 % MA grew extremely slowly, reaching 5–8 mm diam. in one month at 20 °C. Black, hard, elevated colonies developed on this medium. On 3% MA, growth of cultures was moderately fast, reaching ca. 30 mm diam. in one month at 20 °C; colonies were mid to dark grey, with umbonate centre, zonate at the margins, lobed. Aerial hyphae brown, up to 5 μ m wide,



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Fig. 1. A-N. Anguillospora mediocris. A. First-formed conidium detached in premature stage.
B. Percurrent proliferation of a conidiogenous cell. C. Sympodial proliferation of a conidiogenous cell. D-F., G., J., M., N. Detached conidia from pure culture. H., I. Conidia from nature: H., from filter; I., from alder leaf. K. Surface structure of pycnidia of the microconidial state. L. Microconidia. Scale bar = 50 mm.

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walls slightly thickened; inflated globose cells 10–17 μm wide present in chains or clusters. Substrate mycelium forming crusts of pseudoparenchymatous tissue composed of more or less isodiametric or shortly elongate cells 7.5–15 μ m diam. with thin dark walls. Some hyphae bear amorphous brown incrustations. Reverse black, marbly, with several slits. Sporulation after submergence in standing water, below water level, from substrate mycelium. Conidiophores single or grouped, short, unbranched, slightly widening towards the apex, 10–60 \times 2–3 μ m. Conidiogenous cells integrated, proliferations percurrent, rarely sympodial. Conidial initiation integrated. Conidia scolecoform, septate, typically falcate, resembling thin, long conidia of a Fusarium, sometimes more strongly curved and attenuate in the distal part, rarely sigmoid or straight; apex subulate, base slightly bulging, or snake-head-like (with a short percurrent extension) or with a pedicel. The conidial dimensions (50 conidia measured on filter from a filtration performed in November 2000 at the locus classicus) are $62-104 \times 3.3-4.8 \ \mu m$, with an average $81.8 \times 3.9 \ \mu m$. In pure culture (isolate J. Gönczöl 2000/5, submerged in standing distilled water) the conidia measure $70-92 \times 2.8-4.5 \ \mu\text{m}$ with an average $80.5 \times 3.6 \ \mu\text{m}$ (50 conidia measured). The length/width ratio is 20–22. In lactophenol with cotton blue the conidial dimensions are reduced roughly by a tenth. Freshly formed conidia are coated with a thin mucous layer, which disappears after some time. Conidial secession is schizolytic.

Microconidial state (presumed andromorph): coelomycetous, in agar cultures. Pycnidia embedded in the mycelial mat but easily separable, whitish in reflected, brownish in transient light, globose, 50–175 μ m across, wall thin, of more or less isodiametric cells in two (?) layers. Conidiophores or conidiogenous cells not seen. Conidia minute, ellipsoid, or irregularly elongate, $1.5-4 \times 1.5-1.8 \mu$ m.

DISCUSSION

The conidial shape and the percurrent proliferation of the conidiogenous cells point to the anamorphic genus Anguillospora. This is heterogeneous and polyphyletic. The teleomorphs, known in seven species, belong to four orders: Pleosporales (A. longissima (de Wild.) Ingold), Helotiales (A. crassa Ingold, A. furtiva J. Webster et Descals, A. fustiformis Marvanová and Anguillospora sp. anamorph of Loramyces juncicola W. Weston), Orbiliales (A. rosca J. Webster et Descals) and Lulworthiales (marine species A. marina Nakagiri etTubaki). Classification above the genus level is according to Eriksson et al. (2001). Species without known teleomorph are: A. filiformis Greath. and A. rubescens Gulis et Marvanová. All the above species have distinctly longer and often broader or narrower conidia.



Fig. 2. A-D. Anguillospora mediocris. A. Developing conidium. Note the percurrent proliferation of one conidiogenous cells (arrows). B., C. Detached conidia from pure culture, the right one in B turned upside down. Note the snake-head-like bases. D. Crust-like substrate mycelium and inflated cells on the aerial mycelium. Scale bar for A,D = 30 mm, for B,C = 50 mm.



Fig. 3. Spatial dynamics in conidial concentration of Anguillospora mediocris in the October samples at the sampling sites of the four streams studied. M1 - M6 = sites downstream at the Morgó stream, Csö = Csömöle stream, Nc1 - Nc2 = Nacsagrom stream, Ng1 - Ng2 = Nagyvasfazék stream.

A coelomycetous microconidial state is known in *Anguillospora longissima*, but this species differs by longer macroconidia, rhexolytic conidial secession and profuse percurrent growth of basal extension in detached conidia.

There are three poorly known species of Anguillospora, presently considered doubtful: A. curvula S. H. Iqbal, A. gigantea Ranzoni and A. pseudolongissima Ranzoni. A. gigantea has sigmoidly curved conidia of length several times exceeding that of conidia of our fungus. A. curvula has conidia outside the range of dimensions known for our fungus. The colony was described as greyish brown, with age becoming reddish brown. The conidial dimensions of A. pseudolongissima (50–100 × 4.6–6 m) almost match those of our fungus, but as noted by Marvanová et al. (1992) the latter is very probably a species of Filosporella. These authors support their opinion by the drawing in Ranzoni (1953, Fig. 1T) showing conidiophore with branching typical for Filosporella (see under F. versimorpha

Marvanová et al. below). Type material of *Anguillospora pseudolongissima* was not deposited.

Anguillospora sp. 1 reported by Descals (1997, Figs. 2,3) is described in terms which may characterize also our species: colony is dark grey, margin is lobed, conididophores are short, unbranched, proliferation of conidiogenous cells is percurrent or sympodial. Conidia are long cylindrical or subfusiform, typically $45-120 \times 3-4 \mu m$. However, in Anguillospora sp. 1 the characteristic attenuation and curvature of the apex are lacking, the sites of percurrent proliferations are inflated, no pseudoparenchymatous crusts were reported in the colony. We do not think that these two taxa are conspecific.

There are few other taxa known from water, which approximate our fungus in their conidial dimensions: Sigmoidea aurantiaca Descals has scolecoform conidia $25-90 \times 3.5-4.5 \ \mu\text{m}$, but differs by typically sympodial rachis on conidiophore apex and by orange colonies. Filosporella versimorpha Marvanová et al. (1992) whose conidia are of similar length to our fungus, is out of consideration due to its long conidiophores with acrotonous branching. Aquaphila albicans Goh, Hyde et Ho (very similar to Mirandina dactylelloides Matsush.) has whitish colonies, conidia twice as broad and sympodial conidiophore proliferation.

Some fungi described from leaf litter or from moist conditions also have conidia similar to those of our fungus. However, most of these taxa have typically sympodial conidiophore proliferation:

Mirandina flagelliformis Matsush., anamorph of Chaetonectrioides malaysiana Matsush. (Matsushima 1996) has conidia very similar, but of smaller dimensions. It differs also in a denticulate conidiophore apex and pale colony.

Paraarthrocladium amazonense Matsush. has conidia almost matching those of our fungus in dimensions, but with slightly inflated cells. The conidiogenesis was reported as blastic, with up to two conidia appearing at the conidiogenous cell, which collapses after conidial secession (Matsushima 1993).

Trichoconis antillana Castañeda, Kendrick et Guarro (1997) has obclavate, rostrate conidia, overlapping considerably with those of Anguillospora mediocris, but formed on polyblastic denticulate conidiogenous cells.

ECOLOGICAL CHARACTERISTICS OF A. MEDIOCRIS

The importance of this fungus in the hyphomycete communities of the Morgó stream system has become evident since conidial populations in these streams have been studied by membrane filtration. Spatial and temporal distribution patterns of conidia of this species have been statistically analysed at 7 sites in the stream system in the subsequent years from 1996 to 1998. The fungus was reported as *Filosporella* sp. 1. (Gönczöl et al. 1999; Gönczöl and Révay 1999 a) and as *Filosporella* sp. (Gönczöl and Révay 1999 b). The current study on two

further tributaries confirmed our findings on the spatial distribution of this species (unpublished). Some characteristics of the stream habitats where A. mediocris has been detected by membrane filtration are given in Table 1. The spatial dynamics of the conidium concentration of A. mediocris in the stream water in October samples are shown in Fig. 3. The increasing conidial number in downstream direction in the main channel of the stream system suggests a longitudinal distribution pattern that may be structured by environmental heterogeneity. The stream sections of low altitudes seem to be the preferred habitats of this species. The high conidium concentration in one of the tributaries (Csömöle stream) and the extremely high conidial number in an other tributary (Nacsagrom stream) also suggests that A. mediocris may be a typical hardwater species. At higher altitude and/or in softwater stream portions only low conidial concentration or no conidia of this species could be detected.

	Morgó-stream sites					
	1	2	3	4	5	6
altitude (m)	435	345	305	210	175	125
channel gradient %	12.66	5.66	3.07	2.37	1.09	1.51
stream order	second	second	third	fourth	fourth	fourth
temperature (C)	2.2-15.5	1.2-17.1	0.2-15.1	0.2-17.2	0.8-19.1	0.1-19.5
pH	6.9-7.1	7.1-7.3	7.1-7.4	7.2-7.4	7.4-7.9	7.4-8.3
conductivity (µS/cm)	177-188	177-182	192-229	219-309	276-563	317-614
total hardness (^e d)	2.3-4.8	3.3-5.9	4.2-5.9	4.7-7.0	5.6-12.7	7.0-22.0
	Tributary sites					
	Csöm	Nacs 1	Nacs 2	Nagy 1	Nagy 2	
altitude (m)	190	220	150	400	290	
channel gradient %	4.1	1.67	3.88	9.17	3.79	
stream order	second	second	second	second	second	
temperature (C)	0-17	5.5-11.5	7.1-11.5	7.5-9	7.2-11.5	
pH	7.8-8.3	7.4-7.8	8-8.1	7.1-7.6	7.5-7.7	
conductivity (μ S/cm)	400-720	630-770	750-970	175-200	220-290	
total hardness (°d)	13.5-17.5	15-19	19-22	5.0-7.0	6.0-10	

Table 1. Geographical and physicochemical data of 11 sites in the Morgó stream system.Csöm = Csömöle stream, Nacs = Nacsagrom stream, Nagy = Nagyvasfazék stream.

The conidia have been rarely seen in foam samples. It was pointed out that foam samples and membrane filtered water samples taken simultaneously at the same site differed dramatically in the conidium concentration of this species. While

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a considerable proportion of foam samples contained only some or no conidia, the conidium concentration in filtered stream water ranged from some hundred to several thousands conidia/L (unpublished observation). The fungus, growing on submerged leaves, (mostly on alder) is also commonly found in the same distribution area (see above). Very probably the same species (conidial dimensions from nature were 50–90 × 4.5–6 μ m) was reported and illustrated from three hardwater streams in the Aggtelek National Park as *Anguillospora* sp. 1. (Gönczöl and Révay 1992, Fig. 2.).

Outside Hungary the fungus is probably rare. We found only one illustration, which may represent a conidium of A. mediocris (Descals 1998, Fig. 15 K, as unknown). Its dimensions correspond well to those of this species, and the depicted conidium has the characteristic short basal extension. The conidium was collected in the valley of Karrantza (Basque country, Spain), at ca 500 m alt. in foam in a stream flowing through mixed Fagus wood and meadows, with Alnus, Corylus and Salix on the banks. The water chemistry is not given.

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