Studies on *Anguillospora longissima:* morphotypes or different species?

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Abstract. Sigmoid or crescent shaped conidia with acute basal and apical tips, partly resembling those of *Anguillospora longissima* are being encountered in streams in Hungary over the past two decades. Conidia are generally shorter and wider than those described by Ingold. Some of them are with characteristic rostrated distal part. In one of the streams abundant conidia have been observed on several occasions. But their identity with *A. longissima* remained questionable. Monoconidial isolations from the cylindrical, thin, long conidia of *A. longissima* ("longissima") and the short, wide, rostrate conidia ("rostrate") collected in the same stream, yielded different cultures. Conidia from strains differed in dimensions and shapes. The "longissima" strain produced exclusively thin, cylindrical-fusoid, "longissima" conidia exactly fitting those described by Ingold. In one of the "rostrate" strains mostly "rostrate" conidia developed. The other "rostrate" strain produced somewhat longer and thinner conidia. However, all of the conidia in both "rostrate" strains, even if some of them overlapped with conidia in the "longissima" strain, could be distinguished by their different degrees in taper. The spatial and temporal distributions of the two types of conidia in Hungary are also discussed.

Key words: *Anguillospora longissima* complex, taxonomy

Introduction

Scolecoform conidia belonging undoubtedly to the same species have been encountered for many years among the streamborne spores of Ingoldian fungi in different streams of Hungary. These conidia resembled those of *Anguillospora longissima* (Sacc. et Syd.) Ingold, but differed in shape and size from the well-known thin and long conidia (Ingold 1942). These conidia (referred here as "rostrate") were referred to as *Anguillospora* sp. in some papers of the present authors. Conidia in foam and forming on different substrata (leaves and wood) were collected several times from hardwater streams in the Aggtelek Mts. (Gönczöl & Révay 1992). This fungus was pointed out as a late colonist on submerged wood immersed in hardwater portions of the Morgó stream (Révay & Gönczöl 1990; Gönczöl & Révay 1993).

Conidial populations of a hardwater tributary of the Morgó stream, Börzsöny Mts. were sampled by membrane filtration (Gönczöl & Révay 1999). Conspicuously great numbers of the rostrate conidia could be found in the early spring and summer samples. Sleeve-like remains of the separating cell were recognised on some of the conidia and at first resembled *A. longissima.* However, the conidia differed markedly from the thin, long and almost cylindrical conidia (here called the "longissima" type) of *A. longissima* in having different conidial dimensions and in being rostrate distal curvature in most of conidia. We succeeded in obtaining pure cultures from both types, which were found in the same stream.

The purpose of the present paper is (1) to demonstrate the morphological characteristics of the "longissima" and "rostrate" conidia *in vitro* and *in vivo,* (2) to compare their cultures and (3) to discuss some ecological and taxonomic aspects in this „*A. longissima* complex".

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Materials and Methods

Submerged leaves, wood and foam were collected several times from a tributary (Csömöle stream) of the Morgó stream, Börzsöny Mts, Hungary. The leaves and wood were separately incubated in aerated distilled water at 15 °C for some days. Conidia from hyphomycetes colonising these substrata were checked by filtration of the incubation water and/or by harvesting conidia from the surface of the incubation water. Monoconidial primocultures from "rostrate" conidia were made on 2 % MA: on 24 September 2004 (CSR2004) and on 10 October 2006 (CSR2006). One culture from a "longissima" type conidium was established: on 25 November 2004 (CSL2004). Cultures were incubated at room temperature (20-22 °C) and colony pieces were later submerged in sterile distilled water, aerated and incubated at 15 °C in a refrigerator with a glass door. Some of the cultures were incubated in running tap water without aeration. Strips from the cultures (CSR2004 and CSL2004) were separately put into glass vials (50 ml of each). These were closed with a plastic stopper with two borings (water inlet and outlet) in each. One of the borings on the stopper was joined through a thin (4 mm diam) plastic tube to the tap. The water flow rate was set at 200-300 ml/min and checked daily. The water was at 11-12 °C during the experiment in February 2006. These non-axenic cultures in flowing tap water were maintained for some weeks.

Results and Discussion

Anguillospora longissima was first described by De Wildeman (1893) as *Fusarium elongatum,* but as the specific epithet had already been used for another species, Saccardo & Sydow (Saccardo 1899) proposed a *nom. nov., F. longissimum.* Ingold studied in culture the development and release of conidia and proposed a new genus *Anguillospora,* with *A. longissima* (Sacc. et Syd.) Ingold as the type species. He emphasised the presence of the separating cell as a decisive character, distinguishing it from other scolecosporous taxa. Several other authors understood the species more broadly, claiming that the separating cell may not always be present (Ranzoni 1953; Petersen 1962). Webster & Descals (1979) accepted Ingold's original concept including the separating cell and described another scolecosporous species, with conidia of similar shape and dimensions but without separating cell, under the provisional name *Anguillospora furtiva,* validly published later by Descals *et al.* (1998).

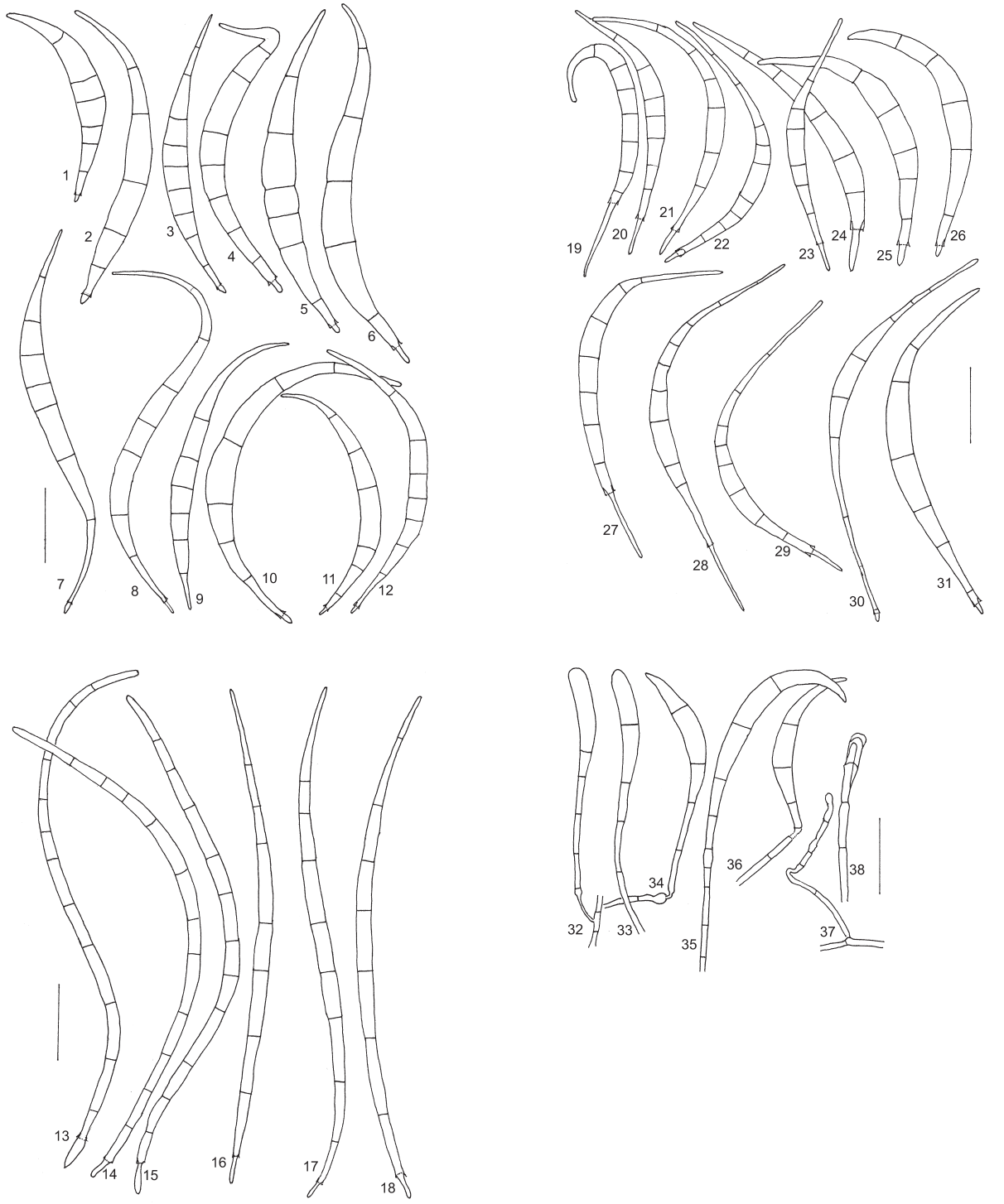
The presence of the separating cell is now accepted as the most decisive diagnostic character, although it may not always be so obvious, either due to optics or to the shallowness of the remnant depending on where circumscission has taken place. Since the description of *A. longissima,* numerous records suggest the worldwide distribution of this species (Ingold 1942; Ranzoni 1953; Petersen 1962; Nilson 1964; Aimer & Segedin 1985). However, observations of conidia in nature and in culture suggested the possibility of much morphological variation of *A. longissima* conidia *in vivo* as well as *in vitro* (Willoughby & Archer 1973; Webster & Descals 1979; Baschien *et al.* 2006). In the original description De Wildeman illustrated conidia clearly tapering both ways, measuring 100-400 × 3-7 µm and with 5-10 septate. Ingold's conidia were 200-350 long and 5-6 µm wide, almost cylindrical conidia, and only moderately tapering towards the basal and apical ends. A number of shorter and wider conidia conspicuously differing from the dimensions given by Ingold have been reported under the name *Anguillospora longissima* or as *Anguillospora* sp. (Table 1).

Because of the significant variation in conidial dimensions, Marvanová is of the opinion that *A. longissima* may be a species aggregate (Baschien *et al.* 2006).

In most of the streams studied in Hungary the "longissima" conidia have frequently been seen, though mostly in low numbers. In some of the streams we have also seen sigmoid or crescent-shaped conidia definitely tapering towards both tips (the "rostrate" type) (Figs 1-12). They were widest in the lower third, with both ends relatively thin, and their upper third was characteristically curved. Both the apical and basal parts of most conidia mounted in lactophenol-cotton blue appeared to be very sharp (Figs 7-12), mostly measuring 120-180 × 7-10 (–13) µm and with 6-10 septa. There was clear evidence of the separating cell remnants in some of them. The widest conidia (13 µm) were seen in water mounts (Figs 5-6, 10). An immediate shrinkage in the width of conidia was seen when lactophenol-cotton blue was added to water mounts. The "rostrate" conidia considerably differed from those described and illustrated by Ingold and were referred to as *Anguillospora* sp. (Table 1). We doubted whether these conidia just belonged to a morphotype of *Anguillospora longissima* or whether we were dealing with an undescribed

Table 1. Conidial dimensions of *Anguillospora longissima* and *Anguillospora* sp. in vivo or in vitro, as reported in some papers

A. longissima **(µm)**	**References**	***Anguillospora*** **sp. (µm)**	**References**
100-400 × 3-7	De Wildeman 1893	140-180 × 7-9 (–11)	Révay & Gönczöl 1990
200-350 × 5-6	Ingold 1942	120-150 × 9-10 (–11)	Gönczöl & Révay 1992
150-350 × 5-6	Ranzoni 1953	130-150 × 8-10	Gönczöl & Révay 1999
150-350 × 6-7	Petersen 1962		
150-290 × 5-7	Willoughby & Archer 1973		
132-220 × 5.5-8	Webster & Descals 1979		
110-230 × 7	Aimer & Segedin 1985		



Figs 1-12. *Anguillospora* sp. "rostrate" conidia in nature, Csömöle stream, Hungary. **Figs 1-6.** Conidia from leaves incubated for 10 days. **Figs 7-8, 10.** Conidia from wood incubated for 10 days. **Figs 9, 11-12.** Conidia filtered out of stream water. Bar = 30 µm. **Figs 13-18.** Comparison of selected conidia with similar dimensions produced in culture. **Figs 13-15.** Conidia from the CSL2004 ("longissima") strain. **Figs 16-18.** Conidia from the CSR2004 ("rostrate") strain. Bar = 30 µm. **Figs 19-31.** Conidia from the "rostrate" culture (CSR2004) incubated in standing water. **Figs 19-26.** The same, after 6 days aerated incubation. **Figs 27-31.** After 8-10 days aerated incubation. Bar = 30 µm. **Figs 32-38.** Conidial development in the "rostrate" culture (CSR2004) after 6 days incubation. **Figs 32-36.** Attached conidia in early stages of development, with (Figs 34, 36) and without (Figs 33, 35) clear evidence of a separating cell. **Figs 37-38.** Spent conidiophores after percurrent development of conidia. Bar = 30 µm

species of *Anguillospora.* The cultures established from the two types of conidia yielded, in part, different characters.

Characterisation of cultures

Primocultures (CSR2004 and CSR2006) derived from "rostrate" type conidia were similar to each other in having a dirty white or pale fuscous colony with very scanty or appressed aerial mycelium. Colony growth: 45-50 mm diam after two months. The dark brown or black pigmentation was lacking or developed only in small isolated patches in young primocultures or appeared only with age (in one- or two-month old cultures). Buff patches appeared also in old cultures. However, the blackish colour appeared sooner (after 10-12 ds) in some of the subsequent subcultures. Reverse brownish with paler marginal ring.

Primoculture (CSL2004) derived from the "longissima" type conidium: 45 mm diam after two months, the dark brown or black pigmentation developing concurrently with colony growth. Aerial mycelium mid- or reddish brown, abundant, woolly, in three to four concentric rings but sparse in the centre. Reverse dark brown with a well-developed brownish-black marginal ring.

Incubation of cultures (CSL2004, CSR2004 and CSR2006) in standing water with or without aeration

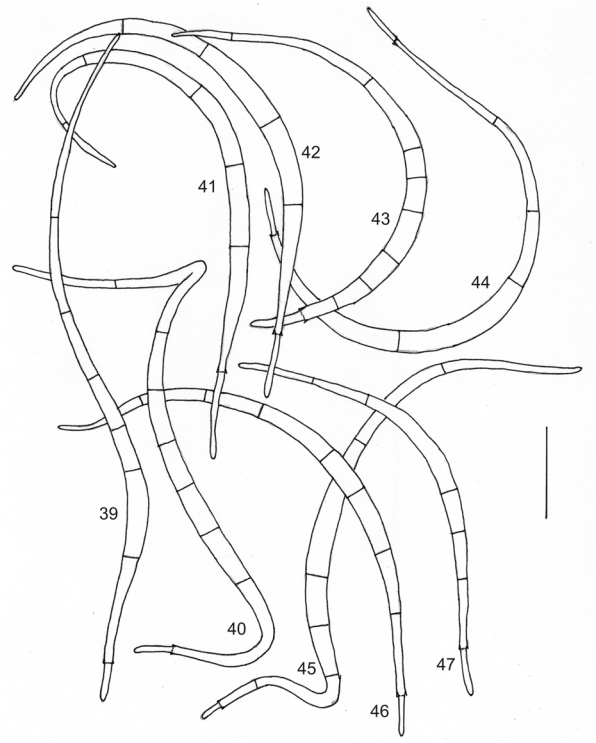
Sporulation was relatively late in the "longissima" culture (CSL2004). Culture strips submerged in standing water produced no conidia during the first month of incubation, irrespective of aeration. Sporulation occurred in the second month and lasted even into the third month of underwater incubation. Many conidia were obtained when the same colony slivers were then half-submerged at the same temperature (15 °C). The conidia obtained (Figs 13-15) were identical both in dimensions and shape with those described and illustrated by Ingold (1942). Some of them were over 200 (and up to 250) µm long, many of them barely shorter than 200 µm (190-200). No conidia were wider than 6 µm (typically 5-6 µm) and all had the long less tapered portion so characteristic in Ingold's illustrations (Ingold 1942; Text-figs 42-43). The only difference was that in most conidia there were more septa (between 10 and 15). We did not find even a single "rostrate" type conidium.

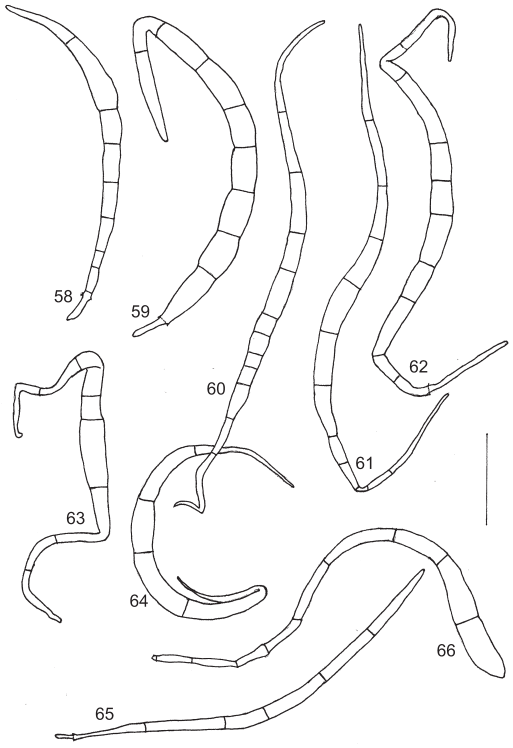
All conidia (Figs 19-31) obtained in the "rostrate" type culture (CSR2004) were of dimensions and shapes noticeably different from the thin, long, cylindrical-fusoid conidia of *A. longissima.* Conidia developed readily after one week's submergence of the colony strips in aerated water. Many of them were typically rostrate, undoubtedly identical with the conidia regularly seen in great numbers on membrane filters and in foam from the Csömöle stream. After six days' incubation of colony slivers in aerated, unchanged distilled water, relatively short (120-150 µm) conidia of various widths (7-10 µm) were mostly seen (Figs 19-26). Incubation for eight to ten days yielded somewhat longer conidia. Especially those harvested from the water surface showed a conspicuous bipolar elongation suggesting the post-release growth (Figs 27-31). Conidia in this culture began to develop as a relatively thick primordium (Fig. 32). They broadened to *ca* 8 µm even at a very early stage of development (Figs 32-34). The characteristic distal curvature and rostrum also developed soon in attached conidia (Figs 35-36). Separating cells were observed in two conditions: probably due to the shrinkage of the separating cell an isthmus appeared between the conidiogenous cell and the conidial base (Fig. 34). The other condition was observed when the separating cell was presumably evanescing, appearing loosely connected to the conidiogenous cell and the conidium (Fig. 36). This second stage was generally seen when conidium release was examined on natural substrata (e.g. leaves).

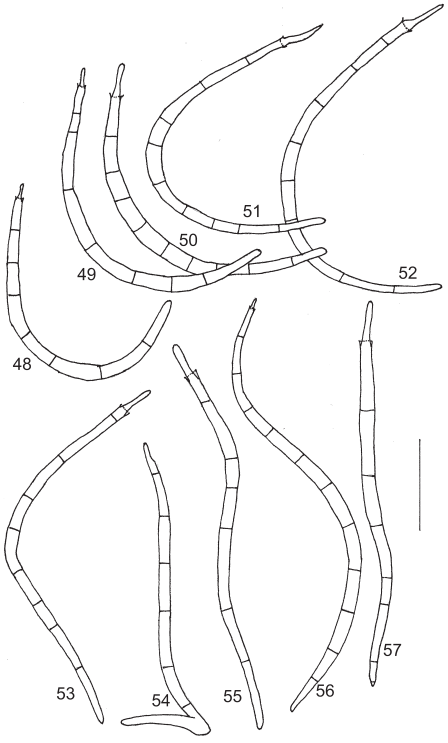
The culture of CSR2006 was established from a "rostrate" conidium selected from the incubation water on leaves from the Csömöle stream. Slivers from a 35-day culture were submerged and aerated for a week. Abundant sporulation was obtained. Conidia were longer and thinner (typically 160-200 × 7-8 µm) than those in the other culture (CSR2004), but retained their main characteristics, viz.: the rostrum and the marked degree in taper (Figs 39-47). We have however seen some additional forms: strongly curved crescent-shaped conidia (Figs 40-45) as well as some atypically long sigmoid forms (Fig. 39). A comparison of morphological and cultural characters of "longissima" and "rostrate" conidia is given in Table 2.

Table 2. Morphology and dimensions of "longissima" and "rostrate" conidia and main cultural characters

Characters	"longissima" CSL2004	"rostrate" CSR2004, CSR2006
Cultures	Dark brown-black, aerial mycelium wholly	Pale fuscous-whitish, aerial mycelium absent to very sparse
Conidia (µm)	160-220 (–250) × 4-6 µm	100-200 (–230) × 6-10 µm
Length/width ratio	Typically above 30	Typically below 30
Septation	Typically 10-15	Rarely above 10
Shape	Thinly fusoid Never with curved rostrum	Fusoid Frequently with curved rostrum
Degree of tapering	Moderate: due to the long cylindrical part	Marked: due to the short cylindrical part







Figs 39-47. Conidia from a 35-day old "rostrate" culture (CSR2006) after one week aerated submergence in standing water. Bar = 30 µm. **Figs 48-57.** Conidia from the "longissima" culture (CSL2004) after one-week incubation in running tap water. Bar = 30 µm. **Figs 58-66.** Conidia from the "rostrate" culture (CSR2004) after one-week incubation in running tap water. Bar = 30 µm

Incubation of non-axenic cultures (CSL2004 and CSR2004) in running tap water without aeration

Sporulation in the "longissima" culture CSL2004 was abundant after one week incubation in running tap water and lasted for weeks. Conidial width was 4-6 µm, quite similar to that in the axenic culture incubated in standing water, but conidia were surprisingly short (80-130 (–160) µm) (Figs 48-57). At the same time, the cylindrical middle portion remained quite similar to those of *A. longissima* with normal lengths. This experiment suggested that water flowing through the culture might induce shortening of conidia but apparently not the width nor the shape of the "longissima" type conidia.

Sporulation of the "rostrate" type culture CSR2004 after one-week incubation was not as abundant as that in aerated standing water. We have seen a number of variously

injured, distorted or "crumpled" conidia (Figs 58-66) which were lacking in standing water. This suggested that water flow disturbed rather than stimulated conidial development in this strain. Nevertheless, all of these conidia retained the main characters (presence of a rostrum and a marked taper towards both ends), and they were well recognisable and distinguishable from the "longissima" type conidia.

The "rostrate" conidia are generally somewhat wider and shorter than the "longissima" ones. In these cases it is easy to recognize the marked differences in morphology (Figs 13-15; 19-31). However, in some cases conidium dimensions may overlap. In Figs 13-18 the six conidia selected were of almost identical width (6 µm) and the length is also very similar (approx. 200 µm). All appear to be very similar to each other, but still distinguishable by their degree of taper. In the "longissima" type this is very gradual, i.e. the 5-10 middle cells hardly tapering at all and appearing as a fairly long tube. However, the "rostrate" type conidia taper more markedly towards both ends, lacking the long, cylindrical portion, or it is otherwise short. The apical part of the "longissima" type conidium is 2-3 µm wide and more or less rounded, while it is only 1.5-2 µm wide (i.e. more sharp) in "rostrate" type conidia. This little difference is measurable with difficulty, but the different taper is well recognisable in most cases. The same is true when cultures incubated in running tap water are compared. Conidia in the "longissima" strain (Figs 48-57) are clearly distinguishable from those obtained from the "rostrate" culture (Figs 58-66). The long-cylindrical portion and relatively blunt ends in the "longissima" type, as opposed to the sharply pointed ends and strong taper in rostrate conidia, appear to be stable morphological characters and are thus of diagnostic value.

The conidia of *Anguillospora longissima* and of the rostrate *Anguillospora* collected in Hungary have been distinguished by us for many years. The spatial distribution of aquatic hyphomycetes was studied in nine streams of the Aggtelek National Park (Gönczöl & Révay 1992) and in seven streams in the catchment area of the Morgó stream system in the Börzsöny Mts. (Gönczöl & Révay 1999, 2003, 2004; Gönczöl *et al.* 1999). Conidia of *A. longissima* sensu Ingold were regularly found in all streams and in all seasons, but their numbers were significantly higher in the softwater streams. Casas & Descals (1997) in a study of aquatic hyphomycetes of two Mediterranean streams in Spain, also found *A. longissima* more frequently in the circumneutral than in the alkaline stream. On the contrary, the conidia of the rostrate-type *Anguillospora* were found exclusively or more frequently in hardwater streams, and were especially numerous in spring and early summer.

The development of the two conidial types is quite similar, but their shape and dimensions both *in vitro* and *in vivo* are different. We believe that *Anguillospora longissima* and the rostrate type *Anguillospora* are distinct species.

To resolve the correct taxonomic position of the above strains inevitably requires molecular studies. Phylogenetic analyses of five strains of *A. longissima* carried out by Baschien *et al.* (2006) did not succeed in separating them taxonomically. However, we do not know if these included the "rostrate" type we observe in Hungary.

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