



***Polylobatispora setulosa*, a new freshwater hyphomycete from Ilhabela, Sao Paulo state, Brazil**

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Abstract

Polylobatispora setulosa sp. nov. is described and illustrated from submerged mixed leaf litter samples collected at streams in Ilhabela, São Paulo state, Brazil. The fungus is distinct in having setulae at the tip of the conidial lobes. *Acumispora verruculosa*, isolated from identical substrate and characterized by the presence of fusiform, acuminate, rostrate, verrucose, 2-septate, hyaline to greenish conidia, is recorded for the third time from the Americas. They are compared with morphologically similar species and identification keys are provided.

Keywords – anamorphic fungi – freshwater fungi – systematics – tropical fungi

Introduction

Among freshwater hyphomycetes, the so called typical aquatic or Ingoldian fungi have been studied extensively in Brazil, mainly in well-preserved areas of the “cerrado” region (Schoenlein-Crusius 2002), the Atlantic rainforest of the state of São Paulo (Schoenlein-Crusius & Grandi, 2003) and also in a few urban waters (Schoenlein-Crusius et al. 2009, 2014). Recently, however, increasing attention has been paid to other groups of freshwater fungi, particularly those hyphomycete genera frequently reported from terrestrial as well as freshwater habitats and known as facultative-aquatic (Goh & Hyde 1996) or inmigrants (Shearer et al. 2007). While the semi-arid “caatinga” biome in the northeast Brazil has proven to be rich in freshwater fungi (Barbosa & Gusmão 2011, Barbosa et al. 2013, Carneiro et al. 2012), other poorly studied or unexplored areas are also believed to harbour a high diversity of this group of fungi.

Between the years 2012 and 2013, an extensive survey of freshwater hyphomycetes inhabiting submerged materials was carried out in the islands off the coast of the state of São Paulo, including the island of São Sebastião in the municipality of Ilhabela. Two interesting anamorphic taxa were collected on mixed submerged leaf litter samples. They are morphologically similar to the genera *Acumispora* and *Polylobatispora*, but the latter was found distinct enough from previously described species to be considered as a new taxon. Taxonomic descriptions and illustrations are presented here along with identification keys for both genera.

Materials and Methods

Sampling and morphology

Samples of submerged mixed leaf litter (approx. 10g) and local water were collected in sterile polyethylene bottles (about 200 ml) and brought to the laboratory. The leaves were cut into pieces in approximately 1 cm diam. and incubated in Petri dishes containing sterile distilled water. They were kept in climatic chambers at around 20°C following Ingold (1975). From the seventh day of incubation and for at least one month, slides of leaf fragments were prepared using polyvinyl alcohol lacto glycerol resin as mounting medium (Morton et al.1993). Fungal structures were observed and measured using an Olympus BX50 light microscope and images were taken with a Leica DM LB2 microscope fitted with a Leica DFC 280 camera.

Isolates

In order to obtain pure cultures of fungi, water droplets containing conidia of the target fungi were captured with a Pasteur pipette and spread over the surface of Malt Extract Agar (MEA) Petri dishes containing Chloramphenicol (final conc. 25mg/L). After 24 h of incubation in climatic chambers at 21°C, the reverse side of the dishes was observed using a stereoscope to verify hyphal growth the position of the conidia was marked with a permanent marker pen. Under aseptic conditions and with the aid of a sterile stainless steel needle, the conidia were removed from the surface of the medium, inoculated onto a new MEA Petri dish and incubated under similar conditions. Permanent slides are deposited in the Herbarium "Maria P. Eneyda Kauffmann Fidalgo" (SP) and fungal cultures are deposited in the Fungal Collection of the Instituto de Botânica, São Paulo (CCIBt).

Scanning electron microscopy

For scanning electron microscopy (SEM), mycelia with conidiophores and conidia grown on MEA were prepared according to the modified method described by Pan et al. (1994) They were fixed overnight in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.4), washed thoroughly with distilled water, dehydrated through an ethanol series (10%, 40%, 60%, 80% and 100%), each series lasting 30 minutes and dried in silica gel. After preparation, the stubs were covered with double-sided carbon tape, and the specimens were mounted on stubs, sputter-coated with gold, and examined using a PHILIPS XL series XL 20, S/W, 5.21. scanning electron microscope at 10KV.

Taxonomy

Polylobatispora setulosa L.B. Moro, G. Delgado & I.H. Schoenlein-Crusius sp. nov. Fig. 1–6
Mycobank MB811045

Etymology – Latin, *setulosa*, provided with *setulae*.

Colonies on natural substrate inconspicuous. *Conidiophores* macronematous, septate, hyaline, forked, bottle-shaped, 10.0–25.74 × 1.43–2.5 µm. *Conidiogenous cells* lageniform, enteroblastic-phialidic, hyaline to greenish. *Conidia* hyaline, triangular, 3-lobed, 8.6–14.3 µm diam., with thick dark walls and a hyaline setula at each tip, 5.7–12.87µm long; with a central, fuscous halo, 5.7–7.0 µm diam.

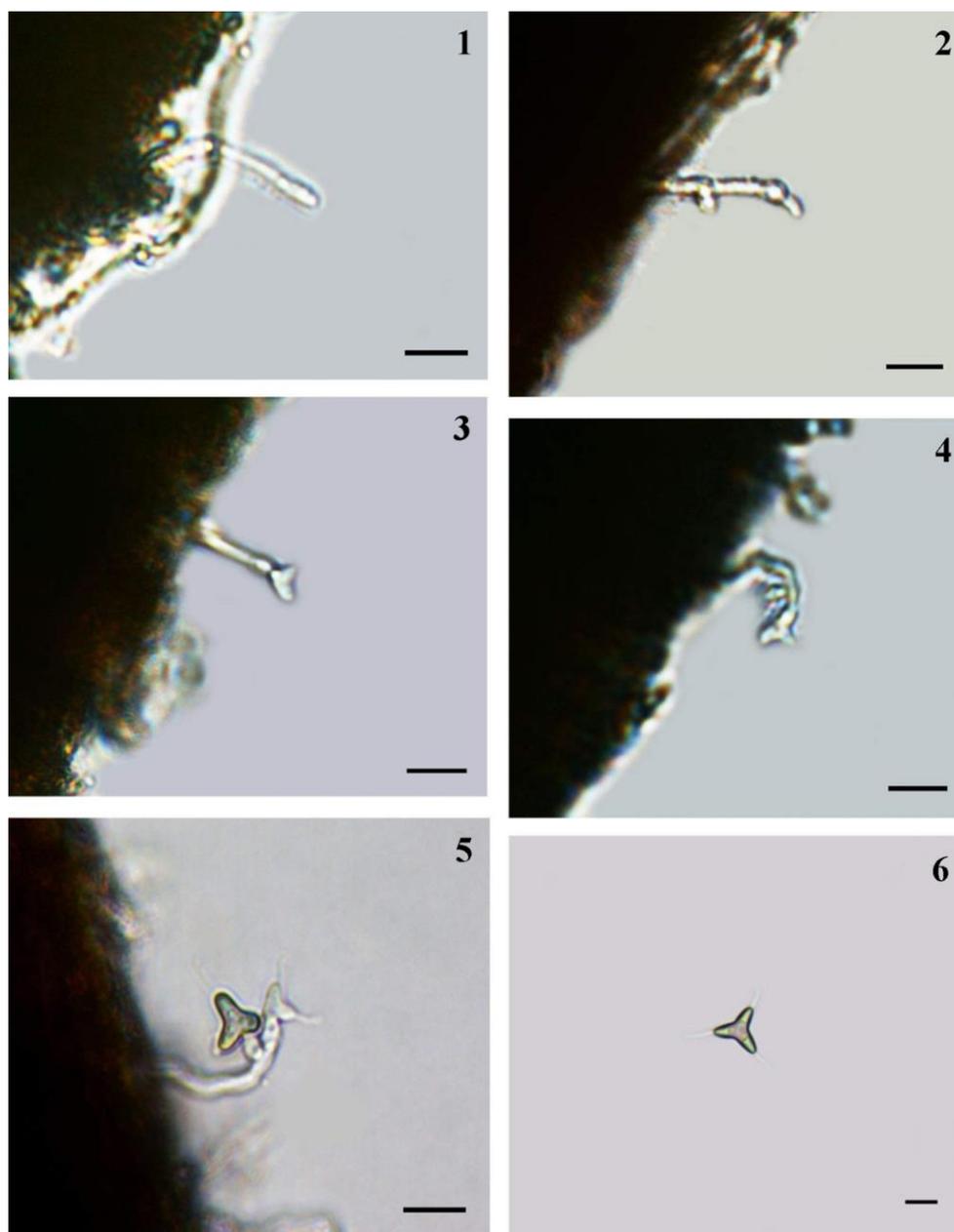
Teleomorph – unknown.

Material examined – Brazil, São Paulo, Ilhabela, São Sebastião island, “Cachoeira do Gato” stream, on submerged mixed leaf litter, 14 May 2013, L.B. Moro (Holotype: SP445-978).

Note – Pure culture of *Polylobatispora setulosa* was not possible to obtain.

Acumispora verruculosa Heredia, R.F. Castañeda & R.M. Arias. Mycotaxon 101: 90, 2007.

Fig. 7–12



Figs 1–6 – *Polylobatispora setulosa*. SP 445-978 (holotype). 1-5 Developmental stages of conidiophores and conidia. 6 Mature conidium with setulae at the tip of each lobe and central halo. Scale bar = 10µm.

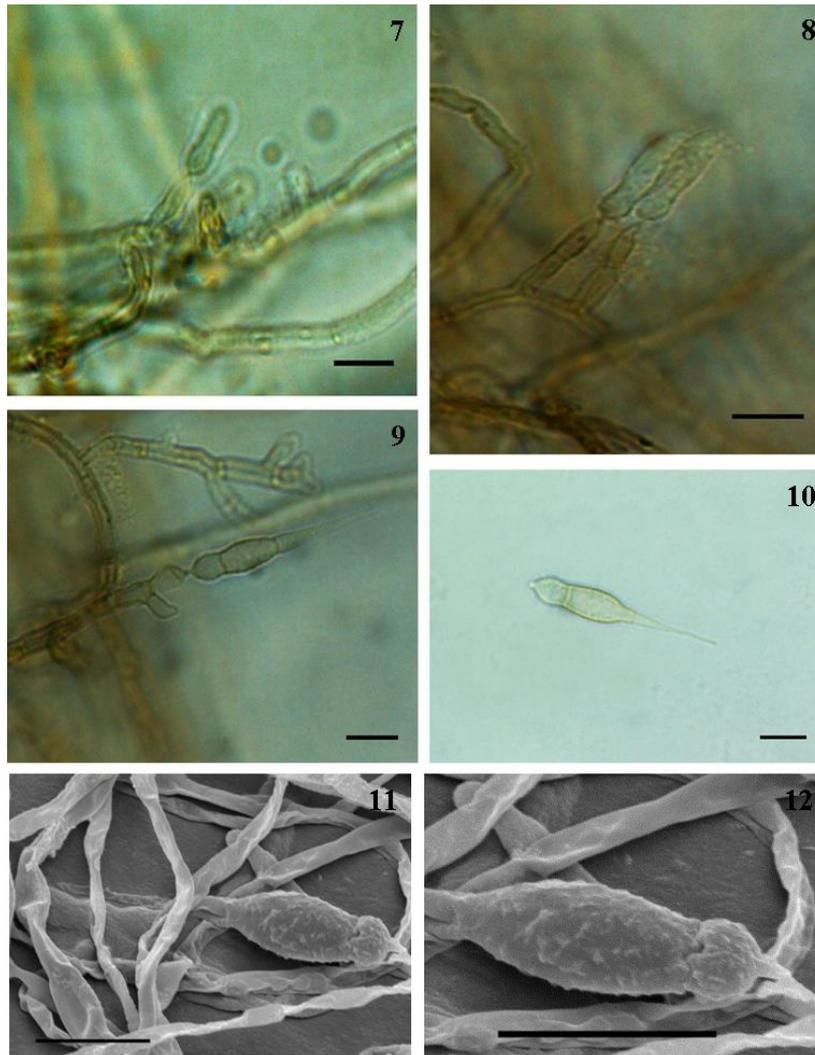
Colonies on MEA dark gray to greenish, velvety, slow growing, reaching less than 2 cm diam. after 2 weeks of incubation at 21 °C. *Mycelium* composed of branched, septate, hyaline to pale brown hyphae. *Conidiophores* macronematous, mononematous, erect, simple or branched, 0–4-septate, light brown to brown, 9.0–51.5 × 2.86–4.30 µm. *Conidiogenous cells* mono- or polyblastic, integrated, terminal and intercalary, proliferating sympodially, denticulate; conidiogenous loci apical or lateral, often protuberant and denticle-like; conidial secession rhexolytic. *Conidia* fusiform, 2-septate, constricted at the septa, with rounded basal cell and slender apical cell, acuminate, rostrate, hyaline to greenish, 30–45 (–50) × 5.0–8.75 µm, verruculose, with the basal cell often bearing a denticle-like detachment scar.

Teleomorph – unknown.

Material examined – Brazil, São Paulo, Ilhabela, São Sebastião island, “Cachoeira do Gato” stream, on submerged mixed leaf litter, 14 May 2013, L.B. Moro (SP445-979, CCIBt 4066).

Discussion

The genus *Polylobatispora* was originally described from leaves collected in Malaysia (Matsushima 1996) with two species: *P. deltoidea* Matsush., the type species, and *P. quinquecornuta* Matsush., based on the presence of cylindrical or bottle-shaped (doliiform), conidiophores and enteroblastic-phialidic, hyaline, conidiogenous cells that produce single, light brown, stauroconidia with a small scar formed after detachment from the conidiophore. *Polylobatispora deltoidea* presents characteristic triangular conidia with three lobes and *P. quinquecornuta* 5-lobed conidia. New records of these species are unknown. The main difference between *Polylobatispora deltoidea*, *P. setulosa* and *P. quinquecornuta* is the number of lobes: the first two species possess 3 lobes and the latter 5 lobes. The 3-lobed *P. setulata* differs from *P. deltoidea* by slightly larger conidia and the presence of flexible setulae, one at the tip of each lobe.



Figs 7–12 – *Acumispora verruculosa*. 7-9 Conidiophores, conidiogenous cells and conidia. 10 Mature conidium. 11 SEM image of mature conidium. 12 Details of the conidia wall. Scale bar = 10µm.

Identification key to species of the genus *Polylobatispora*

- 1a Conidia 5-lobed.....*P. quinquecornuta*
- 1b Conidia 3-lobed.....2
- 2a Conidia 6–8 µm diam., setulae absent.....*P. deltoidea*
- 2b Conidia 8.6–14.3 µm diam., setulae present.....*P. setulosa*

Table 2 Conidial morphology in species of *Polylobatispora*

Species	Conidia			References
	Shape	Diameter (μm)	Presence\absence of setulae	
<i>P. deltoidea</i>	3-lobed	6-8	absent	Matsushima (1996)
<i>P. quinquecornuta</i>	5-lobed	11-15	absent	Matsushima (1996)
<i>P. setulosa</i>	3-lobed	8.6-14.3	present	this paper

Matsushima (1980) described the genus *Acumisporea* to accommodate three species collected on dead leaves in Taiwan: *A. uniseptata* Matsush., the type species, *A. biseptata* Matsush., and *A. phragmospora* Matsush., on the basis of a peculiar acuminate conidial shape and conidia, with different number of septa that secede rhexolytically from monoblastic or polyblastic, sympodially proliferating, denticulate conidiogenous cells on simple or irregularly branched conidiophores. Heredia et al. (2007) described *A. verruculosa* Heredia, R.F. Castañeda & R.M. Arias from submerged leaves collected at the waterfall “Los Tuxtlas” in Veracruz, Mexico, with distinctly rough-walled conidia. This fungus was recently reported for the first time from South America on submerged leaves and branches collected in aquatic environments at the urban area of Belém do Pará (“Ilha do Combu”, “Parque Ecológico Gunma” and “Parque Estadual do Utinga”) located in the Amazon biome (Silva et al. 2013). Our collection is the third from the continent with the conidia slightly larger than in earlier collections indicating that there are variations in conidial size: earlier collections are $25.4\text{--}33.5 \times 4.4\text{--}5.5 \mu\text{m}$ whereas the present collection has conidia measuring $30\text{--}45 \times 5.0\text{--}8.75 \mu\text{m}$. Taxonomic key and diagnostic features of described species in *Acumisporea* are given below.

Identification key to species of *Acumisporea*

- 1a. Conidia with verruculose walls.....*A. verruculosa*
 1b. Conidia smooth-walled.....2
 2a. Conidia 1-septate.....*A. uniseptata*
 2b. Conidia more than 1-septate.....3
 3a. Conidia 3-6 septate.....*A. phragmospora*
 3b. Conidia 2-septate.....*A. biseptata*

Table 1 Diagnostic characteristics of the genus *Acumisporea*

Species	Conidia					References
	Nº of septa	Length (μm)	Width (μm)	Color	Wall texture	
<i>A. uniseptata</i>	1	23-28	5.5-6.5	Hyaline to very light brown	smooth	Matsushima (1980)
<i>A. biseptata</i>	2	16-25	3.8-5	Hyaline to very light brown	smooth	Matsushima (1980)
<i>A. phragmospora</i>	3-6	20-35	4-5	Brown to subhyaline	smooth	Matsushima (1980)
<i>A. verruculosa</i>	2	25.4–33.5	4.4–5.5	Pale brown	verruculose	Heredia et al. (2007)
<i>A. verruculosa</i> (present collection)	2	30-45	5.0-8.75	Hyaline to greenish	verruculose	This paper

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