Phytotaxa 338 (3): 276–284
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.338.3.5

The genus *Tetracladium* in Pampean streams (Buenos Aires, Argentina)

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Abstract

Ingoldian fungi are frequently found in streams of the northern hemisphere, as well as in other regions worldwide. Even though they have high relevance as decomposers of fallen leaves, information on this group of fungi in Argentina is scarce. To assess the presence of ingoldian fungi in Pampean streams, samples of foam from 14 streams of different basins were collected. Four species and a new form of *Tetracladium* were recorded: *Tetracladium breve*, *T. furcatum*, *T. marchalianum*, *T. setigerum* and *Tetracladium* sp. *Tetracladium furcatum* is reported for the first time in South America, while *T. breve* and *T. marchalianum* are first records for Argentina.

Key words: Ingoldian fungi, new form, new records, Pampas region, South America

Introduction

Ingoldian fungi are a cosmopolitan and phylogenetically heterogeneous assemblage from members of the Basidiomycetes and Ascomycetes, which actively grow and sporulate under water (Chang *et al.* 2000). Their spores are highly adapted to dispersion by currents, either free-floating or trapped in foam (Chang *et al.* 2000). These fungi are the main decomposers of leaf litter in forested streams and important mediators of energy flow and nutrient cycling by mineralizing and making leaves more palatable and nutritious to stream detritivores (Bärlocher 1995, Gessner *et al.* 1999, Graça & Canhoto 2006, Wurzbacher & Grossart 2011).

Even though ingoldian fungi are widespread in most temperate streams, studies on this group in Argentina, and particularly in the Pampas region, are scarce (Arambarri *et al.* 1987a, b, c, Cabello *et al.* 1990, 1993, Cazau *et al.* 1990, 1993).

The Pampas region is an extensive territory covered with grasses, with a temperate climate and the developing of intense agricultural activity. Streams in this region are shallow and of variable width, with abundant macrophytes, which are the main source of organic matter for decomposers (Giorgi *et al.* 2005). The riparian vegetation in these streams is predominantly herbaceous, however, in the last few years it has been modified by invasion of woody plants that have introduced new sources of allochthonous organic matter (Feijoó *et al.* 2012, Giorgi *et al.* 2014, Vilches *et al.* 2014). In addition, other human activities such as agriculture, urbanization, and industry have considerably modified the original biological and physicochemical characteristics of Pampean streams.

The genus *Tetracladium* De Wild. (Leotiomycetes, Helotiales) is cosmopolitan and contains 11 valid species (www.mycobank.org): *T. apiense* R.C. Sinclair & Eicker, *T. breve* A. Roldán, *T. ellipsoideum* M.M. Wang & Xing Z. Liu, *T. furcatum* Descals, *T. globosum* M.M. Wang & Xing Z. Liu, *T. marchalianum* De Wild., *T. maxilliforme* (Rostr.) Ingold, *T. nainitalense* (Sati & Arya), *T. palmatum* A. Roldán, *T. psychrophilum* M.M. Wang & Xing Z. Liu, *T. setigerum* (Grove) Ingold (Roldán *et al*. 1989, Sati *et al.* 2009). Species of *Tetracladium* produce holoblastic and staurosporous conidia with digitiform, acicular and filiform appendicular elements developed from a central axis, which help downstream transport (Webster 1959). Conidiogenous cells are polyblastic with sympodial proliferation and conidiophores can be simple or branched and either lateral or terminal (Roldán 1989).

To date, only five species have been reported in South America: *T. breve* and *T. nainitalense* in Brazil; *T. marchalianum* in Chile and Venezuela; *T. maxiliforme* in Chile and Venezuela; and *T. setigerum* in Argentina, Chile and

Venezuela (Fernández da Silva & Smits Briedis 2015, Fiuza & Gusmão 2013, Schoenlein Crusius & Piccolo Grandi 2003). In Argentina, *T. setigerum* has been previously reported only in the Pampas region and southern Patagonia (Godeas 1985, Schoenlein Crusius & Piccolo Grandi 2003, Marano *et al.* 2011). Therefore, the aim of this study was to assess the richness of species of *Tetracladium* in 14 streams from six different basins of the Pampas region, Buenos Aires, Argentina.

Materials and methods

Study area and sampling sites

Fourteen streams that belong to six different basins in the north of the Pampas region were sampled (Fig. 1, Table 1). All sampled sites are located in areas with agriculture and absence of livestock farming.

Sampling and laboratory analysis

Samples of foam and water were collected from the streams during winter and spring of 2016 and 2017. For each stream, the dominant vegetation was characterized and some physical and chemical factors were measured *in situ* using a parametric sensor HACH HQ 40.

Foam and water samples were taken with 60 ml sterile syringes and immediately filtered using cellulose acetate membranes (pore size 5 µm) and a filter holder. In the laboratory, membranes were stained with 0.1% cotton blue in lactophenol and mounted onto microscope slides. Membranes were observed using an optical microscope and conidia of *Tetracladium* spp. were identified using keys and monographs (Roldán 1989, Gulis *et al.* 2005) and photographed with a microscope Nikon Eclipse E200 equipped with a Tucsen built-in camera. A taxonomic key was constructed for the identification of *Tetracladium* species found in Pampean streams.

Samples are kept in the Phytopathology Lab, in the fungal collection of the National University of Luján (UNLu), Buenos Aires, Argentina. Samples have been deposited in the Buenos Aires Fungal Collection (BAFC) (Thiers 2018).

TABLE 1. Location of the streams, according to basin, locality, and coordinates.

Stream	Basin	Locality	Coordinates
La Guardia	Areco river	Carmen de Areco	S 34° 21´ 28.793´´ W 59° 48´ 11.491´´
Giles		San Andrés de Giles	S 34° 27´ 17.284´´ W 59° 27´ 27.91´´
Vagues		San Andrés de Giles	S 34° 17´ 35.167´´ W 59° 27´ 2.48´´
Los Sauces	De la Cruz river	San Andrés de Giles	S 34° 24´ 26.485´´ W 59° 15´ 42.305´´
De la Cruz		Exaltación de la Cruz	S 34° 21´ 39.024´´ W 59° 11´ 29.845´´
S/N1		Luján	S 34° 27´ 50.47´´ W 59° 15´ 1.957´´
Haras	Luján river	Luján	S 34° 31´ 32.862´´ W 59° 10´ 4.461´´
Balta		Mercedes	S 34° 40´ 27.264´´ W 59° 19´ 40.105´´
Frías		Mercedes	S 34° 36´ 38.747´´ W 59° 25´ 32.7´´
Durazno Chico	De la Reconquista river	Marcos Paz	S 34° 48´ 23.04´´ W 58° 58´ 56.179´´
Durazno		General Las Heras	S 34° 47´ 38.416´´ W 59° 02´ 23.764´´
Morales	Matanza-Riachuelo river	General Las Heras	S 34° 27´ 50.47´´ W 59° 15´ 1.957´´
S/N2	Salado river	Navarro	S 34° 58´ 57.645´´ W 59° 13´ 27.088´´
Salgado		Lobos	S 35° 08´ 29.825´´ W 59° 05´ 36.607´´

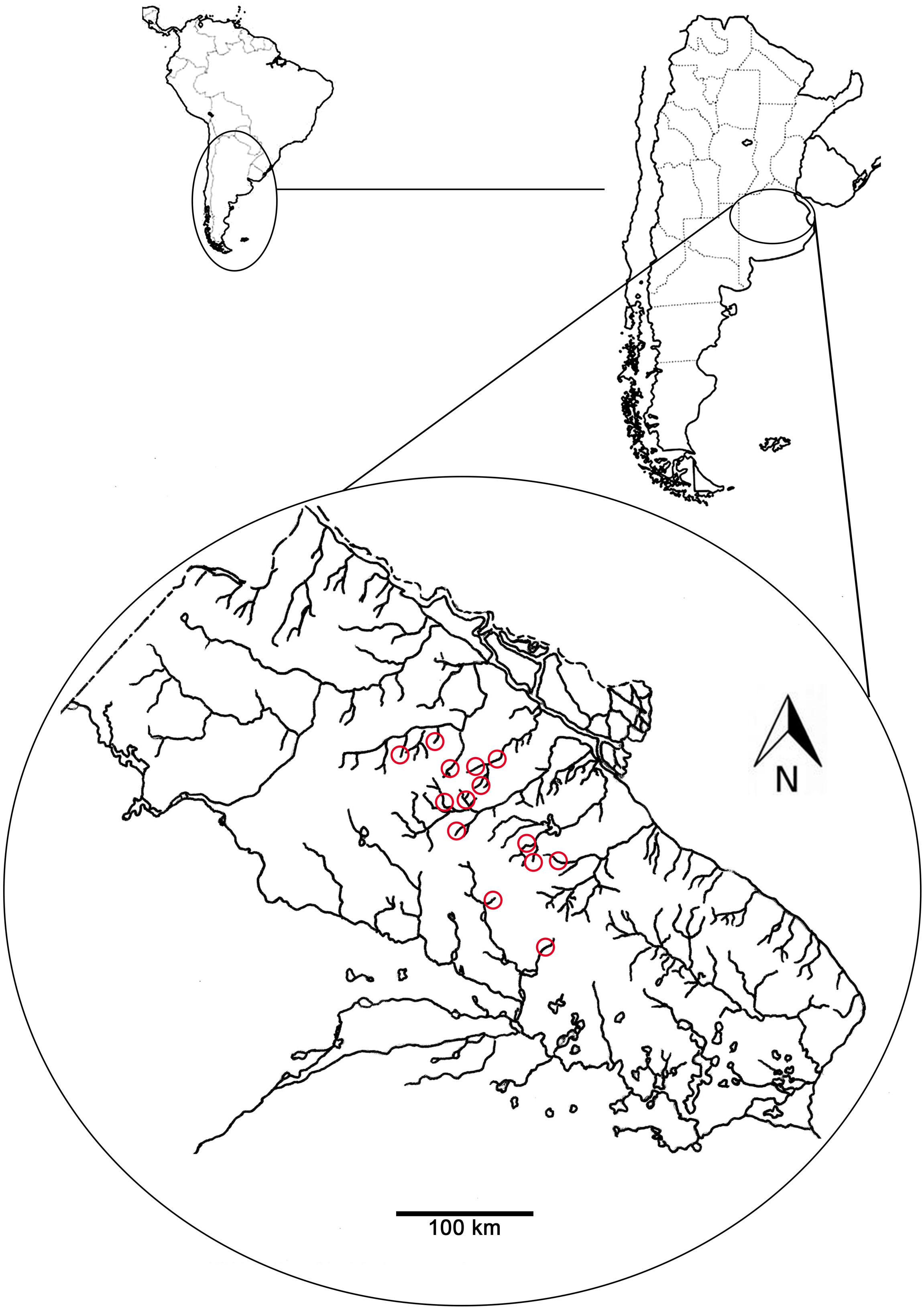

FIGURE 1. Location of the study region in the northeast of Buenos Aires Province, Argentina. References: red circles indicate the sampling sites locations.

Results

We found five conidial forms of *Tetracladium* corresponding to four known and one unknown species, which are briefly described below. All specimens came from foam samples.

Tetracladium setigerum (Grove) Ingold, Trans. Brit. Mycol. Soc. 25: 369 (1942). (Fig. 1a–i)
≡ *Tridentaria setigera* Grove, J. Bot. 50: 16 (1912).

Conidia with three digitiform, one filiform and two acicular elements. Acicular elements slightly longer than the digitiform branches. Central axis 28–42 × 4–4.5 µm, with basal extension and a distal subclavate to digitiform element. The digitiform branch at the second level is arranged opposite to the digitiform element of the first level.

Specimens examined:—Buenos Aires, San Andrés de Giles, Vagues stream, 34° 17´ 35.167´´ S, 59° 27´ 2.48´´ W, 0–50 m, 2 October 2017, *Kravetz, S. 116* (slide BAFC 52781); Luján, S/N1 stream, 34° 27´ 50.47´´ S, 59° 15´ 1.957´´ W, 0–50 m, 17 September 2017, *Kravetz, S. 94* (slide BAFC 52780).

Notes:—*T. setigerum* was previously reported in a Pampean stream (Las Cañas) at early stages of the decomposition of submersed leaf litter of *Ligustrum lucidum* Ait. and *Pouteria salicifolia* (Spreng.) Radlk. (Marano *et al.* 2011).

Tetracladium marchalianum De Wild. Ann. Soc. Belge Microscop. 17: 39 (1883). (Fig. 2a–i)

Conidia with one or two globose cells and two or three filiform elements. Central axis 28–46 µm long, without basal extension and globose to ellipsoid distal cell (seldom flame-shaped). Primary branches at one level; secondary acicular branch inserted below the second globose cell.

Specimens examined:—Buenos Aires, Luján, Haras stream, 34° 31´ 32.862´´ S, 59° 10´ 4.461´´W, 0–50 m, 15 September 2017, *Kravetz, S. 88* (slide BAFC 52782); *ibid., Kravetz, S.* (slide UNLu 89); Mercedes, Frías stream, 34° 36´ 38.747´´ S, 59° 25´ 32.7´´ W, 0–50 m, 7 August 2017, *Kravetz, S. 48* (slide BAFC 52783); San Andrés de Giles, Los Sauces stream, 34° 24´ 26.485´´ S, 59° 15´ 42.305´´ W, 0–50 m, 15 September 2017, *Kravetz, S. 100* (slide BAFC 52784); Mercedes, Balta stream, 34° 40´ 27.264´´ S, 59° 19´ 40.105´´ W, 0–50 m, 17 September 2017, *Kravetz, S.* (slide UNLu 101).

Notes:—This is the first report of the species in Argentina. Our specimens are in agreement with Roldán (1989), but they did not have a basal extension.

Tetracladium breve A. Roldán, Mycol. Res. 93: 455 (1989). (Fig. 3a–i)

Conidia with three digitiform, two acicular and one filiform elements. Filiform element sharp and conspicuously longer than the digitiform branches. Central axis 14–22 × 1.5–2.5 µm, with basal extension and a distal digitiform element. The digitiform branch at the second level may appear indistinctly to the right or to the left of the axis.

Specimens examined:—Buenos Aires, Exaltación de la Cruz, De la Cruz stream, 34° 21´ 39.024´´ S, 59° 11´ 29.845´´ W, 0–50 m, 15 September 2017, *Kravetz, S. 98* (slide BAFC 52785); General Las Heras, Morales stream, 34° 27´ 50.47´´ S, 59° 15´ 1.957´´ W, 0–50 m, 28 August 2017, *Kravetz, S. 72* (slide BAFC 52786); Carmen de Areco, La Guardia stream, 34° 21´ 28.793´´ S, 59° 48´ 11.491´´ W, 0–50 m, 7 April 2017, *Kravetz, S.* (slide UNLu 41); Lobos, Salgado stream, 35° 08´ 29.825´´ S, 59° 05´ 36.607´´ W, 0–50 m, 15 November 2016, *Kravetz, S.* (slide UNLu 30).

Notes:—This is the first report of the species in Argentina. In general, most of the characteristics of our material are in agreement with Roldán (1989). *Tetracladium breve* has conidia similar to *T. setigerum*, although smaller, and the digitiform branch at the second level may appear indistinctly to the right or to the left of the axis (Roldán 1989).

Tetracladium furcatum Descals, Trans. Brit. Mycol. Soc. 80: 70 (1983). (Fig. 4a–c)

Conidia with two digitiform, and two or three acicular elements. Central axis 18–35 × 2.5–3.5 µm, without basal extension and a distal digitiform element. The digitiform elements form a v-shaped fork. Primary branches at one or two levels; secondary branch acicular, inserted on the digitiform branch of first level.

Specimens examined:—Buenos Aires, General Las Heras, Plommer, Durazno stream, 34° 47´ 38.416´´S, 59° 02´ 23.764´´W, 0–50 m, 28 August 2017, *Kravetz, S. 79* (slide BAFC 52787); *ibid., Kravetz, S.* (slide UNLu 80); Marcos Paz, Villars, Durazno Chico stream, 34° 48´ 23.04´´ S, 58° 58´ 56.179´´ W, 0-50 m, 28 August 2017, *Kravetz, S. 77* (slide BAFC 52788); Navarro, S/N2 stream, 34° 58´ 57.645´´ S, 59° 13´ 27.088´´ W, 0–50 m, 28 August 2017, *Kravetz, S.* (slide UNLu 64).

Notes:—This is the first report of the species in South America. In general, all the characteristics of our material are in agreement with Roldán (1989), except for the basal extension wich was not present in our material.

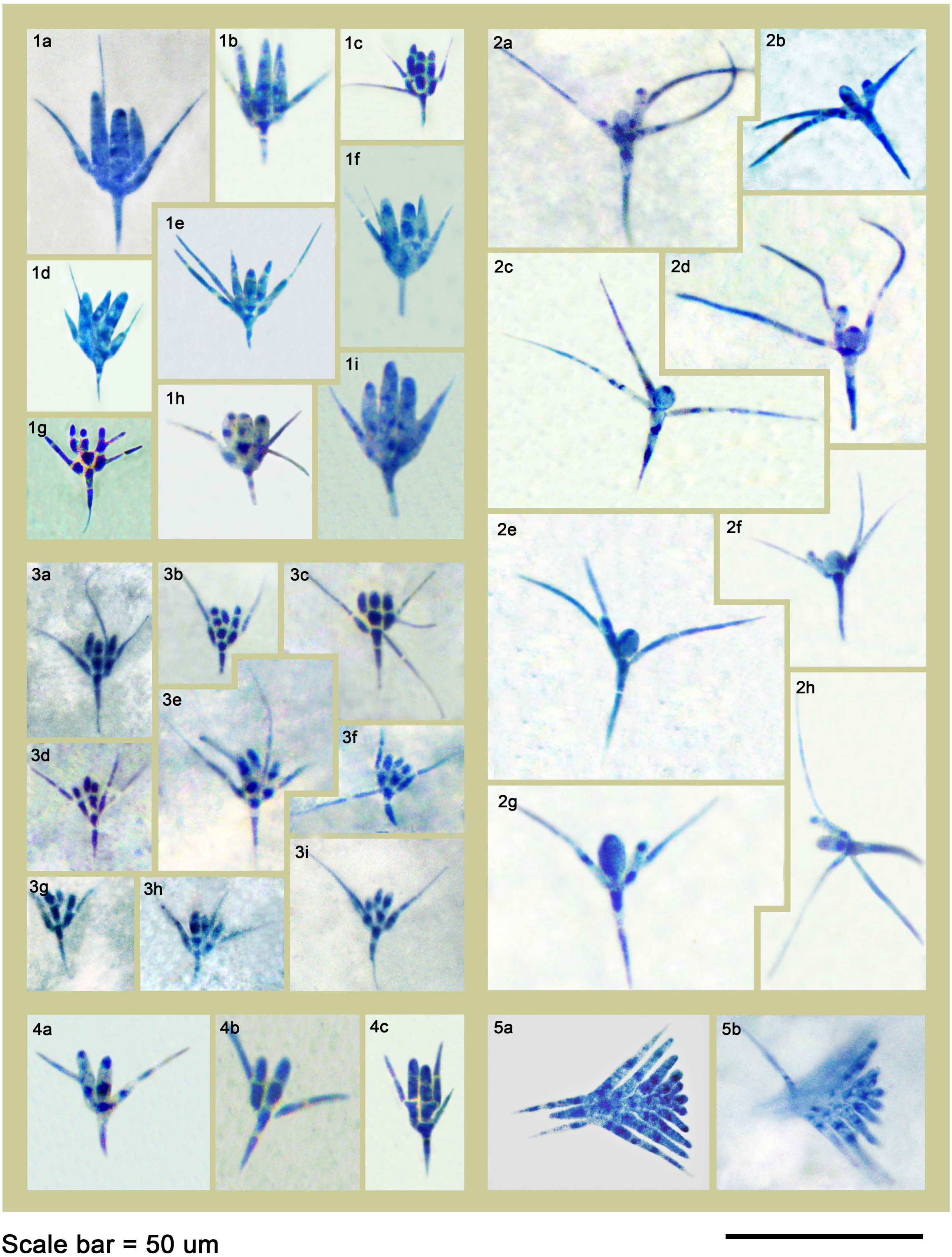

Figure 2. 1a–1i. Conidia of *T. setigerum* (Giles stream, Vagues stream, De la Cruz stream, SN1 stream, Haras stream, Durazno Chico stream, Durazno stream, Morales stream and SN2 stream, respectly). **2a–2h.** Conidia of *T. marchalianum* (Vagues stream, Los Sauces stream, De la Cruz stream, SN1 stream, Haras stream, Balta stream, Frías stream and Durazno stream, respectly). **3a–3i.** Conidia of *T. breve* (La Guardia stream, Giles stream, Vagues stream, Los Sauces stream, De la Cruz stream, Haras stream, Balta stream, Durazno stream, Morales stream and Salgado stream, respectly). **4a–4c.** Conidia of *T. furcatum* (Durazno Chico stream, Durazno stream and SN2 stream, respectly). **5a–5b.** Conidia of *Tetracladium* sp. (Giles stream). Scale bar = 50 µm.

***Tetracladium* sp.** (Fig. 5a–b)

Conidia with digitiform, acicular, globose and filiform appendicular elements developed from a central axis (32–48 × 2.5–3.5 µm). Primary branches arising at four or five levels. Two secondary acicular branches, inserted in the base of the acicular elements at first level (22–45 × 0.7–1.7 µm). At the axis base, stinger-shaped cell (9–13 × 1.3–2.5 µm) without basal extension, then in each following cell on the central axis, two appendicular elements arise that develop in the same plane in opposite form: at the first cell arise two acicular elements 3–4-septate (27–33 × 2–2.7 µm), and perpendicular to a third filiform element also 3–4-septate; at the second level of branchings arise two obclavate-acicular elements 2–3-septate (19–22 × 2.5–3.7 µm); from the third level two digitiform elements of 1–2-septate (15–18 × 2–2.5 µm) are developed. In the fourth level the digitiform elements (8–11 × 2–2.3 µm) are bicellular. In the fifth level the globose elements are unicellular (2.5–3.5 × 1.5–2.7 µm). Distally with globose cell (2.7–3.5 × 1.5–2.7 µm). Its symmetry with respect to the central axis gives the appearance of a candelabrum.

Specimens examined:—Buenos Aires, San Andrés de Giles, Giles stream, 34° 27´ 17.284´´ S, 59° 27´ 27.91´´ W, 0–50 m, 17 September 2017, *Kravetz, S.106* (slide BAFC 52789); *ibid., Kravetz, S.* (slide UNLu 105).

Notes:—Due to their morphological characteristics, these conidia can undoubtedly be attributed to *Tetracladium* but do not match those of the species described so far. The most similar species is *T. palmatum,* however, the number of appendices and levels of branches of the axis in the conidia of our form is greater than in *T. palmatum.*

Key for the identification of *Tetracladium* species from Pampean streams

1 Symmetric conidia with branches arranged in five levels on the axis .... ***Tetracladium* sp**.
1´ Asymmetric conidia with branches arranged in fewer than three levels on the axis **2**
2 Conidia with two globose and two or three acicular elements .... ***T. marchalianum***
2´ Conidia without globose or acicular elements........ **3**
3 Conidia with two digitiform elements that form a v-shaped fork and two or three acicular elements (4–5 apices)........ ***T. furcatum***
3´ Conidia with three digitiform, two acicular and one filiform elements **4**
4 Axis ≤21 µm, digitiform branch at the second level of the axis arranged on the same side as the digitiform element at the first level .... ***T. breve***
4´ Axis ≥28 µm, digitiform branch at the second level of the axis arranged opposite to the digitiform element at the first level .... ***T. setigerum***

Half of the 14 sampled streams had exclusively herbaceous vegetation on their banks while the other half had invasive arboreous species, with the dominance of *Gleditsia triacanthos*. In the sampled streams, the temperature ranged between 12.5 and 24.8 °C; the pH was alkaline, while the concentration of dissolved oxygen and conductivity were high except for the concentrations of oxygen in the Salgado stream (Table 2).

The most frequently observed species were *T. setigerum* and *T. breve*, which were found in all basins. *Tetracladium marchalianum* was found in five of the six basins analysed while *T. furcatum* was present in Durazno, Durazno Chico and Navarro streams; these three streams belong to two different basins that are geographically close. The new morphotype *Tetracladium* sp. was only recovered in Giles stream. Among the sampled streams, Durazno and Giles had the most species (Table 3).

TABLE 2. Dominant vegetation and physicochemical factors of the sampled streams.

Stream/Factor	Dominant vegetation	Dissolved oxygen (mg l^{-1})	Temperature (°C)	Conductivity (µS cm^{-1})	pH
La Guardia	No arboreous vegetation *Cortaderia selloana, Cynodon* sp., other grasses	11.64	20.5	1649	8.14
Giles	*Populus nigra, Gleditsia triacanthos*, *Celtis tala, Cortadeira selloana*, *Bromus unioloides*	10.23	21.6	915	7.97
Vagues	No arboreous vegetation *Cynodon* sp., *Paspalum* sp., other grasses, *Juncus* sp.	8.80	15.7	803	7.7

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TABLE 2. (Continued)

Stream/Factor	Dominant vegetation	Dissolved oxygen (mg l^{-1})	Temperature (°C)	Conductivity (µS cm^{-1})	pH
Los Sauces	No arboreous vegetation *Verbena* sp., *Paspalum* sp., other grasses	10.82	17.9	899	7.7
De la Cruz	*Gleditsia triacanthos*, *Ligustrum lucidum, Celtis tala,* grasses	8.23	17.4	871	7.65
S/N1	No arboreous vegetation*, Juncus* sp., grasses	9.89	16.1	937	8.09
Haras	No arboreous vegetation *Cynodon* sp., *Paspalum* sp., other grasses	9.62	15.5	1001	7.46
Balta	*Gleditsia triacanthos,* grasses	7.84	20.4	1202	7.88
Frías	*Gleditsia triacanthos*, *Ligustrum lucidum,* grasses	8.70	24.8	1109	8.29
Durazno Chico	No arboreous vegetation, *Cynodon* sp., *Bromus unioloides, Juncus* sp.	8.82	16	682.2	7.61
Durazno	No arboreous vegetation, *Cynodon* sp., *Bromus unioloides, Juncus* sp.	9.14	17	907.2	8.17
Morales	*Gleditsia triacanthos*, *Cynodon* sp. *Paspalum dilatatum*	11.05	16	1289	7.53
S/N2	*Gleditsia triacanthos, Juncus* sp., grasses	8.3	12.5	1396	7.61
Salgado	*Celtis tala*, *Juncus* sp., *Cortaderia selloana*	4.38	17.1	4406	8.65

TABLE 3. Species of *Tetracladium* found in the streams analyzed.

Stream	*T. setigerum*	*T. marchalianum*	*T. breve*	*T. furcatum*	*Tetracladium* sp.
La Guardia	-	-	+	-	-
Giles	+	+	+	-	+
Vagues	+	+	+	-	-
Los Sauces	-	+	+	-	-
De la Cruz	+	+	+	-	-
SN1	+	+	-	-	-
Haras	+	+	+	-	-
Balta	-	+	+	-	-
Frías	-	+	-	-	-
Durazno Chico	+	-	-	+	-
Durazno	+	+	+	+	-
S/N2	+	-	-	+	-
Morales	+	-	+	-	-
Salgado	-	-	+	-	-

Discussion

In this study, we found four of the 11 described species of *Tetracladium*, plus an unidentified species, which might correspond to a new species. Three of the species are recorded for the first time in Argentina, one of which is also the first record in South America.

Tetracladium spp. are widespread in the studied basins and have a wider distribution in Pampean streams than previously thought. In addition, the high species richness and ubiquitous distribution of *Tetracladium* in the streams analyzed suggest that these fungi might have a relevant role in the decomposition of allochthonous materials. In the last few years, invasion of arboreous plant species has occurred in many Pampean stream banks, which has significantly modified litter inputs and consequently the dynamics of detritus-based food webs (Gantes *et al*. 2011, Giorgi *et al*. 2014).

Species of *Tetracladium,* as most ingoldian fungi, appear to prefer clean, rapidly flowing, and well-oxygenated streams (Bärlocher 1992) and are believed to be sensitive to pollution. Human activities affecting Pampean streams may lead to species losses and, therefore, continuous research is urged to provide a more comprehensive overview of the diversity and distribution of *Tetracladium* in Argentina.

Although most *Tetracladium* spp. are primarily aquatic, some species have been detected with high abundance in soil (Domsch *et al.* 1993, Ryberg *et al.* 2009, Klaubauf *et al.* 2010) and as root endophytes in diverse plant species (Nemec 1969, Watanabe 1975, Sati *et al.* 2009). It is therefore essential that studies not only continue to increase but also include unexplored habitats, in order to gain a better understanding of the ecological roles played by members of this genus. This is particularly relevant in the Pampas region, where streams frequently flood wide areas.

Acknowledgements

The authors wish to thank Dra. Andrea Romero, INMIBO (UBA-CONICET), for review of the manuscript and suggestions. Eduardo Zunino, CONICET, for collaboration in the sampling.

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