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Research Article

First record of the invasive diatom *Didymosphenia geminata* (Lyngbye) Schmidt in a Patagonian Andean river of Argentina

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Abstract

The Futaleufú River, Argentina, was monitored monthly from June 2010 to August 2011, in order to detect the invasive diatom *Didymosphenia geminata*, which was previously observed in Chile. Plankton and periphyton samples were taken from 10 sites. *Didymosphenia geminata* was not found until late winter. In August 2010, the species was first detected at isolated points of the river but in spring and summer the algal coverage extended along several kilometers. The coverage of blooms, spread to deeper areas (pools) with varying depths, reached more than 2 m, invading epiphytic and epilithic substrates. In autumn the bloom diminished its coverage area and there were only small isolated patches. During the next winter, blooms were not observed until late August, when it was recorded again. The observations showed that *D. geminata* appeared, colonized and invaded the bed of the river at low flow situations in spring and summer and its blooms declined in autumn -when the flows increase- after having been dried in summer when flows were the lowest.

Key words: biological invasion; diatom; freshwater environment; Andean area; South America; bloom spread; temporal dynamic

Introduction

Invasions occur when introduced species become established outside their native ranges and cause ecological and economic damage (Alpert et al. 2000). Microalgae are capable of forming harmful blooms in rivers, lakes and oceans, under certain conditions, but are rarely considered invasive. The freshwater diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt, which has recently received attention because of expansion of its geographical range and detrimental effects on river ecosystems (Spaulding and Elwell 2007), is an exception.

The original distribution of this invasive species, commonly known as "didymo" or "rock snot" is assumed to be the cool temperate waters of the Northern Hemisphere, including rivers of Europe, Asia and North America (Cleve 1894 – 1896, Hara and Sahin 2000; Kociolek et al. 2004, Krammer and Lange-Bertalot 1997; Li et al. 2003; Patrick and Reimer 1975). In the last 10 years, the species has expanded its worldwide distribution at an alarming rate (Spaulding and Elwell 2007). In 2004, it was found in New Zealand and this finding was considered the first record of the species in the Southern Hemisphere (Kilroy 2004). However, the species was cited by Asprey et al. (1964) in Chile, South America, who detected it in the Cisne River (Region XI) and Sarmiento Lake (Region XII). Only in 2010, was it observed again in this country.

D. geminata has the ability to form massive blooms, as in the Lake District, Chilean Patagonia (CIEP 2010). The cells attach to rock or plant substrates and grow into the water column on stalks composed of extracellular carbohydrate material. In suitable conditions the organism

forms dense soggy carpet-like layers, several inches thick, lining the bottom of the aquatic environments where it lives, altering biological and physical conditions of the habitat (Draheim 2009). Kilroy et al. (2008) point out the invasive nature of this species.

The record of this species in the Futaleufú shared basin between Argentina and Chile, prompted the authorities of the Ministry of Environment of the Province of Chubut to monitor areas susceptible to being invaded. The goal of this work is to document the first record of *D. geminata* in Argentina, in the Futaleufú River, Chubut Province.

Materials and methods

Study location

A total of 60 sampling sites were selected from the basins in the mountain range in the province of Chubut, between parallels 42º S and 46º S. Each one of them was monitored monthly. These are Puelo River, Futaleufu River, Engaño River, Corcovado River, Pico River, Senguer River and Simpson River basin.

The Futaleufú River Basin -shared with Chile- is located in the western sector of the province of Chubut in the Cushamen and Futaleufú departments. It is located between 42º17' and 43º25' S and 70º58' and 72º11' W, and covers an area of 7,630 km^2 including the Alerces National Park with an extension of 263,000 ha. This has the lushest and richest forest in Andean Patagonia, whose principal component is *Fitzroya cupressoides* (Molina) I.M.Johnst, the Patagonian cypress.

The Futaleufú headwaters are glacier-fed. A large number of tributaries flow into it through a series of connected lakes that regulate the river regime. Flows increase during spring due to rainfall at the beginning of winter and snow melt in spring.

The Futaleufú or Grande River -regulated by the Futaleufú dam-, is the outfall end of this system that flows to the southeast from the southern end of Amutui Quimey reservoir all the way to the Pacific Ocean.

Along its course it receives, from the left bank, the inflow of the Corintos River and some streams such as Baggilt and Blanco all of them in Argentine territory. Then, it continues towards the Argentine-Chilean border along a wide valley and crosses between the border marker No. VII-6 and VII-5 (Figure 1).

Sample collection and analyses

The study was carried out between June 2010 and August 2011 on the Futaleufú River, which was monitored monthly. Sampling sites were located with GPS and photographed. A thorough visual exploration was carried out first in order to determine presence/absence of *D. geminata* colonies. At each site we then performed a visual estimation of the area covered by *D. geminata* and collected two kinds of samples: benthic samples, which were scrapings from rocks, submerged and emerging plants and macroalgae; and drift samples (where didymo was not detected visually), which were obtained by filtering river water through 25 micron plankton net for 20 minutes.

The samples were placed in 500 ml plastic containers, fixed with 4% formaldehyde and labelled. Three subsamples of each sample were analysed with an Olympus microscope CX41 and a Leica inverted microscope DMIL with phase contrast.

In order to avoid further spread of the alga along the river, upstream sites were sampled first. Following each survey, all equipment was disinfected by means of a hypersaline solution (10%) sodium chloride at each station.

River flow fluctuation data were provided by the Futaleufú hydroelectric company.

Samples in which *D. geminata* was identified were subjected to oxidation treatment of organic matter through the Hasle and Fryxell (1970) method and sent for observation by means of SEM at the Microscopy Service of the La Plata Museum. Samples were placed on glass slides and coated gold-palladium (Ferrario et al. 1995). Several measurements were taken from the photographed samples as well as other taxonomic features of the species.

Results

Morphology of Didymosphenia geminata

Figure 2 shows the species *D. geminata* (Futaleufú River population), Figure 2A and 2C in valve view and Figure 2B in girdle view.

In Futaleufú River cell samples ranged from 114.4 to 126.2 µm in length and from 32.0 to 35.2 µm in width. The number of striae per 10 µm fluctuated from 9 to 10, the number of areolae per 10 µm were from 10 to 11 and the number of stigmata in the centre field ranged from 1 to 5 (mostly 2 or 3).

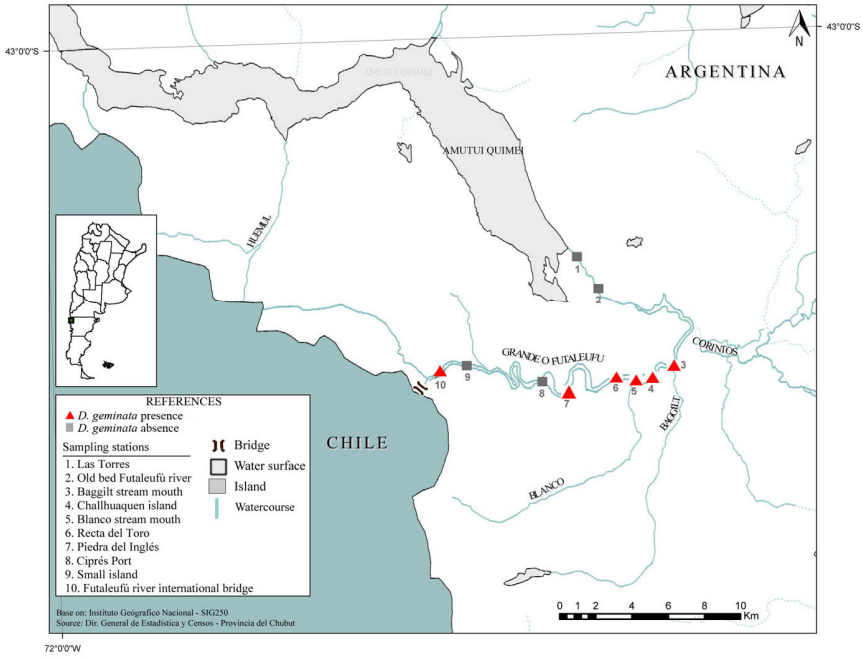

Figure 1. Sampling stations and presence-absence records of *Didymosphenia geminata* in the Futaleufú River, Province of Chubut.

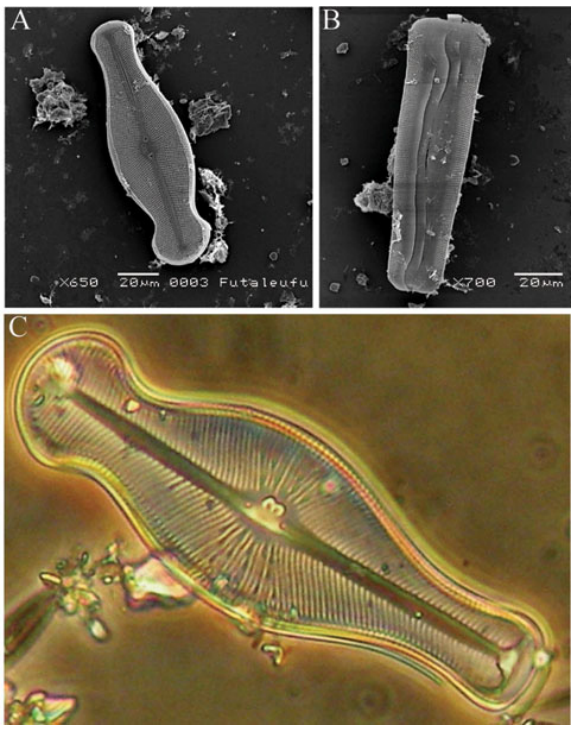

Figure 2. *Didymosphenia geminata* from Futaleufú river. A) Valve view with SEM. B) Girdle view with SEM. C) Valve view with OM (Photograph by AVS).

Cells possess one large, H-shaped chloroplast positioned against one side of the girdle and lapping under both valves (Cox 1996). The chloroplast contains a central lenticular, dorsal pyrenoid. Raphes are present on both valves and are dorsally deflected.

Macroscopically, although didymo looks slimy, it feels like wet cotton wool. It forms dense intertwined mats, often completely smothering the riverbed (Kilroy 2004; Kelly 2009).

Didymosphenia geminata in the Futaleufú River

From the sixty sampling sites, *D. geminata* was detected only in those located on Futaleufú River lower basin, downstream from Corintos River (Figure 1, Appendix 1).

Didymosphenia geminata was not found in the samples collected before late winter. In late August-early September 2010, the species was first detected at isolated points of the river (Piedra del Inglés and Futaleufú River international bridge) only on inundated substrates at depths varying between 0.30 and 1.50 m (Stage 2: Initial Detection in Figure 3).

By late spring, in December 2010, the algae coverage had extended along the entire western sector of the Challhuaquen Island (Stage 3:

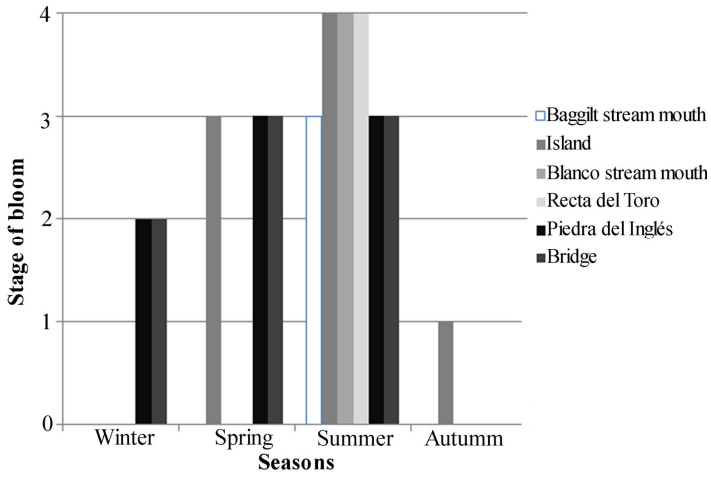

Figure 3. Stages of invasion of *Didymosphenia geminata* in the annual cycle 2010-2011 in the Futaleufú river. 0: No detection; 1: Declination; 2: Initial detection; 3: Colonization; 4: Invasion.

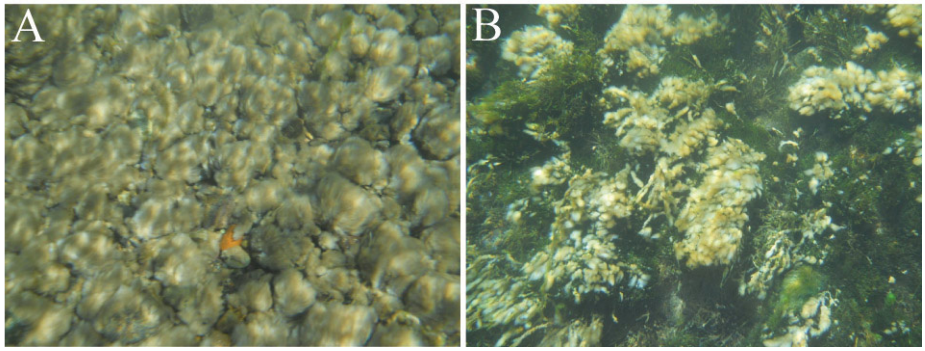

Figure 4. *Didymosphenia geminata* growing on riverbed substrate. A) on rocky bottom. B) on macrophytes (Photograph by GAB)

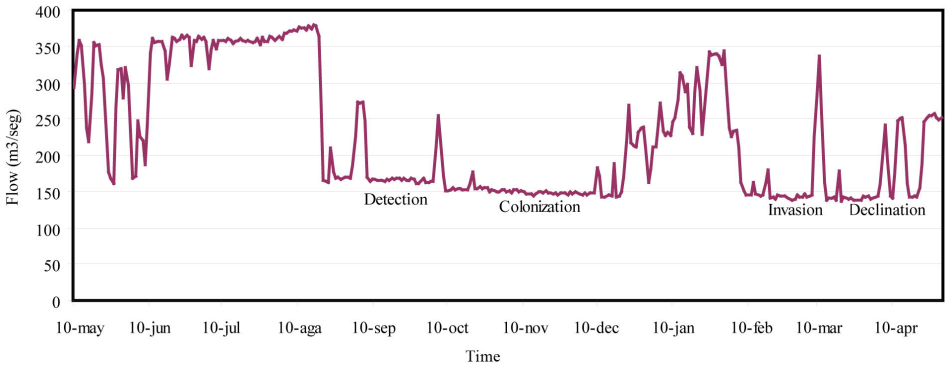

Figure 5. Development of *Didymosphenia geminata* blooms relating to Futaleufú river flow variations.

Colonization in Figure 3). In summer -February 2011- *D. geminata* was widespread, being present at both sides of Challhuaquen Island extending downstream to the mouth of the Blanco stream (Stage 4: Invasion in Figure 3). The coverage of blooms, spread to areas (pools) more than 2 m in depths (Figure 4), invading epiphytic (Figure 4A) and epilithic substrates (Figure 4B). It was also recorded on the macrophytes of genus *Myriophyllum* and bryophytes at the mouth of the Baggilt stream, 1.5 km upstream from the tip of the island.

The site Recta del Toro located downstream of the Challhuaquen island, had the highest cover of *D. geminata*. In this site didymo appeared mainly on rocky substrate, invading different sizes of rocks.

In autumn, April 2011, the bloom diminished its coverage area and there were only small isolated patches in the Challhuaquen island site (Stage 1: Declination in Figure 3). During the winter, blooms were not observed until late August, when it was recorded again.

During the period analyzed, the highest estimated percentage of coverage of the river by *D. geminata* was 30% of the total surface.

Flows of the Futaleufú River (Figure 5) have large variations due to the water flow regulation of the Futaleufú dam carried out by the Futaleufú hydroelectric company (Hidroeléctrica Futaleufú S.A.). During the study period, maximum flow was 378.7 m^3/s and its minimum of 138 m^3/s (average 197.1 m^3/s ± 110.9 m^3/s). *D. geminata* appeared and invaded the river bed during low flow. The blooms declined in autumn when flows increased.

Discussion

The morphology of *D. geminata* cells found in the Futaleufú River matches that depicted by other authors (Cox 1996; Kilroy 2004; Kelly 2009).

This is the first record of initial detection and colonization of the invasive diatom *Didymosphenia geminata* in Argentina, in the Futaleufú river basin. How this alga found its way into this river is still unknown but it was observed five month after was first discovered in Chile, downstream in the same river basin. The translocation of used fishing or water recreation equipment is considered the primary means for didymo introduction and also secondary spread (Kilroy and Unwin 2011; Bothwell et al. 2009) but the natural wildlife vectors, including birds, mink, and fish are also possible vectors. Migrating Chinook salmon (*Oncorhynchus tschawytscha* Walbaum 1792) has significantly expanded its distribution range in the last decade (Correa and Gross 2008). This may be a possible vector for *D. geminata* spreading upstream in Patagonian rivers (CIEP 2011).

These are some of the dispersal means proposed by Blanco and Ector (2008) and it could have been the way it spread in the Futaleufú River.

Previous studies on biological and hydrochemical parameters indicate that Futalaufquen Lake (which is part of the Futaleufú basin) is oligotrophic (Pizzolon et al. 1995). This basin has the appropriate environmental characteristics for the growth of *D. geminata*: low temperature, low nutrient content (oligotrophic), and fast flowing, highly turbulent streams (Sapaulding and Elwell 2007).

The success of the initial colonization of *D. geminata* and the potential for blooms is clearly defined not only by introduction of cells, but also by the suitable habitat window for survival (Cullis et al. 2012). In particular for this alga, the regulation of Futaleufú River flow rate may make up this window. Colonization and invasion occurred in situations of very low flows during the spring and summer. In late summer, flow reduction to below 150 m^3/s left the blooms exposed to drying for several days and finally, in autumn, higher flows could have removed the algae. This behaviour may be explained by the threshold event developed by Cullis et al. 2012.

Before this first record, South American rivers were considered to be especially vulnerable to *D. geminata* invasion (Spaulding and Elwell 2007, Kumar 2008). McNyset and Julius (2006) proposed a global distribution map based on ecological niche models which show suitable stream habitats for *D. geminata* on every continent except Antarctica. Argentinean rivers were particularly identified as at risk of new introduction and invasion.

Currently, in order to determine *D. geminata* presence and distribution in Andean Patagonian Rivers, a monitoring program (Res. 030/10 MAyCDS and Res. 264/10 SP) has been recently implemented through an agreement between the Chubut government and the National University of Patagonia San Juan Bosco. This programme allows the study of the biology and ecology of the algae, and the detection of early stage blooms in order to take control measures when these early stages are detected. The recommendations of Spaulding and Elwell (2007) are taken into account to develop biosecurity protocols.

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Supplementary material

The following supplementary material is available for this article.

Appendix 1. Records of *Didymosphenia geminata* in Futaleufú River (Argentina).

Appendix 1. Records of *Didymosphenia geminata* in Futaleufú River (Argentina)

Record No. (map ref.)	River	Sample Station	Record coordinates		Record date	Reference
			Latitude	Longitude		
3	Futaleufú	Baggilt stream mouth	43°10′07.7″S	71°34′46.9″W	25 February 2011	Present study
4	Futaleufú	Challhuaquen island	43°10′23.4″S	71°35′41.1″W	20 December 2010 25 February 2011 29 April 2011	Present study
5	Futaleufú	Blanco stream mouth	43°10′26.6″S	71°36′23.4″W	25 February 2011	Present study
6	Futaleufú	Recta del Toro	43°10′40.0″S	71°37′48.1″W	25 February 2011	Present study
7	Futaleufú	Piedra del Inglés	43°10′44.9″S	71°39′07.7″W	31 August 2010 2 and 7 September 2010 20 December 2010 25 February 2011	Present study
10	Futaleufú	Futaleufú river International bridge	43°10′04.3″S	71°44′15.6″W	31 August 2010 2 and 7 September 2010 20 December 2010 25 February 2011	Present study