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**First record of the red alga *Compsopogon caeruleus* (Balbís ex C. Agardh) Montagne 1846 in the High Paraná River, Argentina-Paraguay**

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**Abstract**

The presence of a freshwater red alga (Rhodophyta), *Compsopogon caeruleus*, was recorded for the first time in the High Paraná River. It was detected in 2016 and 2017 at five points along 290 km of the border between Argentina and Paraguay. High densities of filaments of the red alga were recorded in the summer months, forming masses flowing through the middle of the riverbed and banks, and not recorded in the main body of the Yacyretá Binational Reservoir (Argentina-Paraguay). We identified the species both morphologically and using molecular methods. The samples were characterized by having short, thorn-like branches, which resembled the morphology reported for *Compsopogon aeruginosus*, nowadays a synonym of *C. caeruleus*. Partial DNA sequences from cytochrome *c* oxidase subunit *I* gene confirmed species identification as *C. caeruleus*. Because of its adaptability to a wide range of environmental conditions, we speculate that the species could establish in the High Paraná River and potentially rapidly increase its distribution downstream throughout temperate regions of South America.

**Key words:** alien algae, freshwater habitats, mitochondrial gene, South America

**Introduction**

Red algae (Rhodophyta) are mostly macroalgae which, compared to green or blue-green algae, are scarcely represented in freshwater environments (Peerapornpisal et al. 2006). They inhabit rivers and streams, principally being encountered in soft, clear, pure waters in shallow riverbeds with rocky substrate and variable current velocity, and generally indicate good ecological quality (Eloranta and Kwandrans 2004), although they can also be found in waters of moderate quality or even polluted waters (Peerapornpisal et al. 2006). Due to their long and complex life cycle, they require hydrologically stable environmental conditions, and are generally weak competitors for the substrate with filamentous algae and bryophytes (Eloranta and Kwandrans 2004; Tomás et al. 2013).
The genus *Compsopogon* Montagne 1846 belongs to the Compsopogonaceae family, of the Compsopogonales order, with 6 to 13 morpho-species recognized for freshwater environments (Krishnamurthy 1962; Yadava and Kumano 1985; Vis et al. 1992; Kitayama 2011). Recent molecular studies have shown most of them belong to a single species, *C. caeruleus* (Balbis ex C. Agardh) Montagne, 1846 (Rintoul et al. 1999; Necchi Júnior et al. 2013; Nan et al. 2016). This genus is comprised of plants that are either free-floating or adherent to a substrate, characterized as being filamentous macroalgae, branched, with one principal axis (uniaxial), formed by large central cells surrounded by small cortical cells in a variable number of layers, and young lateral uniseriate branches with rounded terminal cells. The predominance of pigments such as phycoerythrin and phycocyanin lend them striking colors such as dark red, violet, blue, olive green, reddish brown, or gray (Tomás et al. 2013).

*Compsopogon* is a sub-cosmopolitan genus, with ample distribution in freshwater and brackish habitats around the world in Europe, Asia, Africa, and Oceania, principally in tropical and subtropical regions, occasionally extending into temperate regions (Krishnamurthy 1962; Nakamura and Chihara 1983; Necchi Júnior et al. 1990, 1999; Rintoul et al. 1999; Tomás et al. 2013; Nan et al. 2016). A recent reconstruction of the geographical area of origin for *Compsopogon* suggested that its speciation occurred on the American plate, most likely in North America (Nan et al. 2016). Despite this, it was indicated that members of *Compsopogon* are to be considered alien algae in the temperate regions of North America and Europe (Ceschin et al. 2013 and references therein). In addition, *C. caeruleus* was recognized as an invasive taxon in Belgium (Stoyneva et al. 2006). In South America, the genus has been recorded in Argentina, Brazil, and in French Guiana (Oliveira Filho and Pereira 1973; Leyes and Daga 2015). In Brazil, four morpho-species that are currently regarded as synonyms of *C. caeruleus* (i.e. *C. caeruleus*, *C. leptoclados* Montagne, 1850, *C. aeruginosus* (J. Agardh) Kützing, 1849 and *C. chalybeus* Kützing, 1849) have been recorded in Rio de Janeiro and in various tributaries of the Amazon River, as well in the Conchas, Guandú, Guarai-Mirim, Muquém, Preto, and Upper Paraná Rivers (Oliveira Filho and Pereira 1973; Necchi Júnior et al. 1999, 2013; Rodrigues and Bicudo 2001).

Records of *Compsopogon* in Argentina have been few, and consequently knowledge of them has been fragmentary. In this country, three morpho-species have been recorded: *Compsopogon argentinensis* Pujals, 1967 in the Paraná de las Palmas River delta, Buenos Aires Province (Pujals 1967; Coradeghini and Vigna 2011); *Compsopogon occidentalis* Tracanna, 1979 in the Mistra Stream, Tucumán Province (Tracanna 1979), and *Compsopogon caeruleus* in Córdoba Province (Leyes and Daga 2015). This study reports for the first time the occurrence of *Compsopogon caeruleus* in the High Paraná River, Misiones Province, Argentina in the region bordering Argentina and Paraguay.
Materials and methods

Samples of *Compsopogon caeruleus* were collected as part of the *Water Quality Monitoring Program* of the Yacyretá Binational Reservoir during phytoplankton and fish samplings carried out in the High Paraná River between 2016–2017, with bimonthly frequency and in thirty sampling stations, which included the principal channel of the river, numerous tributaries, and the Yacyretá Binational Reservoir. The Paraná River is one of the fastest-flowing rivers of South America, with a flow rate of approximately 14,000 m³/s, in which four sections (namely, the Upper Paraná, the High Paraná, the Middle Paraná, and the Lower Paraná) can be distinguished with different hydrological and geomorphological features (Bonetto 1986). The Paraná River, becomes the High Paraná River from the former Guairá Falls along the Brazil-Paraguay border, now flooded by the Itaipú Reservoir, until the confluence with the Paraguay River (Bonetto 1986; Vogler et al. 2016). In the stretch between Puerto Iguazú and Candelaria, Misiones Province, Argentina (between 25°35′47.63″S; 54°35′41.19″W and 27°26′42.52″S; 55°44′49.31″W) the High Paraná River has a single channel up to 55 m deep, and runs over a basaltic riverbed, with a stepped profile without floodplain development. Upon passing Candelaria locality the valley grows wider, profundity is diminished, alluvial deposits appear along the margins, with the development of a floodplain (Peso et al. 2013). Sample collection was performed following two methodologies: the live samples were extracted manually in plastic recipients, while the samples to be fixed were collected with a phytoplankton net with a mesh width of 25 μm and fixed *in situ* in 4% formaldehyde solution. Several physical-chemical parameters were measured *in situ* at three sampling stations: water temperature, dissolved oxygen (water analyzer YSI, Pro20), conductivity, pH (multi-parameter water analyzer EcoSense EC300A), and water transparency (Secchi disk). Water samples were taken for laboratory analysis of total phosphorous concentration (APHA 1992). The sites where the analyzed specimens were encountered are presented in Figure 1. Samples of living specimens were placed in absolute ethanol, and voucher material was deposited at the Universidad Nacional de Misiones herbarium, Argentina (voucher number MNES 2351).

In the laboratory, red algae samples were separated under a Leica Z45V stereoscopic microscope, and firstly identified based on morphology following Krishnamurthy (1962), Oliveira Filho and Pereira (1973), Eloranta et al. (2011) and Breton (2014). Morphological characteristics were analyzed from the samples fixed in formaldehyde with a Leitz SM-LUX microscope equipped with camera lucida and micrometric lens of variable magnification. Samples in absolute ethanol were used for molecular identification. DNA was isolated from 1 mg of stems from two locations using a cetyltrimethylammonium bromide (CTAB) protocol, as follows: the
Compsopogon caeruleus in the High Paraná River


Figure 1. Map of the Misiones Province, northeastern Argentina. Circles indicate the locations where Compsopogon caeruleus was recorded in the High Paraná River (for details see Supplementary material Table S1).

Tissues were ground up in mortar and pestle with 2 ml of CTAB buffer (2% [w/v] CTAB, 1.4 M NaCl, 0.2% [v/v] 2-mercaptoethanol, 20 mM ethylenediaminetetraacetic acid, 100 mM Tris pH 7.5). A total volume of 1 ml of ground tissues were centrifuged for 10 min at 12,000 g. The supernatant was washed once with phenol at pH 7, and once with chloroform-isoamyl alcohol (24:1). DNA was precipitated with cold isopropanol at −20 °C and air dried. The DNA was finally resuspended in TE buffer 1X (10 mM Tris pH 8, 1 mM EDTA pH 8).

Subsequently, a 664 bp segment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was PCR amplified using primers LCO1490 and HCO2198 (Folmer et al. 1994). Amplifications were performed following Vogler et al. (2014). Successful amplifications were verified by agarose gel electrophoresis, purified using an AccuPrep PCR Purification Kit (Bioneer, Korea), and bidirectionally sequenced by Macrogen Inc. (Seoul, Korea). The resulting sequences were compared to reference sequences in GenBank through the use of the BLASTN algorithm (Altschul et al. 1990) to identify similar sequences. Genetic distances were investigated in the MEGA 7.0.26 software (Kumar et al. 2016) using the number of differences (p) and the K2P substitution model to explore divergence among individuals from the High Paraná River to those of other C. caeruleus specimens from various locations available in GenBank.

Results

The genus Compsopogon was first registered here in the High Paraná River in the 4th sampling station at the end of spring in 2016 (November). In December
of the same year, it was found in other sections of the river: stations 1, 2, and 3 (Figure 1). In samplings carried out during the summer in 2017 (February) high densities of these algae were recorded (Figure 2), forming dense masses of intertwined filaments carried along by the river, between stations 1 and 5 along a trajectory of approximately 290 km, while being recorded neither in the tributary streams nor in the stations of the principal body of the Yacyretá Binational Reservoir. The high density of the filaments obstructed fishing nets, rendering impossible the tasks of ichthyic fauna monitoring (Figure 2B, C). No fixation structures or rhizoidal filaments were observed on the thalli bases (Figure 2D). The High Paraná River along the section studied had an elevated rate of flow (approximately 14,500 m³/s) owing to intense rains in the river basin. It also had a high concentration of dissolved oxygen (6.36–7.90 mg/l), low conductivity (55–60.60 μS/cm), neutral or slightly alkaline pH (6.9–7.6), high temperatures (24.60–28.50 °C), moderate water transparency (90–210 cm), and an elevated concentration of total phosphorous (10–200 μg/l).

Based on morphological characters, our specimens were identified as *Compsopogon caeruleus* with the *C. aeruginosus* morphology (Figure 3), characterized by the formation of short branches or spiny protuberances in
the cellular cortex of the older filaments, a characteristic exclusive to this morphotype. The total length of the filaments was variable reaching up to 18 cm and maximum diameter of 250 μm, with hues from violet, dark red, olive green, to bluish. Irregularly branched axes were observed, diminishing in diameter with each progressive branching. The young lateral branches were uniseriate, with rounded terminal cells at the apex.
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(Figure 3A), forming an acute angle with the principal axis, composed of cells ranging from 8 to 18 μm in length and from 20 to 66 μm in diameter (Figure 3B). Mature branches measured from 140 to 250 μm in diameter and were composed of an axial cell surrounded by a layer of small cortex cells, polygonal or irregular in shape, from 14 to 22 μm in width and from 16 to 32 μm in length in superficial view (Figure 3C). The short ramifications, similar to spines, were present on the mature principal axes and on older filaments, presenting a longitude between 22 and 204 μm (Figure 3D, E). On mature corticate filaments, monosporangia were found with small monosporangia (12–20 μm in diameter), separated by a curved wall (Figure 3F).

A total of three novel 664 bp DNA sequences were obtained from two sampling stations (Yabebiry Stream, n = 1; Candelaria, n = 2). The COI marker contained no sequence variation among the three sequenced samples, thus a single haplotype was identified. These partial sequences have been deposited in GenBank under the single accession number per locality, as follows: MH638995, MH638996. The identification of the sequences through the BLASTN algorithm confirmed their specific identity as *C. caeruleus*, as they showed 100% sequence identity to available sequences in GenBank, including the complete mitochondrial genome for this species from China (KY083068). When novel sequences generated here were compared with those from other geographic regions available in GenBank, we found the haplotype from the High Paraná River was identical to that previously recorded in Australia, Austria, Brazil, China, Federated States of Micronesia, Spain, USA (continental USA, Guam, Hawaii), Vanuatu, and Venezuela; and differed from other sequences from Brazil by a sequence divergence of 0.2% (genetic distances: $p = 0.002$, $K2P = 0.002$), and from continental USA (Louisiana State) by a divergence of 1.6% (genetic distances: $p = 0.016$, $K2P = 0.016$).

**Discussion**

In this work, we recorded for the first time the occurrence of *Compsopogon caeruleus* in the High Paraná River in northeastern Argentina bordering on Paraguay, in the stretch shared by both countries. Over 23 years of continuous monitoring of the aquatic communities in the influence area of the Yacyretá Binational Reservoir, and even before the filling of the dam, this algal species was never documented (Meichtry de Zaburlín 1999; Meichtry de Zaburlín et al. 2010, 2013). To our knowledge, this is the fourth record of the occurrence of the genus *Compsopogon* for Argentina and the first confirmed record for Paraguay. There is a mention of the presence of *C. argentimensis* for the lower part of the Paraná River from about 50 years ago, with a summary description, although the species has not been recorded since that date (Pujals 1967; Coradeghini and Vigna 2011).

The morphological characteristics of the specimens from the High Paraná River are consistent with the morpho-species *C. aeruginosus,*
nowadays a synonym of *Compsopogon caeruleus*, although opinions on taxonomic validity of *Compsopogon* species differ among authors. Krishnamurthy (1962) mentions that *C. aeruginosus* is very similar to *C. caeruleus*, from which it can be distinguished by comparatively smaller monospores, as well as by the presence of short spiny branches on the older principal axes. This last characteristic was also observed in the specimens collected in India and Japan (Nakamura and Chihara 1983; Yadava and Kumano 1985). Despite that Sheath and Sherwood (2002) synonymized *C. aeruginosus* with *C. caeruleus*, based on studies by Vis et al. (1992) and Rintoul et al. (1999), and that Ratha et al. (2007) indicate *C. aeruginosus* as an ecotype of *C. caeruleus*, Breton (2014) follows Kumano (2002) and Eloranta et al. (2011) and considers *C. aeruginosus* to be a morphologically distinct species identified by the presence of short, thorny twigs on the main axes. However, other studies have shown that the morphological characteristics of *Compsopogon* species are highly variable and therefore are of little taxonomic value for species identification, given that the morphology within the genus is highly plastic (Shyam and Sarma 1980; Necchi Júnior et al. 1990, 2013). Morphologically, the material from the High Paraná River analyzed showed the characteristics mentioned as typical for *C. aeruginosus* and the measurements fall within the range established for this morpho-species. However, our molecular studies confirmed its identity as *C. caeruleus* and to our understanding, this is the first time that the *C. aeruginosus* morphology has been genetically characterized. This morphotype found in the High Paraná River is different from the *leptoclados* morphology previously informed for the Upper Paraná River at about 420 km upstream (Rodrigues and Bicudo 2001). On the other hand, the haplotype from the High Paraná River was identical to a cosmopolitan haplotype previously identified for the *caeruleus* and *leptoclados* morphologies across very distant countries including Austria, Brazil, China, Federated States of Micronesia, Spain, USA and Vanuatu. Our genetic characterization of the *C. aeruginosus* morphology supports evidence from other authors that suggests the genus is monospecific worldwide under the *C. caeruleus* denomination (Necchi Júnior et al. 2013; Nan et al. 2016).

Because of the wide geographical distribution of the haplotype found in the High Paraná River, the origin of the species in Argentina and Paraguay remains unknown. The introduction could have occurred from Brazil passively down the Upper Paraná River or through an important tributary, such as the Iguazú River, a river that presents a propitious environment for the growth of this species. Nonetheless, it is not to be ruled out that monospores may have been carried by migratory birds or through anthropic intervention, as the introduction of *Compsopogon* into new areas has often been associated with the introduction of aquatic plants and
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Environmental data published suggest that the genus Compsopogon is tolerant to a variety of conditions in terms of current, water temperature, pH, and nutrients (Ratha et al. 2007; Necchi Júnior et al. 2013). This tolerance contributes to an omnipresent distribution of the genus in tropical and subtropical regions, through which it is found to be geographically extended (Sheath and Hambrook 1990; Vis et al. 1992; Necchi Júnior et al. 2013). Because of its adaptability to a wide range of environmental conditions, we speculate that C. caeruleus could establish in the High Paraná River and potentially rapidly increase its distribution downstream throughout the temperate regions of South America.

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References


Meichtry de Zaburlín N (1999b) La comunidad fitoplanctónica durante las primeras etapas de llenado del embalse de Yacyrétá, Argentina. Revista de Ictiología 7: 15–26


Shyam R, Sarma YSRK (1980) Cultural observations on the morphology, reproduction and cytology of a freshwater red alga Compsopogon Mont. from India, Nova Hedwigia 32: 745–765


Tracanna BC (1979) Contribución al conocimiento de las algas del Noroeste Argentino, II Compsopogon occidentalis sp. nov. (Rhodophyta). Lilloa 35: 35–37


Supplementary material

The following supplementary material is available for this article:

**Table S1.** Records *Compsopogon caeruleus* in the High Paraná River.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2019/Supplements/BIR_2019_deZaburlín_etal_Table_S1.xlsx