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New record of *Pithophora roettleri* (Roth) Wittrock, 1877 (Chlorophyta: Pithophoraceae) for the São Francisco River basin and its potential geographic distribution in Brazil

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Abstract: This paper presents a new record for a heterotypic synonym of *Pithophora roettleri* (*P. oedogonia*) for the São Franscisco River basin. *Pithophora* are green filamentous algae, branched, with intercallary akinetes of variable (*P. roettleri*) or invariable (*P. oedogonia*) forms in the same individual. The species was found in a temporary pond, which has high electrical conductivity and temperature, and is localized in a semiarid area (Caatinga biome). The soil has median natural fertility and high concentration of dissolved salts. A short diagnosis with illustrations and two models of the potential geographic distribution for the species are included.

Key words: algae; intermittent pond; northeastern Brazil

Pithophora is a genus of branched filamentous algae, which have interspersed barrel-shaped akinetes, cylindrical and irregular and are physiologically adapted to subtropical regions (Wittrock 1877). *Pithophora* was described in 1877 by Wittrock as a basionym of *Ceramium roettleri* Roth (1806). The first species described for this genus was *Pithophora roettleri*, which is native to Asia (Wittrock 1877). Due to the morphological plasticity of *P. roettleri*, several taxa described and typified in the late nineteenth century and early twentieth century (e.g., *Conferva oedogonia* Montagne, *Cladophora sumatrana* Martens, *Cladophora zelleri* Martens, *Pithophora oedogonia* (Montagne) Wittrock, *Pithophora cleveana* Wittrock, *Pithophora sumatrana* (G.Martens) Wittrock, *Pithophora kewensis* Wittrock, *Pithophora aequalis* Wittrock, *Pithophora polymorpha* Wittrock, *Pithophora zelleri* (G.Martens) Wittrock, *Pithophora affinis* Nordstedt, *Pithophora microspora* Wittrock, *Pithophora reineckei* Schmidle, *Pithophora clavifera* Schmidle, *Pithophora radians* W. & G.S. West, *Pithophora pachyderma* Schmidle, *Pithophora variabilis* Schmidle, *Pithophora varia* Wille, *Pithophora macrospora* F. Brand, *Pithophora mooreana* Collins, *Pithophora heterospora* M.Howe & Hervey, *Pithophora pragensis* J. Sula, *Pithophora chinensis* Skvortsov, *Pithophora tropica* Woodhead & Tweed) were indicated as heterotypic synonyms of *P. roetlleri* according to molecular data (Boedeker et al. 2012). This confusion emphasizes the delay in the phylogenetic and taxonomic studies on the genus *Pithophora*.

Up to now, only four records of *P. roettleri* and heterotypic synonyms are valid for Brazil (i.e. publications or herborized specimens with information on the geographic coordinates). *Pithophora sumatrana* was reported to occur in lakes of the Pantanal, in the vicinity of Cuiabá, state of Mato Grosso (MT) (Schmidle 1901). *Pithophora oedogonia* was found in an urban lake in Porto Alegre, state of Rio Grande do Sul (RS) (Franceschini et al. 2006). *Pithophora roettleri* was recorded in ponds in a fragment of the Atlantic Forest in the Parque Estadual das Fontes do Ipiranga, state of São Paulo (SP) (Bicudo 2014), and *P. oedogonia* was found in the Paraná River, state of Paraná (PR) (Algarte et al. 2015). Records of *P. oedogonia* without biogeographical validation are reported for the states of Alagoas, Paraíba, Ceará, and Rio de Janeiro (Species Link 2015).

Due to the scarce knowledge on the biogeography of *Pithophora* in Brazil, finding and reporting new sites of occurrence of such species are still challenging for botanists and applied phycologists. In this context, the new record presented herein is associated with a model of the potential geographic distribution of *P. roetlleri* (*sensu lato*) in Brazil, thus allowing the recognition of new environmentally appropriate areas for the occurrence of such species.

Two samples collected (November 2014) on the water surface underwent the usual techniques for herborization of macroalgae (Pedrini 2010) and were incorporated to the herbarium HVASF (voucher 22395) at the Universidade Federal do Vale do São Francisco. Additional water samples (n = 10) were collected to evaluate morphometric aspects of microscopic organisms (100 specimens). The morphometric analyses of the algae were performed using a magnifying glass (Medilux), and an optical microscope (Physys) at the magnification of 100×. Photomicrographs were captured using a digital camera (Tucsen Imaging Technology®, model TCA – 1.31 Color) coupled to the optical microscope. Algae were identified by consulting specific publications (Wittrock 1877; Franceschini et al. 2006; Bellinger and Sigee 2010; Daga et al. 2014; Algarte et al. 2015), and the taxonomic classification follows Boedeker et al. (2012).

Optimization procedures were performed to elaborate models of the potential geographic distribution of *P. roetlleri* by correlating places of occurrence of the species and its heterotypic synonyms (*P. sumatrana* and *P. oedogonia*) in Brazil with bioclimatic layers (temperature and precipitation), obtained from the database WorldClim7 (Table 1). In order to avoid interpretation conflicts on the taxonomy of *P. roetlleri*, we used the name *P. roetlleri* (*sensu lato*) to represent the species and its heterotypic synonyms. The bioclimatic layers are interpolations obtained from weather stations in the years of 1950 to 2000 (Hijmans et al. 2005).

Before the optimization procedures, we identified both the redundant and collinear environmental variables, and excluded them from the analyses. By excluding collinear variables, we reduced the dimensionality of multivariate analyses, thus decreasing mathematical errors (Legendre and Legendre 2012). Principal Component Analysis (PCA) was performed to identify redundant environmental variables. The PCA analyzed 1000 points generated by the Random Point Generator method within Brazil, with values of 19 different bioclimatic variables. The Broken-stick model (Jackson 1993) was used to determine the number of significant axes in the analysis. More details on these analyses can be found in Diwold et al. (2010) or Eisenlohr (2014).

To identify the collinear variables we performed logistic correlations between the records of *P. roetlleri* (*sensu lato*) in Brazil (response variable) and each bioclimatic variable indicated by the PCA as non-redundant, and used other bioclimatic variables as covariates. For the optimization procedure we selected the variables which were correlated with the response variable ($p < 0.05$), but were not collinear when compared to the other variables. The logistic correlation analyses were performed using the software Maxent version 3.3.2, which has great accuracy indices in exploratory modeling studies (Phillips et al. 2006).

Two models of the potential geographic distribution of *P. roetlleri* (*sensu lato*) in Brazil were generated. The first used points recorded for the states of RS, SP, MT and PR; the second used the same points plus the new record reported herein. The models allowed comparison of the patterns of the potential geographical distribution of *P. roetlleri* (*sensu lato*) before and after the new record, and expanded the areas of possible occurrence of the species.

A cutting limit was defined to separate the appropriate and inappropriate environments for the occurrence of *P. roetlleri* (*sensu lato*) in the research area. Those pixels (1 km^2) with values above the threshold (0.5) were considered to be possible areas of occurrence (Pearson et al. 2007). The AUC values above 0.75 indicated that models were satisfactory (Elith et al. 2006).

Binomial tests (software BioEstat 5.0) (Ayres et al. 2007) were used to determine if the performance of the models differed from chance. The optimization procedures, the establishment of cutting limits, and AUC values for the models were obtained using Maxent 3.3.2. (Phillips et al. 2006). The PCA analysis and the Broken-stick model were performed using Past (Hammer et al. 2001); the randomization of the 1000 points of the PCA, and the production of the models were made using ArcGis version 10.0. (ESRI 2006).

The new record of *Pithophora* occurred at the margin of an intermittent pond on the Campus de Ciências Agrárias (CCA) of the Universidade Federal do Vale do São Francisco (UNIVASF; -09.323078, -040.562387, datum WGS84), Petrolina, PE. This location is in the São Francisco River basin, northeastern Brazil, in the Caatinga biome (Figures 1 and 4).

The morphology and morphometrics of the specimens is in accordance with the description of *P. oedogonia* (Franceschini et al. 2006; Algarte et al. 2015) and its original description (Wittrock 1877, pages 55 to 58): Branched macroscopic thallus, 6–10 cm in height,

Table 1. Climatic variables and their measure.

Initials	Variables	Measure
Bio1	Annual temperature average	°C
Bio2	Mean Diurnal Range (Mean of monthly (max temp – min temp))	°C
Bio3	Isothermality (BIO2/BIO7) (* 100)	°C
Bio4	Temperature Seasonality (standard deviation *100)	°C
Bio5	Max Temperature of Warmest Month	°C
Bio6	Min Temperature of Coldest Month	°C
Bio7	Temperature Annual Range (BIO5–BIO6)	°C
Bio8	Mean Temperature of Wettest Quarter	°C
Bio9	Mean Temperature of Driest Quarter	°C
Bio10	Mean Temperature of Warmest Quarter	°C
Bio11	Mean Temperature of Coldest Quarter	°C
Bio12	Annual Precipitation	mm
Bio13	Precipitation of Wettest Month	mm
Bio14	Precipitation of Driest Month	mm
Bio15	Precipitation Seasonality (Coefficient of Variation)	mm
Bio16	Precipitation of Wettest Quarter	mm
Bio17	Precipitation of Driest Quarter	mm
Bio18	Precipitation of Warmest Quarter	mm
Bio19	Precipitation of Coldest Quarter	mm

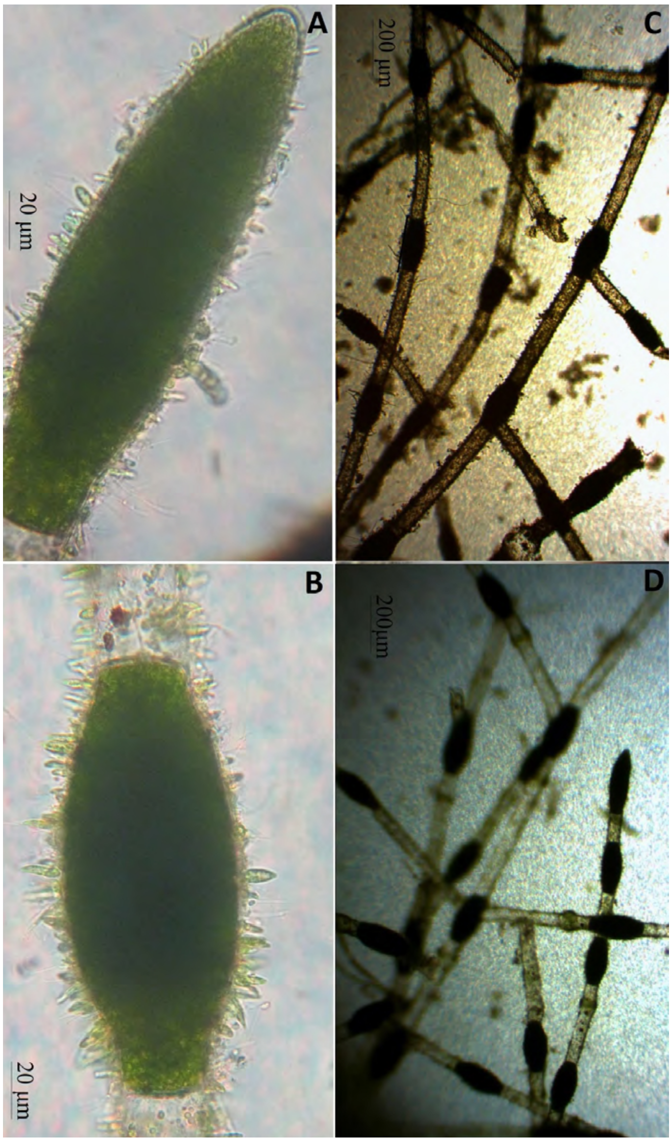

Figure 1. Morphological aspects of *Pithophora oedogonia*, heterotypic synonym of *P. roetlleri*, recorded at São Francisco river basin: apical akinetes (A, D); branching degree varies from first order, unilaterally and bilaterally, opposite and/or alternate; intercalary akinetes barrel-shaped (isospores) (B, C, D); numerous pyrenoids (A, B). Scale bar: 20 µm or 200 µm.

similar to hair strands; branching degree varies from first to third order (more frequently in first order ~65% of cases), unilaterally and bilaterally, opposite (more frequently) or sometimes alternate; cylindrical vegetative cells, 500–850 µm long, 55–90 µm width (thicker in the principal filament and thinner in branches of the 1st, 2nd and 3rd order). Intercalary akinetes barrel-shaped (isospores) 120–200 µm long, 70–95 µm width, apical akinetes solitary, conical 150–230 µm long, 75–90 µm width, chloroplast 1, reticulated, numerous pyrenoids. Planktonic or periphytic habit in rocky regions of shallow waters (between 10–50 cm). Because *P. oedogonia* is a heterotypic synonym of *P. roettleri* (according to morphological and molecular data, Boedeker et al. 2012) we chose to use the valid and correct name (*P. roettleri*) for the new record (Figure 1).

According to Bellinger and Sige (2010), *Pithophora* is likely to be misidentified as *Cladophora*, although *Pithophora* is easily distinguished by the presence of barrel-shaped interspersed akinetes.

The location of the new record is subject to few human activities, other than grazing and trampling by cattle. Previous records of *P. roettleri* occurred within conservation areas such as the Parque Estadual das Fontes do Ipiranga, SP (Schoenlein-Crusius et al. 2006; Bicudo 2014) and the Paraná River, PR (Algarte et al. 2015), indicating that the species occurs in environments with low levels of degradation. However, the record of *P. oedogonia* in an urban lake (Moinhos dos Ventos pond) does not support this tendency, even though this lake is little affected by anthropogenic eutrophication (Franceschini et al. 2006).

The characteristics of climate and soil at the site of the new record are particular to the Caatinga biome: negative water balance, intense light irradiance (18.2 MJ m^{-2} day^{-1}), poorly drained soil, with median natural fertility, and high concentration of salts (EMBRAPA 2010). As a result of these characteristics, aquatic environments in this region are warm and have high conductivity, which was confirmed by empirical data (conductivity 110 µS cm^{-3} and temperature 30.3°C). The limnologic description of the site of the new record is similar to that of the Paraná River (temperature about 31°C, mean alkalinity 320.9 mEq·L^{-1}, mean conductivity 49.6 µS·cm^{-1}), where *P. oedogonia* was previously reported. However, *P. oedogonia* was reported from environments with lower temperature in Brazil (< 25°C *sensu* Franceschini et al. 2006), confirming that the distribution of *Pithophora* also includes subtropical environments (Wittrock 1877).

The Broken-stick model indicates that only the first two axes of the PCA were significant. Thus, the variables with vectors that formed angles of approximately 45 degrees with the axes 1 and 2 of the PCA (Bio2, Bio5, Bio12, and Bio19) were redundant when compared to the others, and were excluded from the optimization procedures (Figure 2). Only the variables Bio4 ($p < 0.001$; Z = 0.19), Bio6 ($p < 0.0001$; Z = –0.24) and Bio13 ($p < 0.0001$; Z = –0.22) were correlated with the dependent variable, i.e., the records of *P. roettleri* (*sensu lato*) in Brazil, and they were not collinear (Figure 3). The fact that the occurrence of *P. roettleri* (*sensu lato*) correlates both directly with the seasonal variation of temperature (Bio4) and inversely with temperature in the cooler months (Bio6) allows us to infer that *P. roettleri* is adapted to colder environments, although this new record is in a semiarid region (Caatinga biome). These results corroborate the work of Wittrock (1877) that mentions the adaptation of *P. roettleri* to cooler subtropical regions. The negative correlation between the occurrence of *P. roettleri* (*sensu lato*) and precipitation during the wettest months (Bio13) shows that the

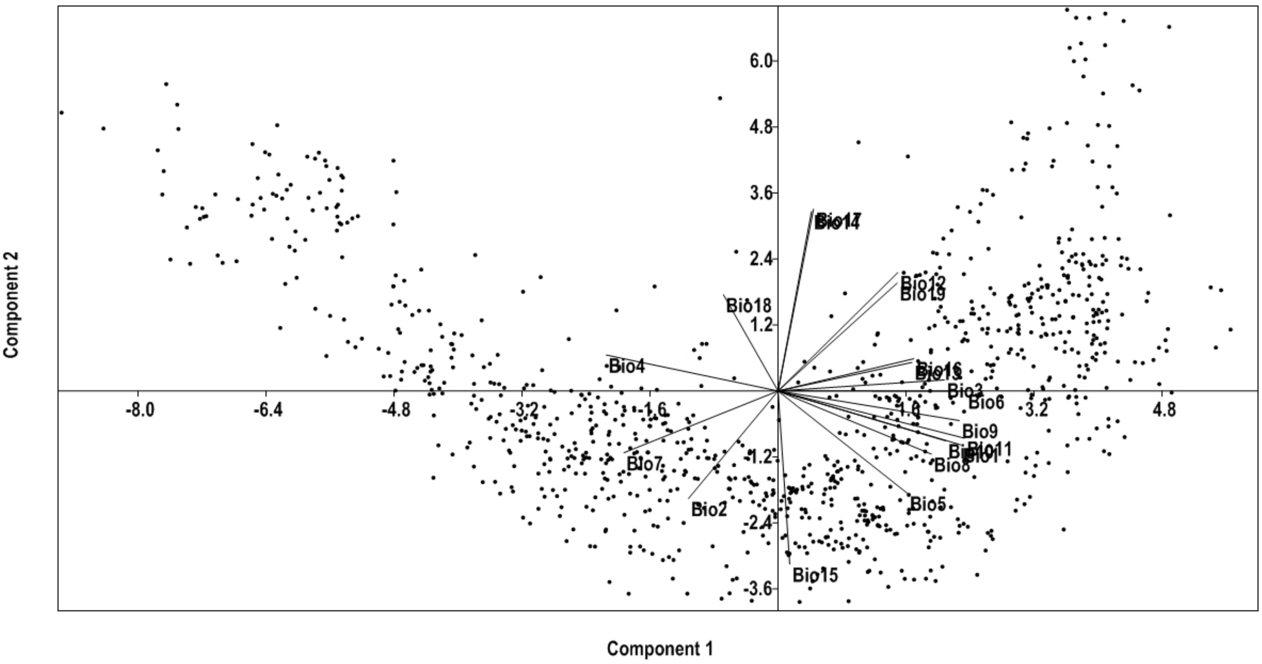

Figure 2. Principal component analysis (PCA) calculated from the randomization of bioclimatic variables data extracted from WorldClim7 for 1000 points in Brazil. The graph shows Bio2, Bio5, Bio12 and Bio19 variables little correlated with significant components (axes). Bioclimatic variable abbreviations are shown in the Table 1.

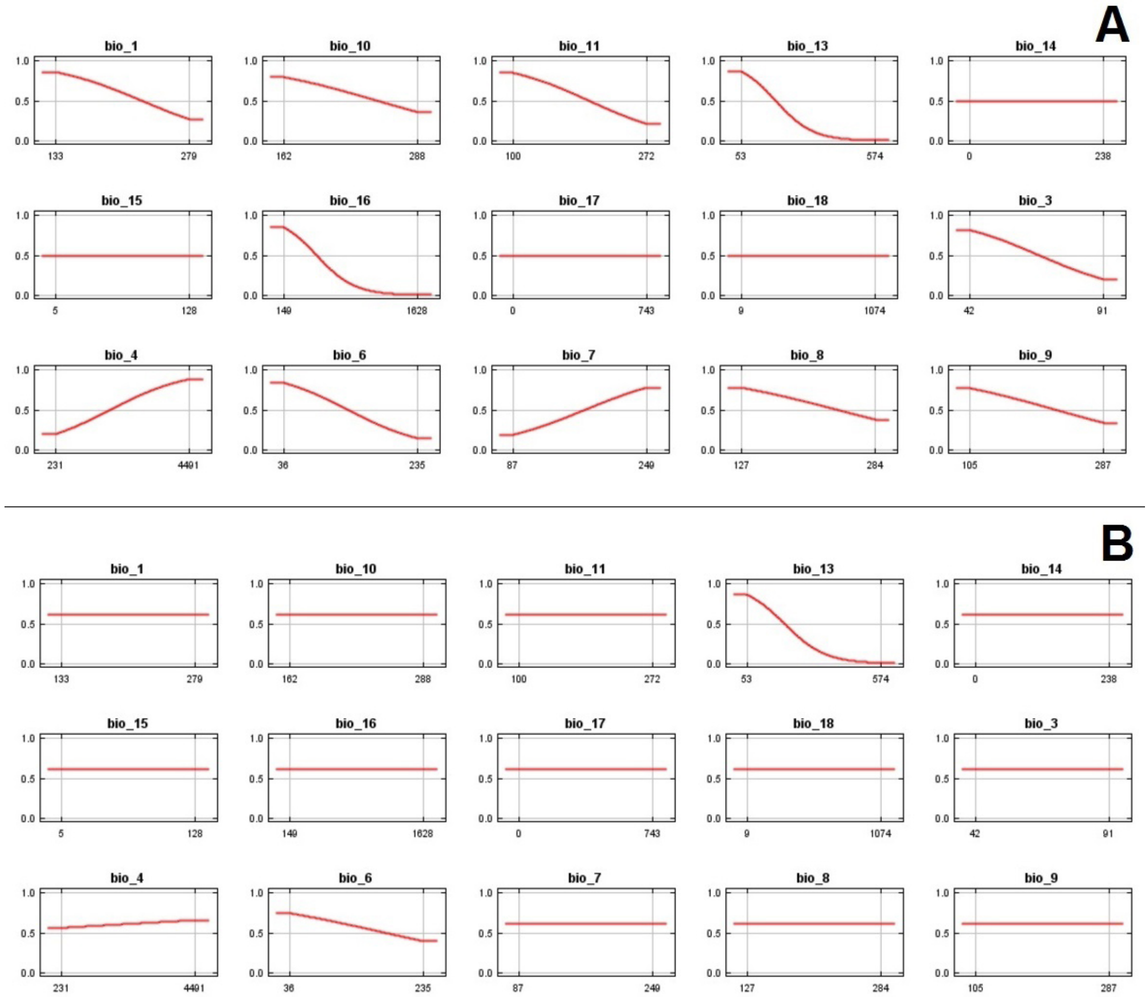

Figure 3. Logistic correlations between the points of occurrence of *P. roetlleri* (*lato sensu*) in Brazil and the bioclimatic variables (WorldClim7). (A) non-redundant variables indicated by the PCA; (B) non-redundant variables indicated by the PCA, under the influence of covariates. Only the variables Bio 13, Bio 4 and Bio 6 correlate to the response variable (records of *P. roetlleri*) and do not present collinearity to the other variables. Bioclimatic variable abbreviations are shown in the Table 1.

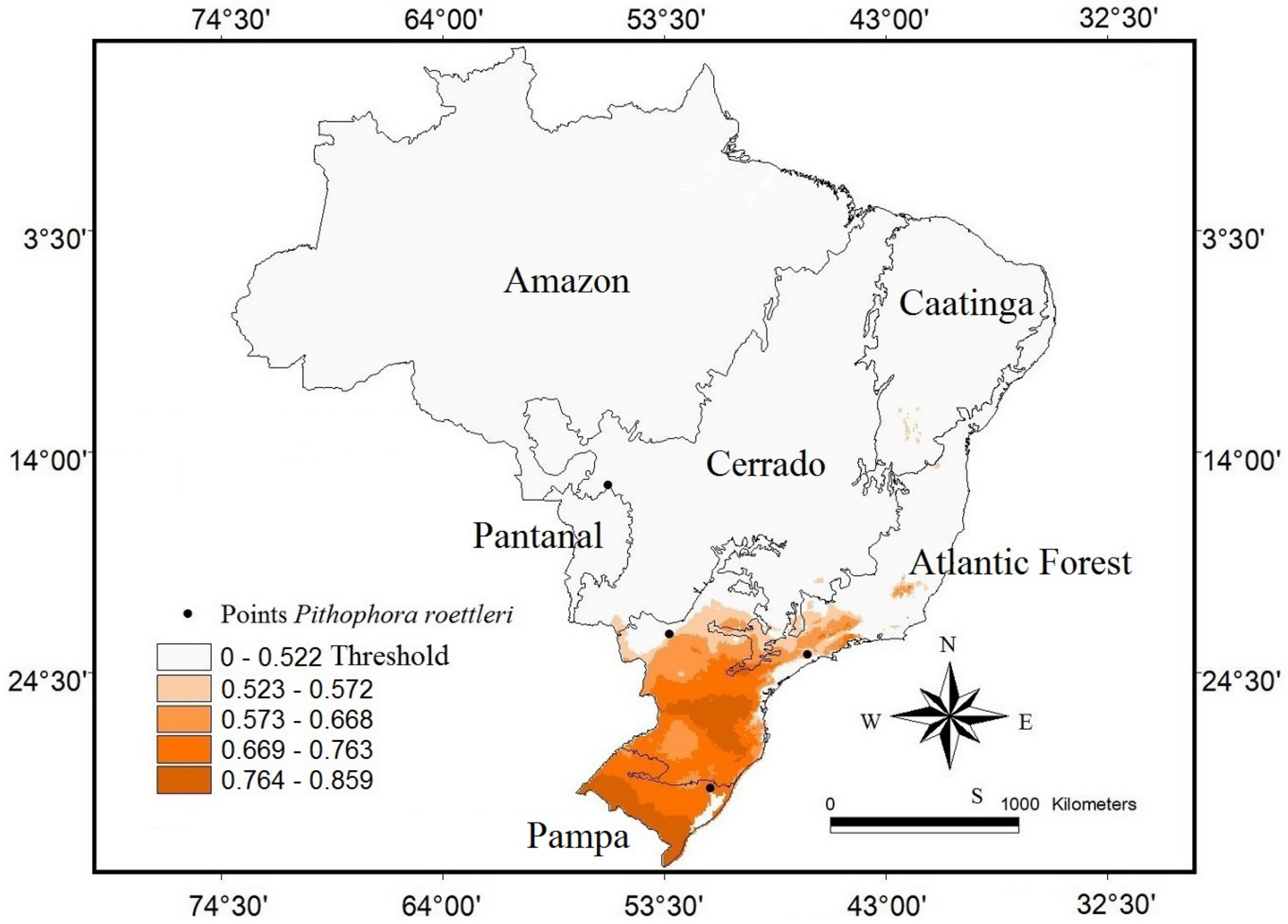

Figure 4. Potential geographical distribution model (Maxent algorithm) for *Pithophora roettleri* (*sensu lato*) in Brazil, excluding the new record to the São Francisco Hydrographic basin. (A) (Threshold = 0.522% and AUC = 0.923).

species prefers environments with little rainfall, which produces lower turbidity and greater transparency in aquatic environments. Rainfall is an important factor in the dynamics of the physical characteristics of aquatic environments (e.g., height, flow, turbidity and transparency) and therefore influences the occurrence of algae and/or macrophytes (Esteves 2011).

The two generated models had satisfactory performance (AUC 0.900). The results of binomial tests confirmed the good performance of the generated models ($p < 0.05$ / $t > 0.5$). This shows that the models were different from chance, and that the optimization procedures between the points of occurrence of the species, and the predictor variables were significantly able to define potential areas for the occurrence of the species in Brazil.

The initial model delimited an area of 9,760 km^2 as environmentally appropriate for the occurrence of *P. roettleri* (*sensu lato*) in Brazil. This area covers patches of the Atlantic Forest and Pampa in the states of São Paulo, Paraná, Rio de Janeiro, Mato Grosso do Sul, Minas Gerais, Espírito Santo, and Rio Grande do Sul (Figure 4). The initial model also identified a small area suitable for the occurrence of the species in the Cerrado and Caatinga biomes. However, the small size of this area and the low probability of records of *P. roettleri* (*sensu lato*) there (~55%) suggests that its occurrence is unlikely (Figure 4).

The final model indicated large environmentally suitable areas for *P. roettleri* (*sensu lato*) in the middle and lower São Francisco River, predicting further records for this river basin. The São Francisco River basin includes aquatic microhabitats known for their potential of colonization by macrophytes (Moura-Júnior et al 2011; Campelo et al. 2012). The model also indicated potential locations in the Parnaiba River basin, and other Atlantic river basins (Figure 5).

The final model has expanded the areas of possible occurrence of the species in Brazil, and confirmed the occurrence of *P. roettleri* (*sensu lato*) in the Cerrado, which was questionable according to the interpretations of the initial model (Figure 5). The final model indicated a potential area of 19,526 km^2 for the occurrence of the species in Brazil (100.06 % greater than the initial model), including two new areas of Cerrado as environmentally appropriate for the occurrence of *P. roettleri* (*sensu lato*).

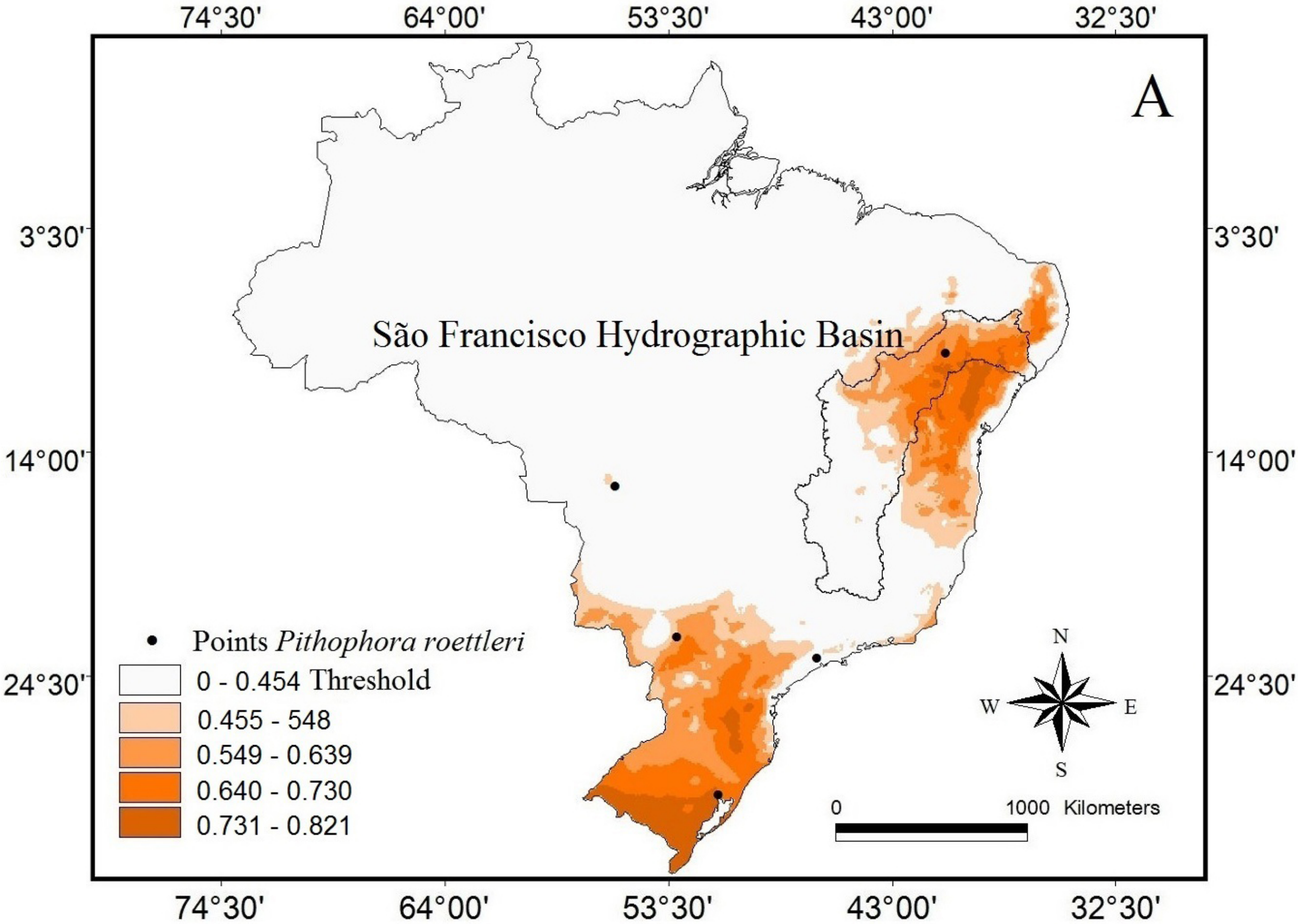

Figure 5. Potential geographical distribution model (Maxent algorithm) for *Pithophora roettleri* (*lato sensu*) in Brazil, including new record to the São Francisco Hydrographic Basin (A) (Threshold = 0.454 and AUC = 0.906).

Both areas are mainly located in the state of Bahia, but also extend to the states of Minas Gerais and Piauí.

In summary, we observed that *P. roettleri* (*sensu lato*) occurred in an environment with high conductivity and little degradation caused by human activities. The species tolerates waters at different thermal conditions. The models presented herein are important to understand the distribution patterns of *P. roettleri* (*sensu lato*), especially in the Caatinga biome. It is noteworthy that the specimens collected in the Caatinga were the first to be deposited in botanical collections in northeastern Brazil, and emphasize the importance of this publication for the biodiversity of Brazil.

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