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Algae in phytotelmata from Caatinga: first record of the genus *Rhopalosolen* Fott (Chlorophyta) for Brazil

Geraldo José Peixoto Ramos,[1] Carlos Eduardo de Mattos Bicudo,[2] Carlos Wallace do Nascimento Moura[1]

1 Universidade Estadual de Feira de Santana, Programa de Pós-Graduação em Botânica, Av. Transnordestina, s/n – Novo Horizonte, CEP 44036-900, Feira de Santana, BA, Brazil. **2** Instituto de Botânica, Núcleo de Pesquisa em Ecologia, Av. Miguel Estéfano, nº 3687, CEP 04301-902, São Paulo, SP, Brazil.

Corresponding author: Geraldo José Peixoto Ramos, geraldojpr@gmail.com

Abstract

The first record of the genus *Rhopalosolen* Fott (Chlorophyta) for Brazil is presented here. Taxonomic studies on phytotelm algae are, in general, scarce, but especially are so in semi-arid regions such as the Caatinga. Here, we sampled 60 bromeliads in the Itaberaba's municipality, Bahia state, and we identified *Rhopalosolen cylindricus*, a green algae species not previously recorded for Brazil. Additionally, our data provide an expansion on the known distribution of the species and their morphological and reproductive aspects as well as microhabitat conditions and frequency of occurrence.

Key words

Bahia; bromeliads; drought; microcosms; Trebouxiophyceae.

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Introduction

Caatinga is an exclusively Brazilian biome characterized by a vegetation mosaic that covers the semi-arid region of northeast Brazil, and bromeliads are 1 of the main components of the flora (Andrade-Lima 1981, Giulietti et al. 2004). The rosette arrangement of various Bromeliaceae leaves allows accumulation of water usually rich in nutrients, which forms an aquatic microcosm, known as phytotelm, where several organisms may be found, including algae (Picado 1913, Varga 1928, Laessle 1961). Tank bromeliads form an "oasis" within the semi-arid area, as these plants usually are the only source of water available to many organisms that live nearby (Islair et al. 2015). Most studies in these environments are targeted to the fauna, especially invertebrates, and little is known about algal diversity in these microcosms (Sophia 1999). The relationship between bromeliads and algae is still a largely unexplored and poorly understood topic (Marino et al. 2011).

Some recent studies have helped understanding the ecology of algae in tank bromeliads. As an example, a few studies demonstrated that algae may be an alternative energy source for some bromeliad food webs (Brouard et al. 2011, Farjalla et al. 2016). Other studies have shown that different algal communities may be found in different plants that are near in the same area (Carrias et al. 2014), which may be related to plant architecture (Marino et al. 2011) and the exposure to sun (Sophia et al. 2004,

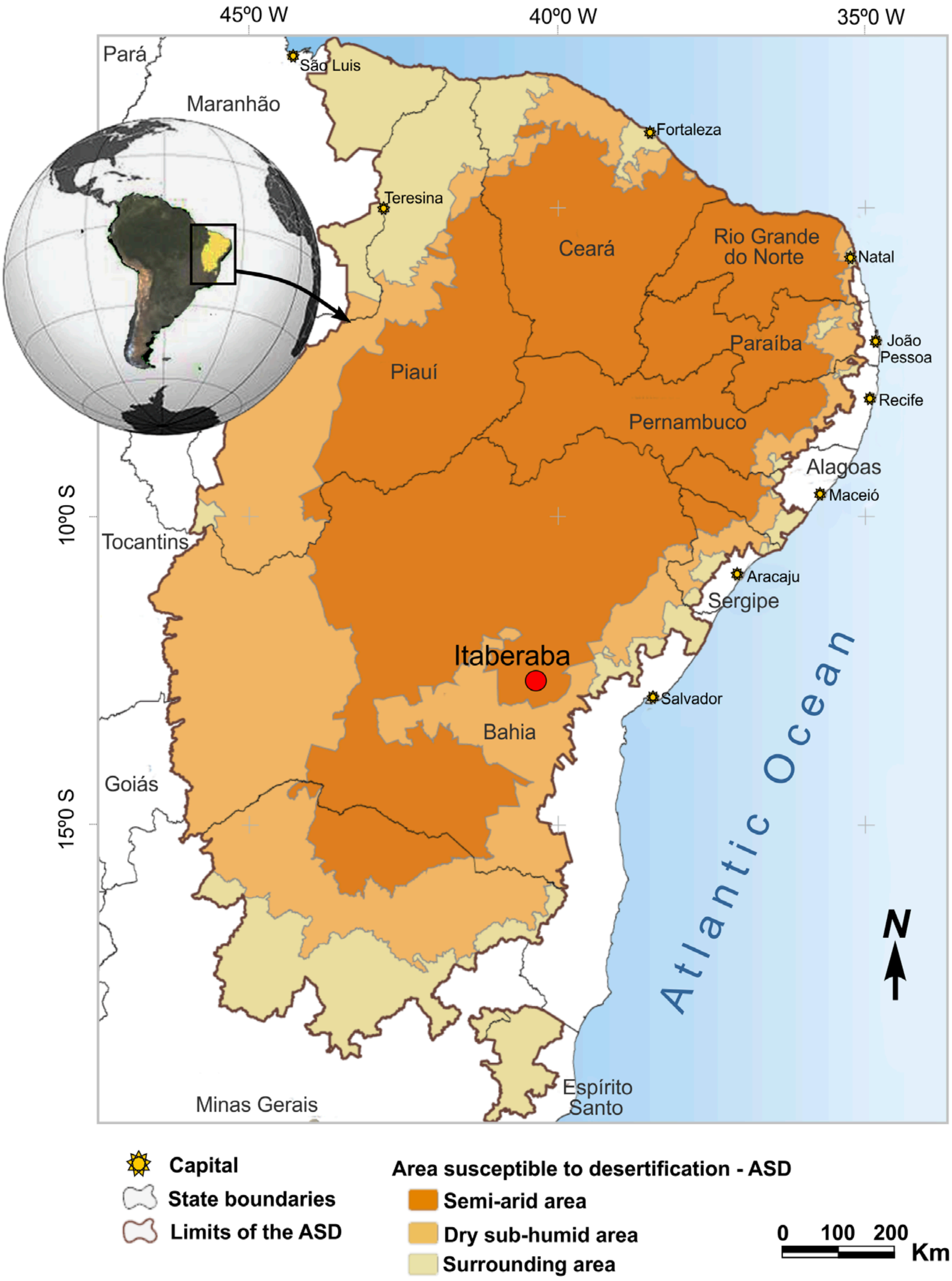

Figure 1. Map of study area. Red dot represents the local of the new record in the Caatinga biome.

Brouard et al. 2011, Carrias et al. 2014, Farjalla et al. 2016). Among the main algal groups occurring in Brazilian phytotelmata are the diatoms (Lyra 1971, 1976), coccoid green algae (Nogueira 1991), dinoflagellates (Ramos et al. 2016) and desmids (Sophia 1999, Sophia et al. 2004, Ramos et al. 2011, 2017).

Studies on phytotelm algae are scarce in semi-arid regions such as the Caatinga. During a survey of algae from bromeliads in Bahia state, we found a representative of *Rhopalosolen* Fott (Chlorophyta). Here we report the first record of *Rhopalosolen* for Brazil. Additionally, our data provide an expansion on the known distribution of *Rhopalosolen cylindricus* highlighting their morphological and reproductive aspects as well as microhabitat conditions and frequency of occurrence of the species.

Methods

This study was conducted in the Fazenda Itaberaba (12°30′ S, 40°04′ W), Itaberaba municipality, Bahia state, northeast Brazil (Fig. 1). The average annual temperature at Itaberaba is 25.3 °C with an average rainfall of 62.2 mm, while the driest month is September (15 mm) and the wettest 1 is December, with an average fall of 115 mm (Climate-data.org 2016). The study area is located in a semi-arid area of the Caatinga biome, and recently, was considered as extremely high priority for biodiversity conservation and very high urgency for susceptibility to desertification (MMA 2016).

We sampled 60 tank bromeliads (*Aechmea* cf. *lingulatoides* Leme & H.E. Luther) (Fig. 2) selected at random on each of 3 months (20 bromeliads per month): January

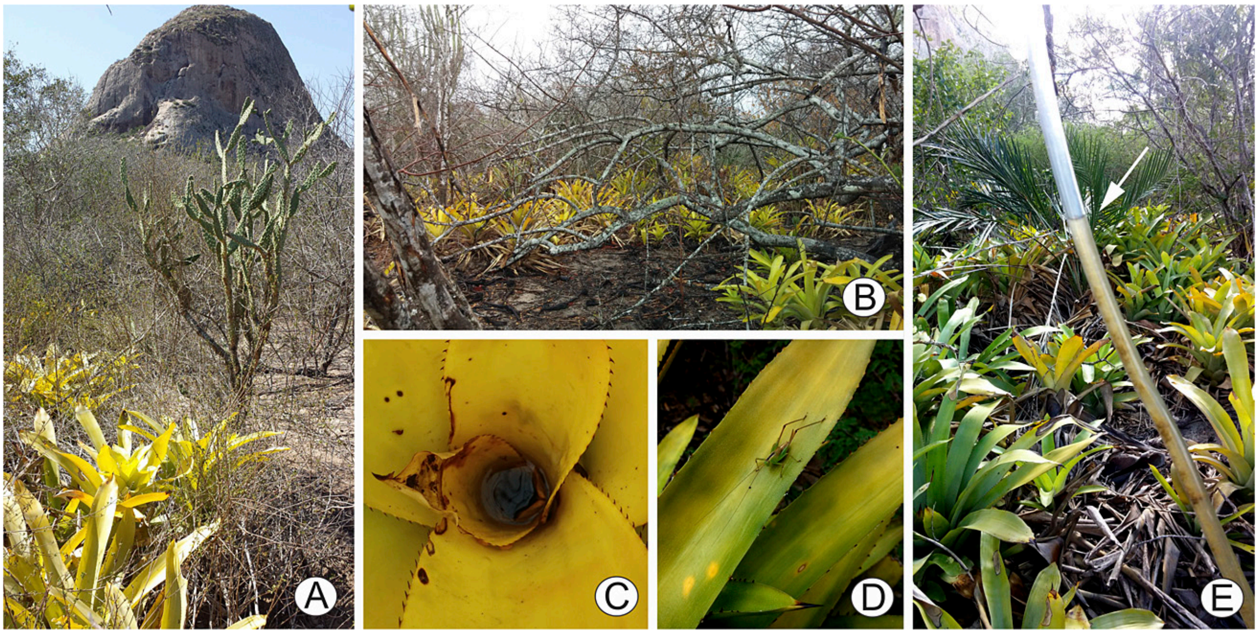

Figure 2. A–B. General aspect of the bromeliads from Fazenda Itaberaba. **C.** Detail of the central tank. **D.** Insect commonly found in the bromeliads. **E.** Detail of the hose with water collected (arrow).

(dry), May (rain), and August (dry) 2015. All stored water in the tank bromeliads was collected with a syringe (50 ml) coupled with a polyethylene hose. The samples collected were preserved in Transeau solution (Bicudo and Menezes 2006), and the vouchers were deposited in herbarium (HUEFS) of the State University of Feira de Santana.

Water variables such as temperature (°C), pH, conductivity (mS cm^{-1}) and total dissolved solids (ppt) were measured with a multiparameter probe Hanna HI98130, and dissolved oxygen (mg·L^{-1}) was measured using a portable digital Instrutherm (MO-910).

Morphological and metric features of both vegetative and reproductive cells were observed with a light microscope (Olympus Model BX-43), and digital images were taken with a 5.0 MP QImaging camera using the software Image-Pro Premier 9.1.4. The present species studied was identified using specialized literature (Korshikov 1953, Fott 1957, 1958, Komárek and Fott 1983) and classification system was checked with Algaebase (Guiry and Guiry 2017).

Frequency of occurrence of algae was calculated in each period, considering the number of samples in which the present species occurred in relation to the total number of samples collected. We follow the Matteucci and Colma (1982) categories: >70% (very frequent, VF); ≤70% and >40% (frequent, F); ≤40% and >10% (occasional, O); ≤10% (rare, R).

Results

Rhopalosolen cylindricus was found in tank bromeliads (phytotelm) growing in different types of substrates such as invertebrate exoskeleton, fungal hyphae, leaf fragments, and foliar trichomes (Fig. 3). Water had acidic pH, low conductivity, and high temperature (Table 1).

World distribution is known to be Argentina, Ivory Coast, Romania, Russia, Sweden, Spain, Hungary, Poland, Ukraine, and United States (Komárek and Fott 1983, Guiry and Guiry 2017). The present work represents the first record of the genus for Brazil (Fig. 5). *Rhopalosolen cylindricus* was a very frequent species (FO = 85%) in tank bromeliads of Itaberaba, occurring in 51 from the total 60 bromeliads sampled. This species was also very frequent in each sampled period: January (FO = 95%), May (FO = 75%), and August (FO = 80%), suggesting it is well adapted to both dry and rainy periods.

Rhopalosolen Fott, 1957

Rhopalosolen cylindricus (F. Lambert) Fott, 1957
Figures 3, 4

Basionym. *Characium cylindricum* F. Lambert (1909): 70.

Description. Solitary cells, cylindrical to clavate, apex rounded, usually epizoic and/or epiphytic (Fig. 3), attached to substrate by a mucilaginous basal disk; young

Table 1. Measurements of water characteristics of the tank bromeliads with *Rhopalosolen cylindricus*. Water temperature = T, Conductivity = C, Total Dissolved Solids = TDS, Dissolved oxygen = DO. Values represent mean (standard deviation).

Variable	January	May	August	All periods
T (°C)	32.9 (± 1.8)	28.6 (± 1.3)	27 (± 1.5)	29.7 (± 2.9)
pH	5.1 (± 1.5)	4.2 (± 0.3)	4.3 (± 0.6)	4.5 (± 1)
C (mS·cm^{-1})	0.5 (± 0.6)	0.1 (± 0.03)	0.1 (± 0.06)	0.2 (± 0.4)
TDS (ppt)	0.2 (± 0.3)	0.03 (± 0.01)	0.03 (± 0.02)	0.1 (± 0.2)
DO (mg·L^{-1})	4.3 (± 1.4)	4.4 (± 1.1)	8.1 (± 3.6)	5.3 (± 2.7)

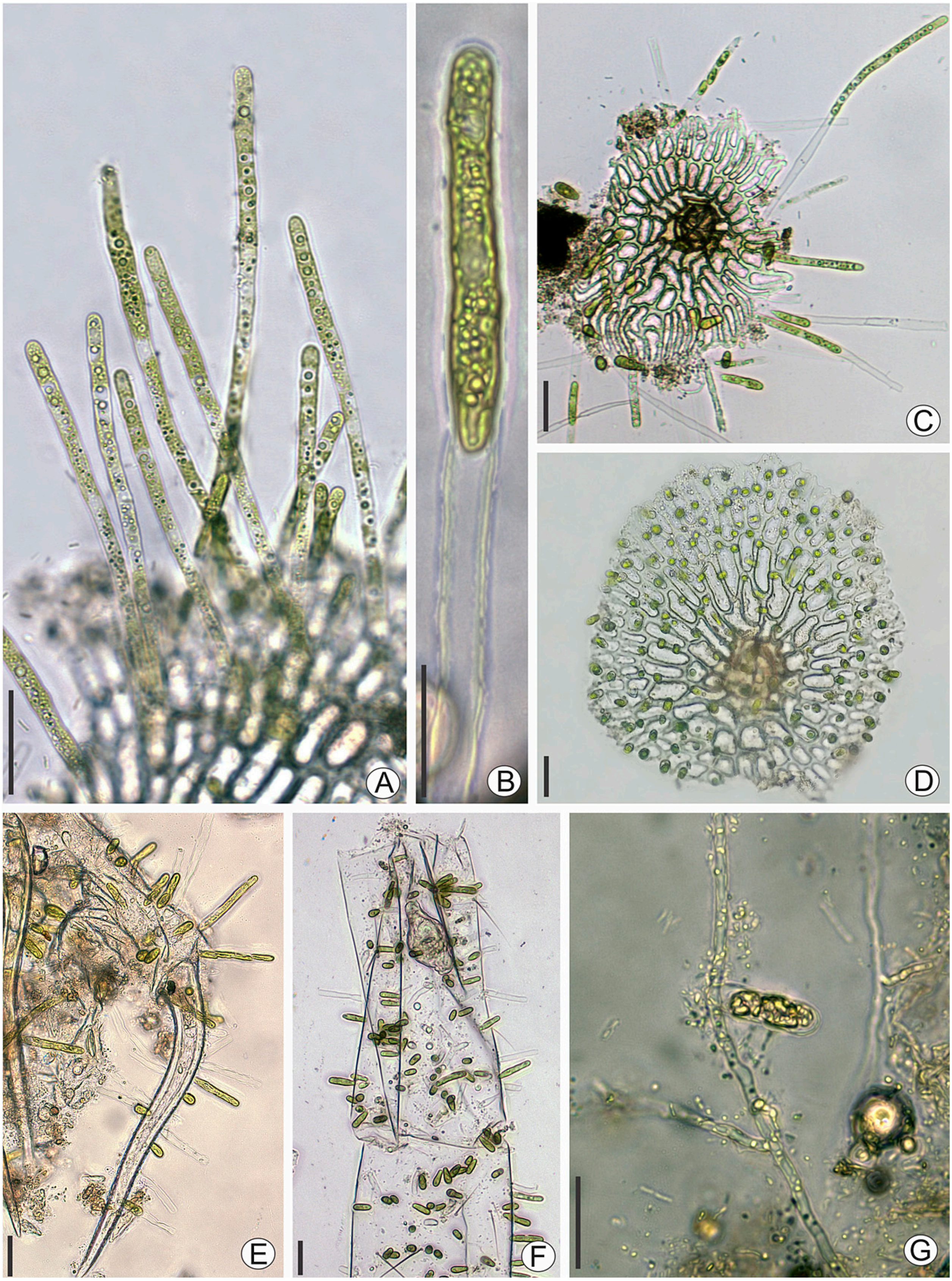

Figure 3. *Rhopalosolen cylindricus*. **A.** General aspect. **B.** Detail of adult cell. **C–E.** Epiphytic growth. **C, D.** Bromeliad trichomes with algae associated. **F.** Epizoic growth. **G.** Epifungal growth. Bars = 20 μm.

cells straight, uninucleate, later becoming multinucleate (Fig. 3A); chloroplast parietal, adult cells straight or slightly curved (Figs 3B–3C), with many pyrenoids. Asexual reproduction by releasing elliptic-ovoid aplanospores (Fig. 4H–4L). Sexual reproduction by globose biflagellate isogametes (Fig. 4B); reproductive structures released through an opening produced at the cell apex by the cell wall dissolution. Adults cells 70–180 μm long,

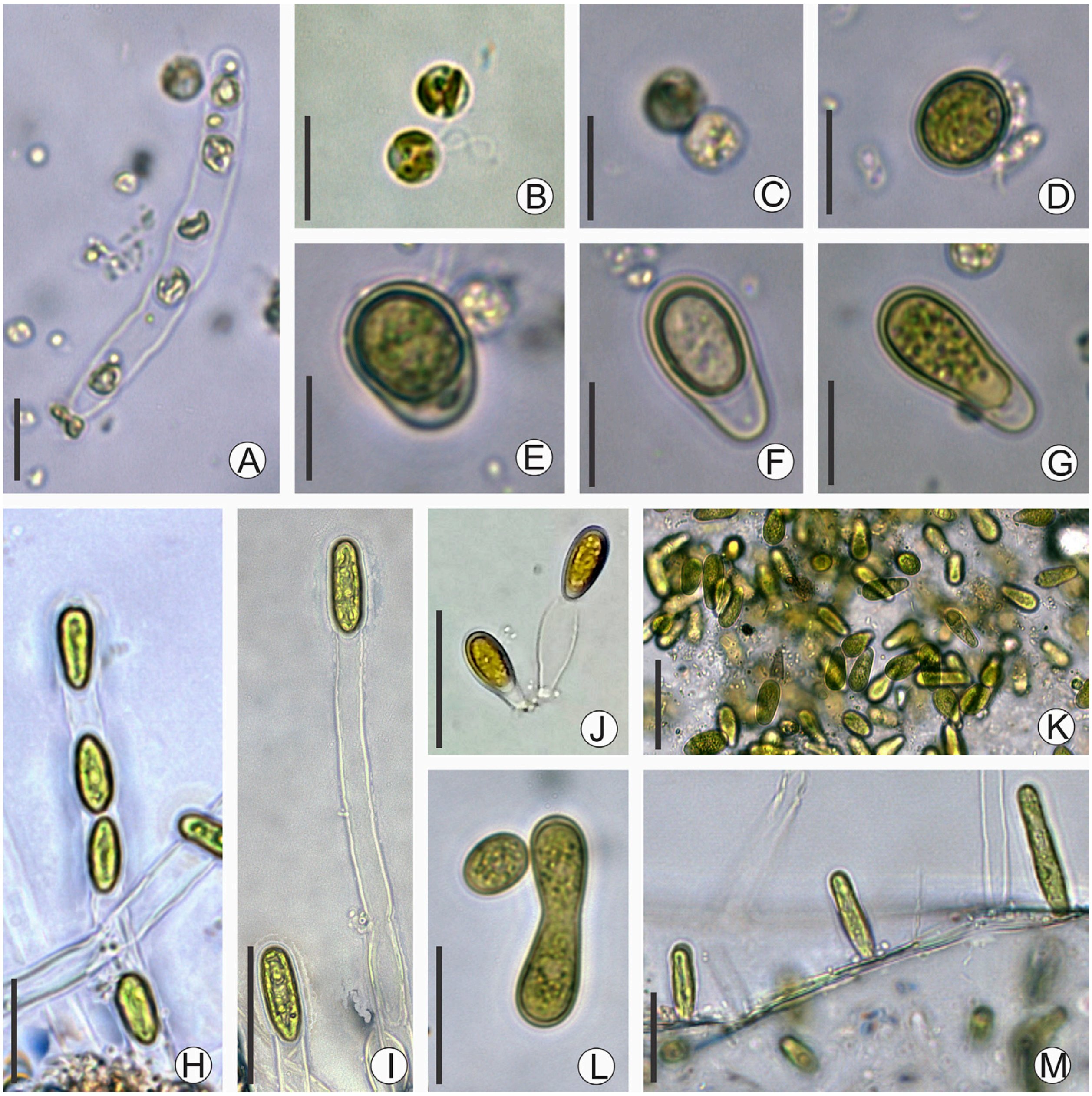

Figure 4. Reproduction of *Rhopalosolen cylindricus* (A–G show sexual reproduction and H–M show asexual reproduction). **A.** Gametes formation. **B** Isogametes. **C.** Fusion of gametes. **D.** Zygote. **E–G.** Development of zygote. **H.** Aplanospores formation. **I–J.** Release aplanospores. **K.** Young cells. **L.** Vegetative reproduction. **M.** Fixation to substrate. Bars = A–H = 10 μm; I–M = 20 μm.

5–10 μm in diameter; aplanospores 13–18.7 long, 5–8 μm wide; gametes 5.1–7.4 μm in diameter.

Material examined. Brazil. Bahia: Itaberaba, Fazenda Itaberaba, 12°30′30.6″ S, 40°04′35.8″ W, 21 January 2015, *G.J.P. Ramos et al. s.n.* (HUEFS 215777); 12°30′29.9″S, 40°04′36.1″W, 08 May 2015, *G.J.P. Ramos et al. s.n.* (HUEFS 155596); 12°30′31.3″S, 40°04′34.7″W, 21 August 2015, *G.J.P. Ramos et al. s.n.* (HUEFS 222992). Full list of examined vouchers is available in Appendix, Table A1.

Discussion

Despite several records of the occurrence of *Rhopalosolen cylindricus* in the northern hemisphere, especially in the European countries, there are few records of that species in the southern hemisphere, with records only in Australia and Argentina (Komárek and Fott 1983, Challagulla et al. 2015). Thus, we report the second record of this species for South America and the first for Brazil. *Rhopalosolen* (Rhopalosolenaceae, Trebouxiophyceae) was proposed by Fott (1957) as a new genus of Chlorophyta to transfer *Characium cylindricum* F. Lambert (substitute name for *Filarszkia* Korshikov, nom. illeg.) and *C. saccatum* Filarsky for *R. cylindricus* (F. Lambert) Fott (type species) and *R. saccatus* (Filarsky) Fott. *Rhopalosolen* is characterized by presenting solitary and long cylindrical cells with widely rounded apices, attached to any type of substrate, usually of animal origin (Fott 1957, Tuno et al. 2006). *Rhopalosolen* has morphological similarity with other green algal genera living attached to a substrate, and is rarely documented in floristic works. According to Guiry and Guiry (2017), this genus is classified by some authors in the subfamily Fernandinelloideae (Characiaceae) or excluded from Characiaceae and placed in Rhopalosolenaceae.

Morphologically, *Rhopalosolen* representatives can be easily confused with others species of *Characium* A. Braun (Chlorophyta) and *Characiopsis* Borzi (Xanthophyceae), however, the first one is different by having an elongated pedicle fixation and cell poles tapered, whereas the second one differs primarily by the golden polygonal chloroplast. Another genus that resembles *Rhopalosolen* is *Characiellopsis* M.O.P. Iyengar (Chlorophyta), but the latter presents as its diagnostic feature the presence of a marked ring in cell wall at the apex of the cell. Moreover, adult cells of *Characiellopsis* are usually shorter than those of *Rhopalosolen*.

There are 3 described *Rhopalosolen* species, *R. cylindricus, R. saccatus,* and *R. sebestyenae* Fott. However, in Algaebase (Guiry and Guiry 2017), only the 2 first species are currently "taxonomically valid". *Rhopalosolen cylindricus* and *R. saccatus* are distinct from each other in the length and shape of the cells, which is straight and about 400 μm long in *R. cylindricus*, and slightly curved at the base and up to 200 μm long in *R. saccatus* (Komárek and Fott 1983).

Sometimes is difficult to separate these species, especially when *R. cylindricus* has some morphological variations. In our study, we observed that shorter *R. cylindricus* cells are usually straight while long cells tend to be slightly curved, which can be confused with *R. saccatus.* Here, the main difference in these species is the base of cells, which is straight in *R. cylindricus* and curved in *R. saccatus. Rhopalosolen sebestyenae*, documented for Europe (Fott 1958, Matviyenko 1972, Komárek and Fott 1983), differs from the 2 preceding ones by its strongly curved cells, especially at the base.

Rhopalosolen cylindricus can reproduce sexually or asexually (Fig. 4). Anisogamic reproduction has been described only for *R. saccatus* (Komárek and Fott 1983), whereas isogamic reproduction was observed in *R. cylindricus* (as *Filarszkia cylindrica* Korshikov). In the present study, isogametes (Fig. 4B) were observed in some samples gathered during rainy season (May).

Asexual reproduction occurs by division of the protoplast to form dozens and even hundreds of zoospores, which are released and quickly lose its flagella to fix in a substrate (Korshikov 1953). However, during the present study, we did not observe zoospores, but only aplanospores. This is the first time that aplanospores are reported for *Rhopalosolen* reproduction (Fig. 4). Aplanospores are produced by the successive divisions of the protoplast, and are released by dissolution of the apex cell wall. In all periods, we found aplanospores, although they were more frequent during dry season.

Sometimes young cells do not reach adult size to release the aplanospores. A mucilage cover is secreted at the basal ending of the new cell, helping its attachment to the substrate. Nevertheless, it was common to find adult cells detached from the substrate. Absence of zoospores and formation of aplanospores is most probably due to the adverse conditions of phytotelm in the semi-arid area such as little water stored at bromeliads which can quickly evaporate due the high environmental temperature. Ramos et al. (2017) considered the adverse environmental conditions (water deficiency in tanks) as the likely cause of algae to produce a large amount of zygospores in bromeliads from a resting area.

It was noted that when the bromeliads accumulate a fair amount of rain water in May, *R. cylindricus* was developing well, usually with well elongated cells forming a biomass attached to plant leaf. On the other hand, in drought periods where the bromeliad accumulated little water it was much more common to find numerous aplanospores on the trichomes, possibly as a reproductive survival strategy. These specialized trichomes located on the inner (submerged) part of leaves play an important role in the water and nutrient's absorption in bromeliads, as well as contribute towards protecting against desiccation in environments with water restriction (Benzing 1976). When the leaf surface dries, the cap cells drain and a vapor lock is established to prevent further water loss from the live stalk cells (Leroy et al. 2015). Thus, these trichomes present basic conditions for the establishment of *R. cylindricus* during the dry periods: substrate and moisture. As the rain water accumulates in the bromeliads the algal cell tends to elongate and disperse in the environment, also growing in other types of substrates. In this study, we did not find another algae species associated with these trichomes. In addition of the reproduction ways described above, during our studies, we observe sometimes young cells stretching and splitting by the vegetative way. Korshikov (1953) observed the same patterns, however, in culture tests.

Rhopalosolen cylindricus was found in acidic waters (usually typical of tank bromeliads, Laessle 1961, Sophia et al. 2004, Guimarães-Souza et al. 2006) whose pH ranged from 3.45 to 7.45 (4.5 ± 1), with the greater variation occurred in January (5.1 ± 1.5) and the less in May (4.2 ± 0.3). *Rhopalosolen sebestyenae* and *R. cylindricus* were reported in ponds from Krakow (Poland) with the pH 6.5 to 7 (Matviyenko 1972) while *Rhopalosolen saccatus* was reported with alkaline conditions (pH 8.8–9.7) in a pond from Nebraska (USA) (Holland & Hergenrader 1981). Recently this latter species was also found in the Fitzroy River (Australia) with neutral pH condition (7.2) (Challagulla et al. 2015).

Temperature is an important variable for the development of algae in tank bromeliads (Lyra 1976, Sophia et al. 2004). In Itaberaba, *R. cylindricus* was found usually in bromeliads with water temperature about 29.7 ± 2.9 °C. Bromeliads were usually exposed to the sun, which naturally contributed for high value of that variable. Recent studies demonstrate that high sun exposure is an essential factor for the presence of algae in tank bromeliads (Brouard et al. 2011, Marino et al. 2011, Carrias et al. 2014).

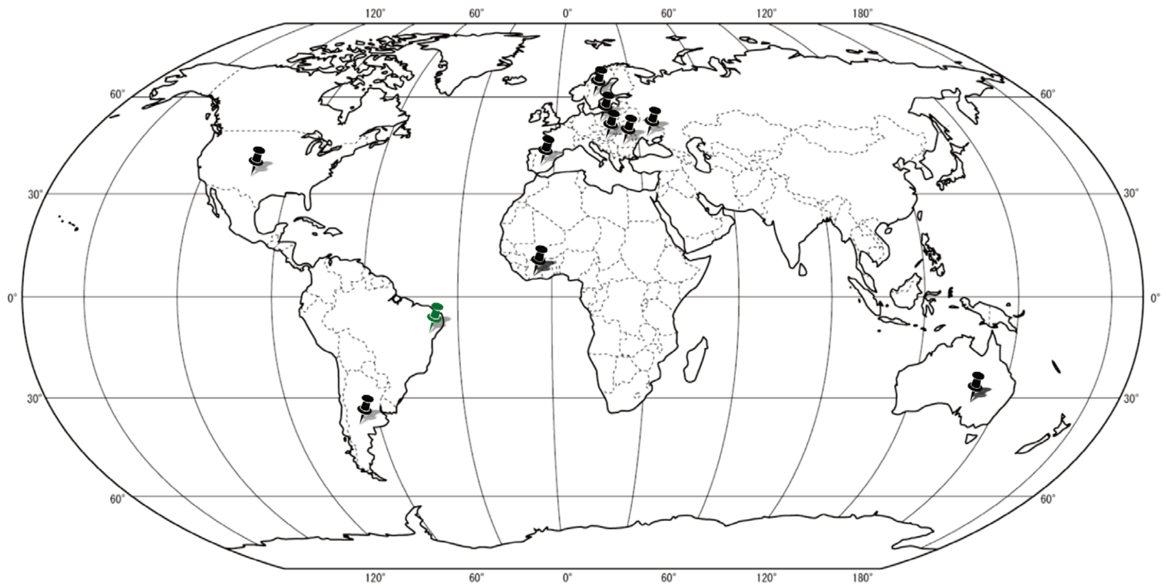

Figure 5. World distribution of *Rhopalosolen cylindricus*. Green marker represents the local of the new record for Brazil and black markers are countries where *Rhopalosolen cylindricus* have been previously reported.

Dissolved oxygen (DO) varied greatly, especially in August reaching quite high values (8.1 ± 3.6 mg·L^{-1}) than January (4.3 ± 1.4 mg·L^{-1}) and May (4.4 ± 1.1 mg·L^{-1}). DO peaks were also reported in the tank bromeliads from Jamaica being such values associated to algae presence (Laessle 1961). *Rhopalosolen cylindricus* was predominant in wider bromeliad (78.2 ± 19.3 cm), which may retain water more effectively, thus favoring the development of the algae. According to Marino et al. (2011), the bromeliad size is an important factor regulating algae biomass.

Although *Rhopalosolen* representatives have the predominant epizoic habit (Fott 1958, Komárek and Fott 1983), it was common to find them growing attached to other types of substrate such as exoskeletons, fungal hyphae, leaf fragments, and especially on the foliar absorption bromeliad trichomes. *Rhopalosolen cylindricus* may be an important resource for the micro- and macroinvertebrates that live in the tank bromeliad. *Rhopalosolen* is usually reported as epizoic in the literature, thus playing a major ecological role in regulating populations of *Anopheles gambiae* Giles (Tuno et al. 2006). Among the main animals used as substrate by *R. cylindricus* in the Itaberaba bromeliads are representatives of rotifers, nematodes, polychaetes, cladocerans, copepods, and mites, the latter usually infested by young cells of algae and possibly one of the main dispersers of *Rhopalosolen* among bromeliads of the area. It is possible that other visitors like insects, birds and frogs can also be considered potential dispersers of algae in bromeliads, especially crickets as they were frequently found in these plants. However, further studies still are needed to clarify the dispersion of algae on phytotelmata. Finally, we also emphasize the need for further research that will provide a better understanding of the biodiversity of algae from bromeliad phytotelmata. This will allow for more effective conservation actions, especially in threatened areas such as the Caatinga.

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Authors’ Contributions

GJPR, CWNM, and CEMB wrote the manuscript. GJPR and CWNM participated in the fieldwork.

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Appendix

Table A1. Voucher numbers (HUEFS) for specimens of *Rhopalosolen cylindricus* from Fazenda Itaberaba, Bahia state, Brazil.

Voucher (HUEFS)	Date	Voucher (HUEFS)	Date	Voucher (HUEFS)	Date	Voucher (HUEFS)	Date
215777	21/01/2015	155590	21/01/2015	155451	08/05/2015	222994	21/08/2015
190772	21/01/2015	155591	21/01/2015	155452	08/05/2015	222995	21/08/2015
164804	21/01/2015	155592	21/01/2015	155454	08/05/2015	222996	21/08/2015
164807	21/01/2015	155593	21/01/2015	155455	08/05/2015	222997	21/08/2015
164809	21/01/2015	155594	21/01/2015	130846	08/05/2015	222998	21/08/2015
164812	21/01/2015	155595	21/01/2015	170355	08/05/2015	222999	21/08/2015
164813	21/01/2015	155596	08/05/2015	222987	08/05/2015	223000	21/08/2015
164815	21/01/2015	155404	08/05/2015	222988	08/05/2015	223001	21/08/2015
164819	21/01/2015	155405	08/05/2015	222989	08/05/2015	155390	21/08/2015
155586	21/01/2015	155441	08/05/2015	222990	08/05/2015	155357	21/08/2015
155587	21/01/2015	155447	08/05/2015	222991	08/05/2015	155361	21/08/2015
155588	21/01/2015	155448	08/05/2015	222992	21/08/2015	155368	21/08/2015
155589	21/01/2015	155450	08/05/2015	222993	21/08/2015		