



First record of *Botryococcus braunii* Kützing from Namibia

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Background: *Botryococcus braunii* is well known from all continents, but it has been sparsely recorded from Africa compared to other continents. The alga recently formed a rusty orange-red bloom in the Tilda Viljoen Dam, situated near Gobabis in Namibia. Blooms of this species are known to produce allelopathic substances that inhibit the growth and diversity of other phytoplankton, zooplankton and fish.

Objectives: The objective of this study was to record the presence of *B. braunii* in Namibia.

Method: Morphological features of the species were compared with illustrations and literature on *B. braunii* found in other continents of the world, particularly North America and Europe. Extensive literature surveys revealed its currently known geographical distribution.

Results: The organism responsible for the discolouration of the water was identified as *B. braunii*. Microscopic examination revealed large colonies that floated in a thick layer on the surface of the water. Literature searches on the geographical distribution of *B. braunii* revealed that this was the first record of this species' presence in Namibia.

Conclusion: The known geographical distribution of *B. braunii* was expanded to include Namibia.

Introduction

Under suitable environmental conditions, *Botryococcus braunii* may form significant blooms. Colony densities as high as 55 300 colonies/L were recorded in Nozha Lake, Egypt (Labib et al. 2014), while a bloom in the Darwin Reservoir, Australia, was estimated to weigh 1500 tonnes (fresh mass; Wake & Hillen 1980). During bloom formation, cells often rise to the surface where they float in a thick layer as a result of the production of lipids (Qin 2005). The bloom in Nozha Lake, Egypt, ranged in colour from green at the start, to orange or red towards the peak and end (Labib et al. 2014). Senousy, Beakes and Hacks (2004) found that some biochemical strains possess the orange-red pigment while others do not and thus appear green.

Significant negative linear relationships between phytoplankton biomass and species diversity have been demonstrated by several authors (e.g. Todd et al. 2014). Decreases in phytoplankton diversity during blooms are, in turn, responsible for decreases in zooplankton diversity (Bockwoldt et al. 2017). In addition, *B. braunii* blooms can influence biodiversity because it has allelopathic toxic effects on a variety of aquatic organisms. Chiang, Huang and Wu (2004) and Wu et al. (2006) investigated allelochemicals secreted by *B. braunii* and found that a mixture of free fatty acids secreted by this species results in decreasing diversity of other phytoplankton, zooplankton and fish. Blooms of this alga are also associated with fish mortalities in several parts of the world, such as Taiwan (Liyu Lake; Chiang et al. 2004), the Philippines (Paoay Lake; Papa et al. 2008) and Egypt (Nozha Lake; Labib et al. 2014).

B. braunii is widespread in fresh and brackish water bodies in most parts of the world. In contrast to its wide distribution in other continents, it was only described from 12 countries in Africa, of which only two, South Africa and Mozambique, were located in southern Africa. During 2017 a dense bloom of *B. braunii* developed in the Tilda Viljoen Dam, Namibia. The bloom was monospecific, and *B. braunii* outcompeted all other phytoplankton species in the dam at the time of the bloom. The bloom rendered a rusty orange-red colour to the water (Figure 2a) and a thick scum (Figure 2b) developed on the surface of the water. Literature searches revealed no known record of the presence of this species in Namibia.

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Study area, research materials and methods

Study area

The Tilda Viljoen Dam (22°26'42.21"S 18°57'26.21"E), a small rock-fill dam with a concrete outlet, is situated near Gobabis in Namibia (Figure 1). It supplies drinking water to Gobabis. The dam was completed in 1964, the dam wall has a height of 14 m and a length of 630 m. The full supply capacity is 1.224 million m³ and it has a surface area of 1.9 km².

Materials and methods

A visible orange-red discolouration of the surface water of Tilda Viljoen Dam (Figure 2a) was observed during November 2017 and it lasted for three months until February 2018, where after it gradually subsided. A 1-L grab sample was taken from the surface of the dam at the onset of the bloom on 15 November 2017 and the unpreserved sample was dispatched to the North-West University, Potchefstroom, for algal identification. Algae were identified to species level with a Zeiss light microscope (×400 magnification), equipped

with a digital camera and eyepiece graticule. Measurements of cell and colony sizes were made by means of a ruler in one of the eyepieces.

Ethical considerations

Permits

Collection of algal material in Namibia is not currently regulated by specific legislation and no permits are required.

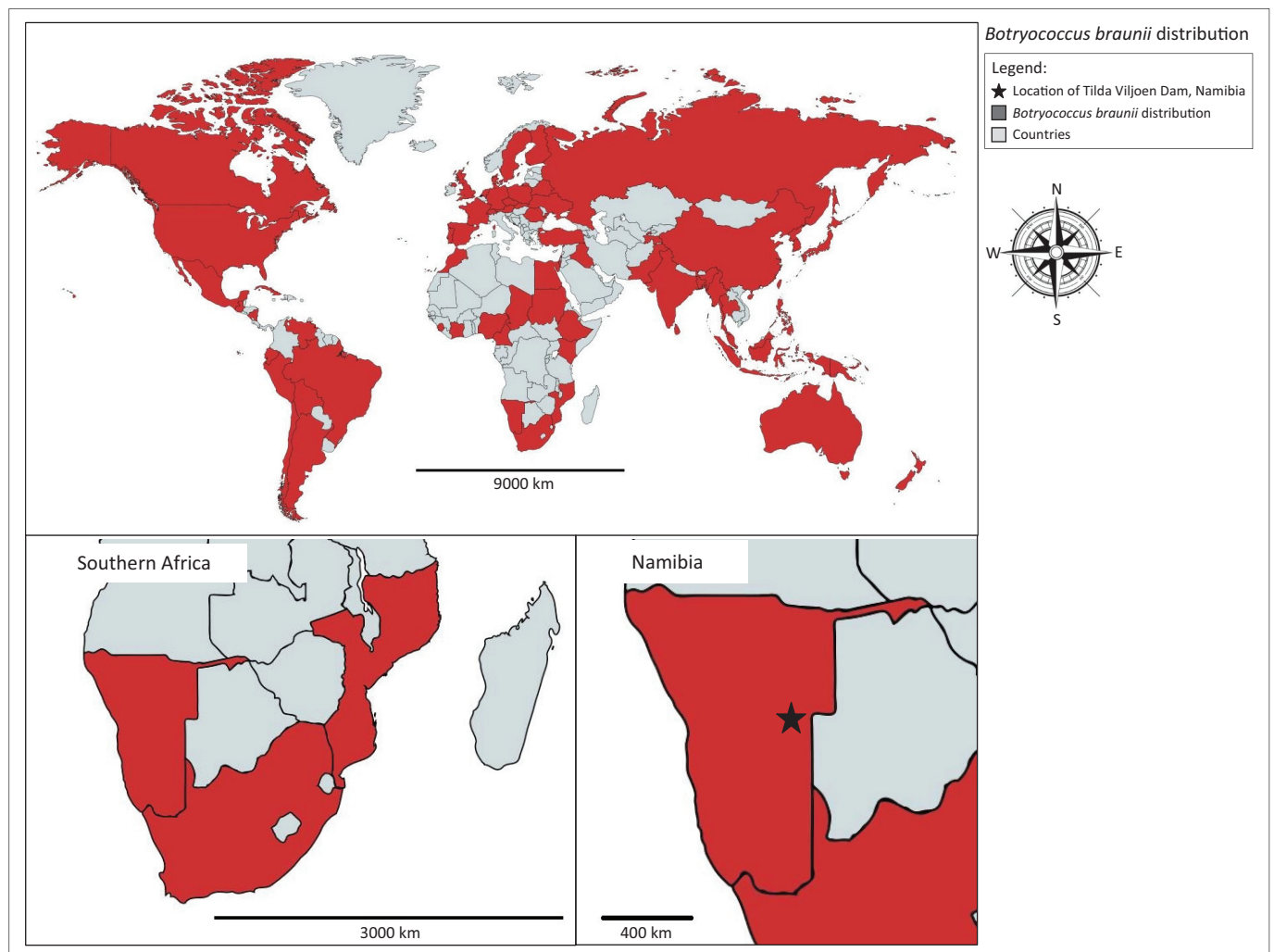
Taxonomic treatment

Botryococcus braunii Kützing in Kützing 1849:892.

Type: SWITZERLAND, in lacu Neoburgense, Helvetiae (Lake Neuenburg, also known as Lake Neuchatel), Kützing 1849:892, Type: A. Braun 115; L? Notes: Isotype: UC 790500.

Description

The genus *Botryococcus* has a long history of study, accompanied by many classification difficulties. It was first included in the Palmellaceae (Kützing 1849), then listed as part of the



Source: Available from <https://mapchart.net/world.html>
km, kilometres.

FIGURE 1: Current known distribution of *Botryococcus braunii*. Map created with mapchart.net.

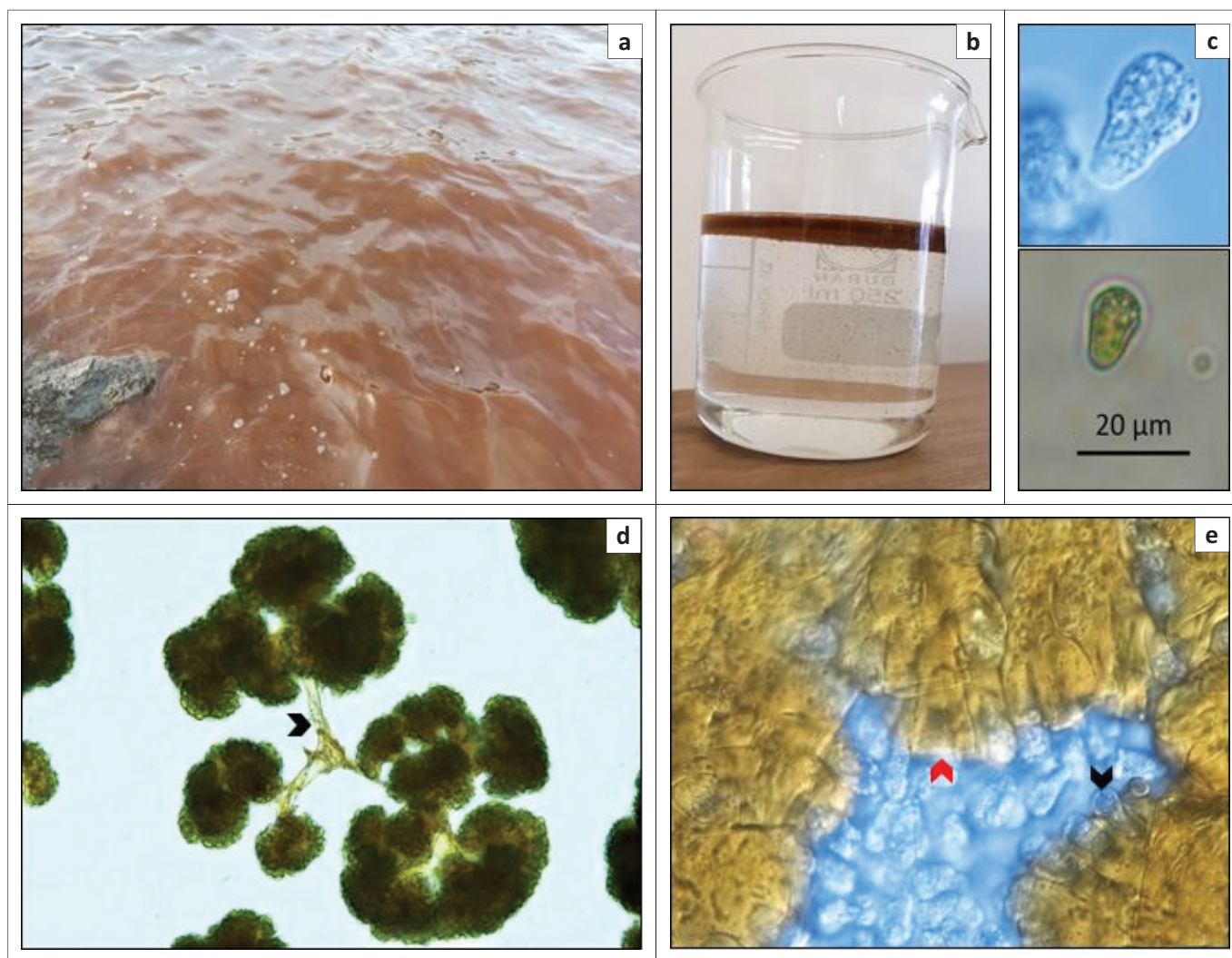


FIGURE 2: Field-, laboratory- and light microscope images of *Botryococcus braunii*. Diagnostic features are indicated with arrows. (a) Surface water of Tilda Viljoen Dam at the peak of the *B. braunii* bloom; (b) macroscopic *B. braunii* colonies floating in a layer on the surface; (c) structure of individual cells; (d) light microscope image of *B. braunii* colonies with subcolonies and linking threads (black arrow); and (e) cells embedded in cup-shaped structures (black arrow) and empty cups after cells have been released (red arrow).

Tetrasporaceae (Klebs 1883, cited in Blackburn 1936) and Xanthophyceae (Pascher 1939). Blackburn (1936) noticed starch inside the cells and that, together with the identification of chlorophyll b (Belcher & Fogg 1955), finalised classification within the Phylum Chlorophyta. Currently, the genus is classified under the Phylum Chlorophyta, Class Trebouxiophyceae, Order Trebouxiales and Family Botryococcaceae (Guiry & Guiry 2018). Komárek and Marvan (1992) established and described differences between at least 13 *Botryococcus* species based on morphology. The identity of several of these species has, however, been questioned based on variability demonstrated in culture studies by Plain et al. (1993). The two most common and widely distributed species are *Botryococcus braunii* and *Botryococcus protuberans*. Besides small differences in cell sizes and colony morphology (Rai et al. 2007), the most apparent difference between these two species is that the cells of *B. braunii* are embedded in the periphery of mucilaginous masses, while those of *B. protuberans* are prominently projecting from it (John, Whitton & Brook 2002).

The colonies of *B. braunii* are free-floating, large (100 μm – 500 μm in diameter), sometimes forming several dense clusters

of subcolonies with indefinite shapes. Subcolonies are often attached to each other by thread-like mucilaginous connections (Figure 2d). Guy-Ohlson and Lindström (1994) described this as ‘branching’ of the colonies. Temperley (1936) found that branching usually occurs when the colony is about 30 μm in diameter and composed of 30 individual cells. In other cases, the refringent threads, linking the clusters, are absent (Metzger & Largeau 2005). The entire colony is imbedded in a mucilaginous matrix composed of fatty acids that add buoyancy to the colony. Individual semi-transparent cells are oval to pyriform in shape (Figure 2e) and surrounded by a cellulose wall. Cell size (length \times width) is in the range of (8–9 \times 5) μm to (13 \times 7–9) μm (Liu, Sun & Gerken 2014). Each cell is embedded in a cup-shaped base (Figure 2e). The cups are at least two thirds of the cell length with the tips of the cells emerging from the cups. The cups are initially very thin, but increase in thickness with consecutive cell divisions (Guy-Ohlson & Lindström 1994).

Distribution and ecology

Living members of the genus *Botryococcus* are described from all continents (Figure 1), except Antarctica, but paleoecological

studies do indicate the presence of fossil forms of *Botryococcus* in the Liddkvarvet Mountains in Antarctica (Guy-Ohlson & Lindström 1994). Figure 1 illustrates that *B. braunii* has been recorded from 12 African countries, of which only three (South Africa, Namibia and Mozambique) are located in southern Africa (Figure 1). This article represents a first record of the presence of *B. braunii* in Namibia.

Botryococcus braunii occupies a variety of aquatic habitats, ranging from ditches, bog pools, open water tanks, ponds and freshwater and brackish lakes (John & Tsarenko 2002). These water bodies are situated in temperate, alpine or tropical zones all over the world (Komárek & Marvan 1992; Wake & Hillen 1980). *B. braunii* can tolerate wide ranges of several physico-chemical parameters, thus accounting for its cosmopolitan distribution. Several studies have proven that the species can tolerate wide ranges of temperature (Labib et al. 2014; Qin 2005), salinity (Ranga Rao et al. 2007) and pH (Dayananda et al. 2005). High concentrations of nutrients and alkalinity were found during a *B. braunii* bloom in the Banglang Reservoir in Thailand (Ariyadej et al. 2004) and it also formed blooms in the eutrophic lake Liyu (Taiwan, Chiang et al. 2004) and Tomahawk Lagoon No. 2 in New Zealand (Mitchell 1975).

Botryococcus braunii blooms are known to excrete a mixture of fatty acids, including α -linolenic, oleic, linolic and palmitic acids, that have allelopathic effects inhibiting the growth of zooplankton (Chiang et al. 2004; Papa et al. 2008) and causing lysis of phytoplankton (Wu et al. 2006), thereby reducing biodiversity of the water body. A visible rusty-coloured bloom, associated by massive fish kills, was described by Labib et al. (2014) in Nozha Lake, Egypt. Fish kills, accompanying blooms of *B. braunii*, were also recorded in Liyu Lake in Taiwan by Chiang et al. (2004). No fish-kills were, however, experienced at the time of the *B. braunii* bloom in Namibia.

Besides the fact that the presence of *B. braunii* blooms may cause alarm among people when high enough densities rapidly change the colour of the water from green to red or burnt orange, people are also sometimes alarmed as these blooms can look very much like a gasoline spill or oil slick, because of the high amounts of lipids secreted by the algae. The bloom in Namibia turned the water a rusty orange-red colour and a thick layer of algae was floating on the surface as a result of density differences caused by the lipids. The orange discolouration of the water may be an indication that the species belongs to the orange pigment-producing chemical 'Race B' strain recognised by Senousy et al. (2004) from Lake Ayamé, Côte d'Ivoire, West Africa. Confirmation of this race would, however, require an 18S rRNA sequence data analysis.

New record

NAMIBIA. Omaheke region: Tilda Viljoen Dam near Gobabis, 22°26'42.21"S, 18°57'26.21"E, 15 November 2017, Almirall 1 (PUC) (Figure 2).

Conclusions

Botryococcus braunii, a species that is potentially damaging to indigenous biodiversity of phytoplankton, zooplankton and fish, has been recorded from a freshwater dam in Namibia. This represents the first record of its presence in Namibia and the third record for a country in southern Africa.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

S.J.v.V. identified the specimen and wrote the text. A.L. conducted the geographical distribution survey.

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