



## Taxonomy and systematics

# Thermophile mats of microalgae growing on the woody structure of a cooling tower of a thermoelectric power plant in Central Mexico

## *Matas de microalgas termófilas que crecen sobre la estructura de madera de una torre de enfriamiento de una central termoeléctrica en el centro de México*

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### Abstract

The aims of this research are to identify and describe a periphyton community of thermophilic microalgae in order to expand our knowledge on biodiversity of a particular environment. Conspicuous biomass of thermophilic microalgae (48 °C) inhabits the cooling towers of the thermoelectric power plant of Villa de Reyes (Central Mexico). Aggregate samples or microalgal mats were taken in three different areas of the top of a cooling tower, for identification. According to the sequencing analysis of 16S and 18S rDNA genes, the community is dominated by 3 species of Cyanoprokaryota: *Chlorogloeopsis fritschii*, *Arthronema africanum* and *Chroococcidiopsis* sp., previously reported as thermophiles. Also, 2 species of the Chlorophyte or green algae *Scenedesmus*. Finally, 12 species of diatoms comprise the microalgal community; diatoms were only microscopically identified within the mats, suggesting that the mats constitute a suitable microenvironment in thermal ambiances. The identified species are of particular interest because their habitat represents an extreme and an artificial biotope. To the best of our knowledge, this is the first report of thermophilic communities of microalgae in Mexico from a power plant; also, this is the first report of *A. africanum* for the country.

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**Keywords:** Microalgal mats; Thermophilic; *Arthronema africanum*; Diatoms; Mexico

### Resumen

Esta investigación tiene por objetivo identificar y describir la comunidad perifítica de microalgas termófilas, para expandir nuestro conocimiento de la biodiversidad en ambientes particulares, como las microalgas termófilas (48 °C) que crecen de manera conspicua en la zona superior de la torre de enfriamiento de la central termoeléctrica de Villa de Reyes (centro de México). Se tomaron muestras de agregados o tapetes microalgales en 3 zonas distintas de la parte superior de una torre de enfriamiento, para su identificación. Una vez realizada la amplificación, la clonación y el análisis de los genes que codifican para las subunidades 16S y 18S del rDNA, se observó el predominio de 3 especies de Cyanoprokaryota: *Chlorogloeopsis fritschii*, *Arthronema africanum* y *Chroococcidiopsis* sp., especies descritas como termófilas en trabajos previos. Además, se identificaron 2 especies de Chlorophyta (algas verdes) del género *Scenedesmus* y 12 especies de diatomeas; la identificación de diatomeas se realizó a partir de observaciones por microscopía electrónica de barrido. Característicamente, las diatomeas solo se observaron dentro los densos tapetes algales que se conforman, sugiriendo que estos tapetes constituyen un microambiente conveniente en ambientes térmicos. Las especies

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identificadas son de particular interés, ya que su hábitat representa un biotopo extremo y artificial. Por lo que sabemos, este trabajo constituye el primer registro de microalgas termófilas que habitan en torres de enfriamiento y *Arthronema africanum* se documenta por primera vez para México. Derechos Reservados © 2016 Universidad Nacional Autónoma de México, Instituto de Biología. Este es un artículo de acceso abierto distribuido bajo los términos de la Licencia Creative Commons CC BY-NC-ND 4.0.

*Palabras clave:* Tapetes microalgales; Termofilia; *Arthronema africanum*; Diatomeas; México

## Introduction

The importance of expanding our knowledge on biodiversity and identifying and characterizing microorganisms, from extreme environments, responds to the social, scientific and technological requirements worldwide (Connolly et al., 2011). This knowledge could serve to develop new and sustainable technologies to obtain food, nutritional supplements, medicines, biofuels, fibers, biopolymers, colorants or other biomaterial, and for bioremediation and biorecovery tasks.

Temperature is one of the main factors determining the distribution and abundance of species due to its effects on enzymatic activities (Aguilera, Souza-Egipsy, & Amils, 2012). Therefore, thermophilic algae have thermal tolerant molecules that constitute their cells, while their metabolism is based on thermostable enzymes (Singleton & Amelunxen, 1973). A good example of a biotechnological use of thermophilic algae is the bioethanol production (Li, Du, & Liu, 2008) and the obtaining of poly- $\beta$ -hydroxybutyrate from *Synechococcus* spp., a compound used to degrade plastics (Nishioka et al., 2002), among other biorefinery products. Another interesting suggestion comes from Ramachandra, Mahapatra, and Gordon (2009): where thermophilic diatoms that harbor symbiotic nitrogen-fixing Cyanoprokaryota for use in solar panels subject to solar heating.

It is well known that thermal ecosystems support microalgal communities dominating total ecosystem biomass and productivity (Aguilera et al., 2012; Hindák, Kviderová, & Lukavský, 2013; Jonker, van Ginkel, & Olivier, 2013; Nikulina & Kocielek, 2011; Reisser, 2013; Stockner, 1967). Since 1969, Castenholz comments that thermal pollution from the water-coolant of power plants (nuclear and conventional) promoted microalgal growth. Previous reports indicated the presence of thermophilic microalgae in thermoelectric power plants, as those overgrowing on the concrete walls of a cooling tower at 4 different plants in the Czech Republic (Hauer, 2010) or in Belchatów, central Poland (Hindák, Wołowski, & Hindáková, 2011), as well as in the woody structure of cooling towers of the thermoelectric power plant of Villa de Reyes in San Luis Potosí (Central Mexico), at 48–50 °C and pH 7.5 (Covarrubias, 2011).

The cooling towers are used to remove the heat from water via their partial vaporization through a heat-exchanger, via convective heat transfer with dry and cold air. Because the towers are exposed to solar radiation, their woody structures are colonized by a vast biomass of thermophilic microalgae. The presence of microalgae is also explained because the cooling systems are

supplied with wastewater from the city of San Luis Potosí. The thermoelectric power plant aims to design a cooling pond for heat rejection as an algae bioreactor pond with the algae that inhabit the top of the cooling towers (*sensu* Leffler, Bradshaw, Groll, & Garimella, 2012), because (1) these algae are removed periodically in order to prevent clogging of filter systems of the water recycler; (2) the algae may fix CO<sub>2</sub> from flue gases, and (3) they seek to use the biomass generated, as biofuel or biofertilizer. Therefore, the objective of this work was to identify the thermophilic microalgae colonizing the top of a cooling tower, a thermal and human-made ambience. We are interested in these conspicuous microorganisms and are removed periodically from the cooling towers, as they may be used in an algae bioreactor pond.

## Materials and methods

Microalgae and water samples were taken in the middle of the Summer (August 2009), in one of the cooling towers (Fig. 1a) of the thermoelectric power plant of Villa de Reyes in Central Mexico (21°48'19" N, 100°56'00" W). The thermoelectric plant is located in a high-altitude zone of the semiarid Mexican Plateau (1,820 m.a.s.l.).

The sampled microalgae were growing as abundant and dense brown-green mats, loosely attached to the wooden floor (Fig. 1b) on the top of a cooling tower, with quite constant temperature and considerably water turbulence (Fig. 1c). Three samples of microalgae mats were collected with a spatula; the mats were submerged and attached to the wooden structure of the cooling tower. The samples were mixed to obtain one mixed sample, which was finally transferred to 2 glass flasks of 250 mL, previously acid rinsed (24 h in 15% HNO<sub>3</sub>) and sterilized. In the laboratory, a flask with the mixed sample of microalgae was used for microscopic observation, and the second flask was stored at –70 °C until use for total DNA extraction. A sample of the water (500 mL) was also collected per triplicate, in the same site where the microalgae were taken. The water samples were transported to the laboratory in a cooler and kept refrigerated until the chemical analysis. In the sampling site, pH (pHmeter Orion 3STAR BenchTop), temperature and light intensity (Photometer EXTECH, 401027) were recorded.

The water samples were prepared for their chemical analysis, which includes the concentration of soluble sulfates (SO<sub>4</sub><sup>2-</sup>) by turbidimetry, carbonates (CO<sub>3</sub><sup>2-</sup>) and bicarbonates (HCO<sub>3</sub><sup>-</sup>) by titration, nitrites (NO<sub>2</sub><sup>-</sup>) and nitrates (NO<sub>3</sub><sup>-</sup>) by spectrophotometry (Shimadzu, model UV-2501 PC); biochemical

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