

Morphological and molecular diversity of microalgae isolated from wastewater bodies and swamp complexes

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ABSTRACT

In this context, the development of technologies aimed at caring for the environment, reusing waste and generating low-cost clean energy has become essential. Among these technologies, microalgae cultures have become very important, as they are considered to be eco-friendly processes, since they efficiently recycle pollutants from liquid and gaseous media. The aim of this study was to isolate and identify taxonomically and molecularly morpho-genera of microalgae present in the Gran de Corozal stream and the San Benito de Abad swamp, in the department of Sucre, Colombia. Water sampling was carried out in two ecosystems of creek and swamp and finally identification at morphological and molecular level. The highest diversity of genera was found in the San Benito de Abad marsh body. The main morpho-genera corresponded to: *Aulacoseira* sp., *Cyclotella* sp., *Chlamydomonas* sp., *Desmodesmus* sp., *Euglena* sp., *Monomastix* sp., *Mychonastes* sp., *Phacus* sp., *Paraphysomonas* sp., and *Thalassiosira* sp. Research with microalgae has reached an enormous importance due, fundamentally, to the combination of uses they can have for the ecosystem in that water bodies and for the community in general.

Key words. Photosynthetic organism, water body, morphogen, molecular and functional diversity.

1. INTRODUCTION

According to the National Plan for the Management of Municipal Wastewater in Colombia, there is a deficit of approximately 80% in the treatment and disposal of wastewater generated by agriculture, industry and domestic wastewater, which has generated significant accumulated pollution in various ecosystems, affecting their integrity and causing problems of health and physical, chemical and microbiological water quality in several regions. This is the case of the department of Sucre, where several cases of pollution have been detected in its water system.

As pointed out by Perez and Causado, (2017), the micro-watershed of the Arroyo Grande de Corozal, located in the department of Sucre, should not be the exception to this privilege. Despite this, it is a notorious fact that this body is in terrible sanitary conditions, negatively affecting the environment and the community that settles around it. For this reason, an interdisciplinary investigation was carried out to analyze the polluting components present in the water, as well as to recognize the effectiveness of the measures ordered by the administrators of justice for its improvement.

The authors also point out that many years ago the Arroyo Grande Corozal was an important water basin in the region, its clean waters were suitable for consumption and also favourable for aquatic life. However, there came a time when its waters began to take on unpleasant colors and odors. The change came with the paving of the streets of the towns, because every time it rained, the water that washed away the dirt and soot from the streets went straight into the stream, which became 'the recipient of the sewage from the sewers of subnormal neighborhoods of Corozal, Sincelejo, Morroa and the Battalion of Fusiliers of Corozal'.

According to Sánchez-Estudillo, (2011), microalgae are microorganisms that form an initial part of the food web of many aquatic beings, providing high quality proteins, pigments and lipids with a high content of polyunsaturated fatty acids, which serve as storage products and energy sources. According to Arredondo and Voltolina, (2007), they are mostly autotrophic organisms, reproducing by binary division, which enhances their growth in controlled environments with sunlight, artificial light or both at the same time. This in turn allows them to grow rapidly when inoculated in a non-limiting culture medium and maintained in suitable conditions.

As stated by Jacob-lopes et al. (2015), these photosynthetic organisms are capable of living in extreme environments, and the study of their adaptive metabolism and the different compounds that they can accumulate in response to physicochemical variations make them of interest to different industries. Among the responses of microalgae to these variations is their antioxidant activity, which leads to the formation of biocompounds (pigments, lipids, phenolic compounds, etc.) that help ensure their survival. According to Gómez et al. (2016), microalgae produce antioxidant compounds as a protective response to damage caused by different types of stress such as UV radiation, temperature variation, excessive lighting, among others. According to Plaza del Moral (2010), physical factors that favors this response include the availability of light, which leads to an increase in carotenoids in the cells, which together with chlorophylls and phenolic compounds are the main antioxidant compounds (Cha et al., 2010; Hu et al., 2008; Wang et al., 2007).

Knowing the importance of microalgae for aquatic ecosystems, the present study aimed to isolate and identify the main genera of microalgae present in the Grande de Corozal stream and in the San Benito de Abad swamp complex.

2. MATERIALS AND METHODS

The present study consisted of two stages: a field stage for the collection of water samples for the isolation of microalgae genera in wastewater bodies and swamp complexes and the other stage consisted of the taxonomic and molecular analysis of the main microalgae genera. The steps and stages of the isolation and molecular identification process are shown in figure 1.

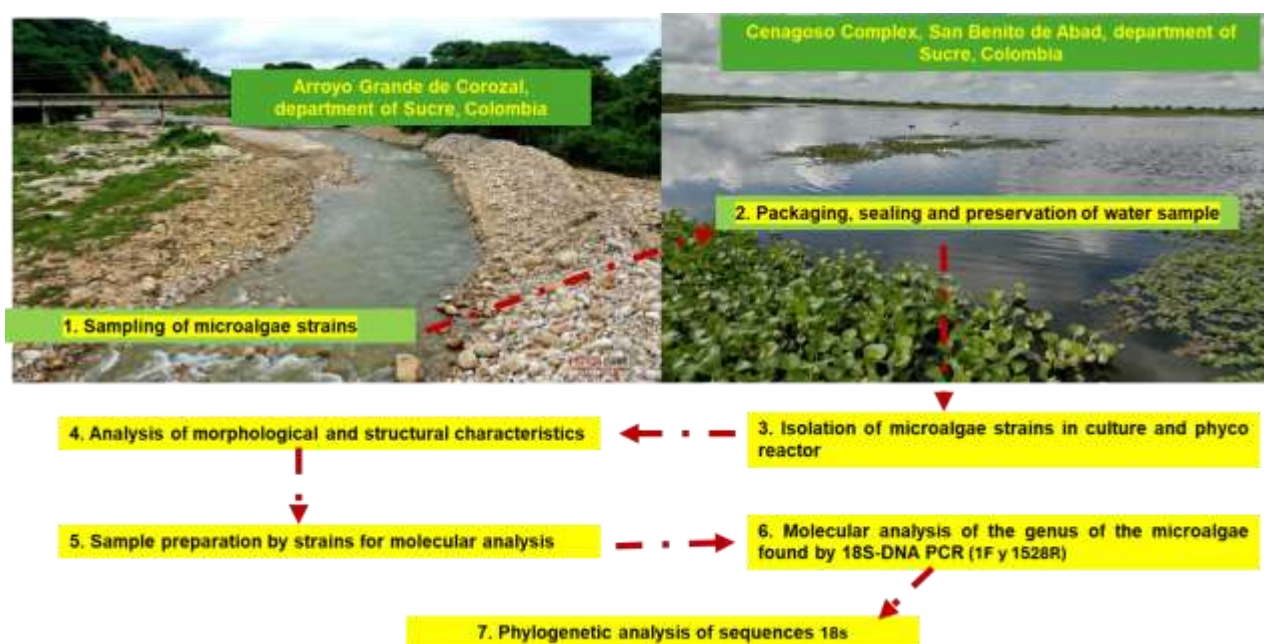


Figure 1. Stages for the isolation, structural and molecular identification of microalgae strains from the water bodies of the Arroyo Grande de Corozal and the San Benito de Abad swamp complex. Source: Arroyo Grande de Corozal (<https://prensaliubreccasanare.com/casanare/41885-jarillun-en-el-rno-chire-evitarb-afectaciones-a-comunidades-campesinas-de-hato-corozal.html>); San Benito Adab Lake Complex (<https://x.com/Corpomojana/status/1810668566099669189?lang=ar&mx=2>).

3. RESULTS AND DISCUSSION

Table 1 shows the diversity of strains isolated and morphologically identified in water from the Arroyo Grande de Corozal and the San Benito de Abad swamp complex in the department of Sucre, Colombia. In the present study, six morpho-genera were identified in the water of the Arroyo Grande de Corozal and seven in the water of the swamp complex. Similar morpho-genera in both water bodies corresponded to *Desmodesmus* sp., *Thalassiosira* sp., and *Aulacoseria* sp.

Table 1. Diversity of strains isolated in water from the Arroyo Grande de Corozal and the San Benito de Abad swamp complex in the department of Sucre, Colombia.

Waste water body	Swamp complex
<i>Cyclotella sp.</i>	<i>Monomastix sp.</i>
<i>Chlamydomonas sp.</i>	<i>Phacus sp.</i>
<i>Desmodesmus sp.</i>	<i>Desmodesmus sp.</i>
<i>Thalassiosira sp.</i>	<i>Thalassiosira sp.</i>
<i>Paraphysomonas sp.</i>	<i>Mychonastes sp.</i>
<i>Aulacoseira sp.</i>	<i>Aulacoseria sp.</i>

Figure 2 describes the main structural and morphological characteristics of the main isolated morpho-genera.

<i>Aulacoseira sp.</i>	Morphology. Most of its cells are tightly bound together forming filaments that may be long, straight, curved or coiled, it has discoid plastids, circular valves with a flat face with scattered poroids, it has a deep valvular mantle arranged at a right angle; between the junction of the face and the mantle it is provided with spines that form junctions, valvular mantle has concave thickening, mantle aureoles may be round or rectangular. Distribution. They have a wide range of trophic conditions, being found in sediments for prolonged periods of time (Kociolek, J.P et al., 2015).	 Image taken under an optical microscope with a 40x objective.
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<i>Cyclotella sp.</i>	Morphology. Structurally, some taxa consist of short, drum-shaped cells, may possess filaments, a large number of discoid plastids, contain circular valves, and a row of areoles running to the center of the valve which are grouped in fascicles. In some species, in the central area, granules or spines are found between the areoles forming patterns. Distribution. Widely distributed in water bodies around the world and found as far as Arctic waters (Kociolek, J.P et al., 2015).	 Image taken under an optical microscope with a 40x objective.
<i>Desmodesmus sp.</i>	Morphology. Single cells or colonies of 2 to 16 cells may form linear or lateral or alternate structures; cells are usually ellipsoidal to ovoid with spines either at terminal cell ends or in central cells parietal chloroplast with presence or absence of pyrenoid. Distribution Genus with cosmopolitan distribution, very abundant in freshwater bodies (Shubert, E. et al.,2015).	 Image taken under an optical microscope with a 40x objective.
<i>Euglena sp.</i>	Morphology. Green cells of various shapes and sizes can be ovoid, fusiform, cylindrical or elongated. Uniflagellates may have chloroplasts with pyrenoid surrounded by paramylon particles in various shapes, either stellate, lobed or spherical, some species have naked pyrenoid. Distribution. They are found in greater proportion in freshwater tributaries, lakes, rivers, ponds, acidified, polluted or muddy waters (Triemer, R.et al., 2015).	 Image taken under an optical microscope with a 40x objective.

<i>Phacus</i> sp.	Morphology. Stiff, flattened or twisted green leaf-shaped cells with multiple parietal chloroplasts around the whole cell, with paramylon granules, no pyrenoid. Distribution. Very common (cosmopolitan) genus of freshwater bodies (Triemer, R. et al., 2015).	 Image taken under an optical microscope with a 40x objective.
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Figure 2. Main morpho-genera of algae in creek and swamp water in the department of Sucre, Colombia. Source: Microbiological Research Laboratory, Faculty of Agricultural Sciences, University of Sucre.

Figure 3 and 4 show the phylogeny of morpho-genera identified by 18s rRNA gene sequencing. The sequencing results indicate that a range of microalgal genera are present in both water bodies and play roles in each ecosystem analyzed. Los diferentes estudios señalan como características las siguientes:

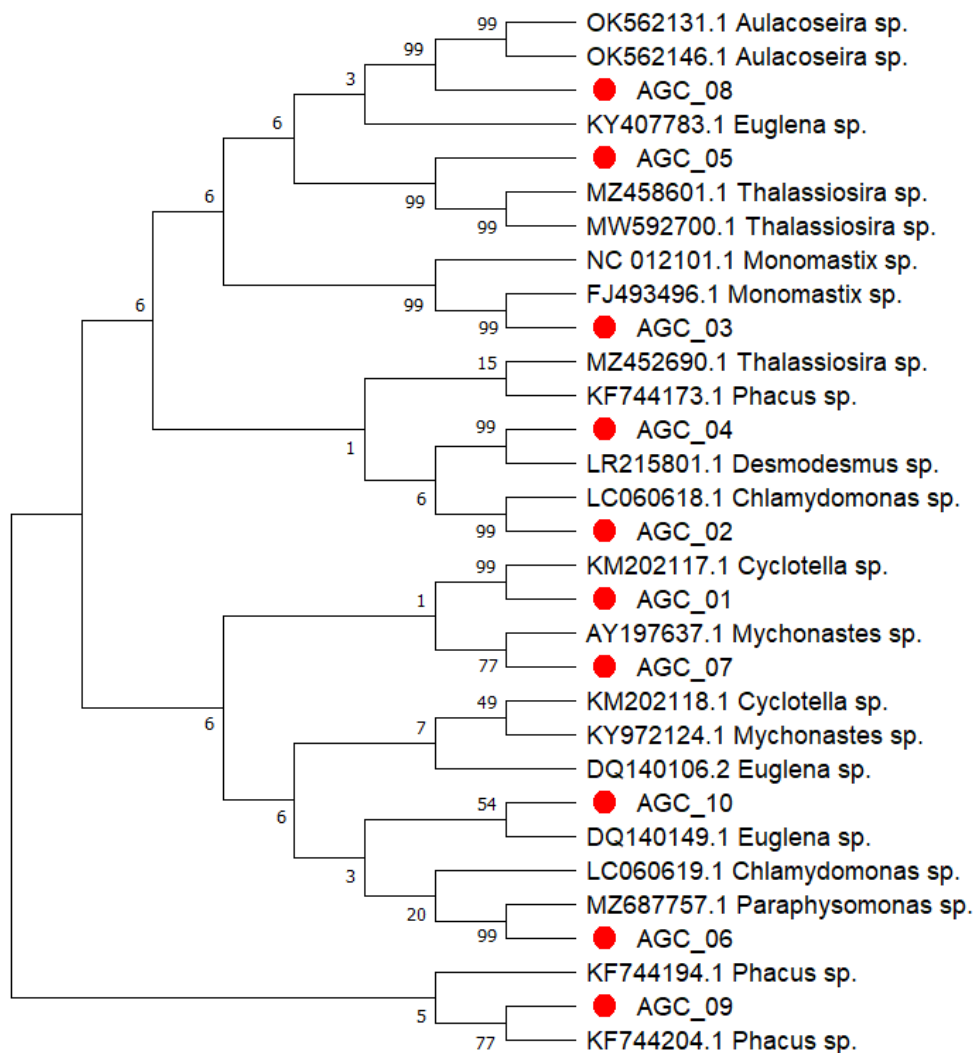


Figure 3. Dendrogram of microalgae from the wastewater body of the creek using the neighbour-joining method from 18S rRNA gene sequences. Source: Microbiological Research Laboratory, Faculty of Agricultural Sciences, University of Sucre.

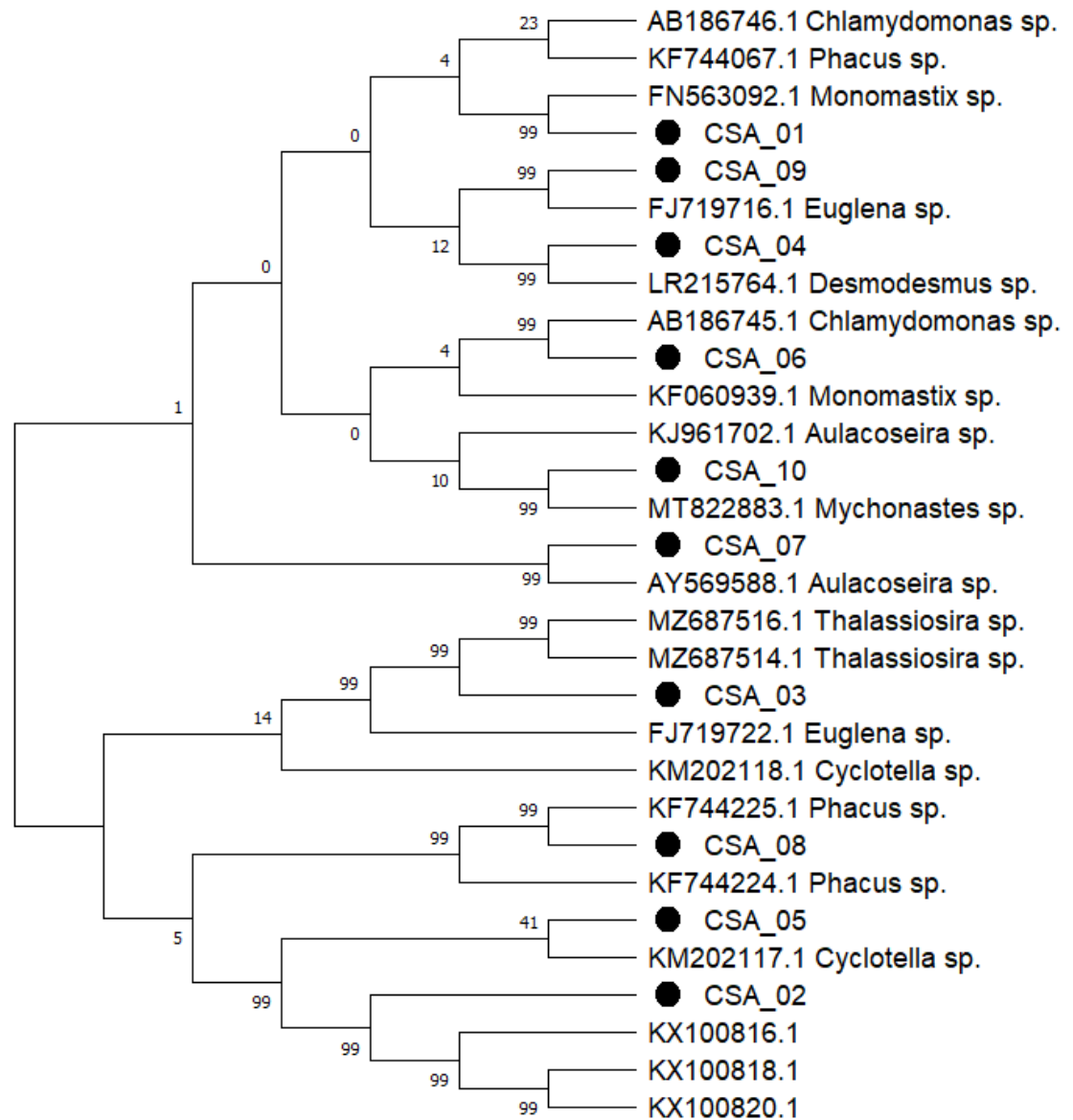


Figure 4. Dendrogram of microalgae from the swamp complex using the Neighbor-joining method from 18S rRNA gene sequences. Source: Microbiological Research Laboratory, Faculty of Agricultural Sciences, University of Sucre.

The genus *Aulacoseira* is a cosmopolitan genus, widely distributed in epicontinental water bodies, whose fossil record dates back to the Late Cretaceous. It produces stamina spores that can survive in the sediment for months or years and re-suspend by turbulence in the water column, defining its species as meroplanktonic or meroplanktonic. The genus *Aulacoseira* has been identified as a diatom belonging to the sediment of Lake Cocibolca, being one of the genera with the greatest presence in the study. This is possibly due to the fact that these points are the closest to the shore where the incidence of organic waste reaching the lake favors the growth of this species, as a response to the alteration of the environment (Trifonova and Davydova, 1983). Andramudio et al. (2016) carried out a complete study of the

succession of bacterial, algal and protozoan components in two environments of the Yahuaraca lagoon system, where they found evidence of the process of colonization and algal succession, with the *Aulacoseira* genus being able to rapidly colonize exposed surfaces, developing under conditions of high radiation.

Cyclotella is a genus of diatoms often found in oligotrophic environments, both marine and fresh water. It is in the family Stephanodiscaceae and the order Thalassiosirales (Kociolek et al., 2020). The genus was first discovered in the mid-1800s and since then has become an umbrella genus for over 100 different species, the most well-studied and the best known being *Cyclotella meneghiniana*. Despite being among the most dominant genus in low-productivity environments, it is relatively understudied (Saros, J.E., Anderson, 2015).

Cyclotella's habitat has traditionally been described as low-productivity mesotrophic or oligotrophic freshwater environments. However, it is unclear whether there is an archetypal aquatic setting for this genus as some species are more cosmopolitan, such as *C. meneghiniana*, which occurs in warm, nutrient-rich environments as well as low-productivity environments (Tanaka, 2007). The high dominance of *Cyclotella* demonstrates a change in environmental conditions. Szczepocka and Szulc (2009) report it as a pollution resistant species. Singh and Gaur (1989), in a stream polluted with oil refinery spills in Digboi (Assam, India), found *Cyclotella* as a diatom capable of degrading oil.

The genus *Chlamydomonas* is one of the most studied for its physiology, due to its rapid adaptation to controlled laboratory conditions, low nutritional requirements, and its potential application in nutrition and commercial uses has been demonstrated (*Chlamydomonas* Collection, 2010; Harris, 2001).

By carrying out the analysis of 18s rDNA sequences, a diversity of microalgae of the genera listed in table 1 was found, according to the sampling site. The results obtained found that the two common strains found in both water bodies correspond to: *Desmodesmus* sp and *Thalassiosira* sp. The phylogenetic relationship of the microalgae genera isolated and identified is related in figure 3 and 4.

Desmodesmus is a genus of green algae in the family Scenedesmaceae (see NCBI web page on *Desmodesmus*, 2007). It is the only chlorophyll-containing organism known to have caused human infections in immunocompetent individuals. All known cases involved open wounds produced in freshwater (Carey-Ann et al., 2015). *Desmodesmus* is found in the plankton of habitats such as ponds and lakes, especially in eutrophic water (Hegewald et al., 2017). It is one of the most common types of freshwater plankton (Shubert et al., 2014). They can also be found in soils and biological soil crusts (Johnson et al., 2007).

Euglena is a genus of unicellular protists (algae or protozoa, depending on whether one speaks of it from botany or zoology) belonging to the Euglenidae group, which may contain numerous lens-shaped or flattened chloroplasts, each with a pyrenoid. They possess a simple light-sensitive organelle called an eyespot, composed of photoreceptors and an adjacent patch of pigment. Organisms that are subjected to darkness for a period of time lose their chloroplasts and feed only heterotrophic, i.e. by engulfing particles or other organisms. If they regain light radiation, they can again synthesize chloroplasts. In other words, they are photosynthetic autotrophs but in the absence of light they are heterotrophs, ingesting the food

present in the surrounding water (Marin et al., 2003; Kim et al., 2015). *Euglena* is a potential source of biofuel.

Monomastix is a genus of green algae in the class Mamiellophyceae (See the NCBI webpage on *Monomasti*, 2007). It is the only genus in the family Monomastigaceae, which in turn is the only family in the order Monomastigales Guiry y Guiry, 2018).

Thalassiosira is a genus of centric diatoms, comprising over 100 marine and freshwater species. It is a diverse group of photosynthetic eukaryotes that make up a vital part of marine and freshwater ecosystems, in which they are key primary producers and essential for carbon cycling (Dreux et al., 2013). *Thalassiosira* occupy diverse habitats, both marine and freshwater. Of note, they are some vital primary producers in temperate and polar regions (Dreux et al., 2013). *Thalassiosira* can thrive in low temperature and light, as well as mixed waters, and are therefore a large part of diatom blooms during spring in temperate regions, such as Canadian and Alaskan waters (Harris et al., 1995). Species in this genus are also capable of assembling defensive threads against zooplankton, allowing them to survive the predation that normally keeps phytoplankton blooms in check.

Mychonastes is a genus of green algae, specifically of the Chlorophyceae. It is the sole genus of the family Mychonastaceae (Fučíková et al., 2013) Species of *Mychonastes* are found in plankton of freshwater or brackish waters, or found in soil. [1] It is a very common genus Krienitz et al., 2012) and is often dominant (Liu et al., 2020).

Thalassiosira species are diverse in both their ecology and physiologies, with variable mechanisms for nitrogen storage or requirements for iron. Iron concentrations, temperature and macronutrient availability have been identified as important factors for the composition of *Thalassiosira* species communities in marine waters (Adl, S.M. et al., 2012).

The genus *Paraphysomonas* comprises solitary, colorless flagellates, spherical to slightly oval cells, covered by numerous spine scales with usually circular, rarely oval, base-plate approximately orthogonal to a long thin central spine. Spine unbranched, unwinged, many times narrower than base-plate even at its base. Base-plate entire or with small perforations, of varying distribution but no large lacunae. Spine length varies from just longer than to several times base-plate width. Separate plate scales generally absent, but if present closely resemble spine-scale base-plate but with spine missing, usually larger in diameter and no distinctive morphology. The cells bear two flagella of unequal length. They are found either as freely swimming cells or attached to the substratum via a slender posterior stalk (Guiry et al., 2021).

Paraphysomonas species feed on various different organisms, such as bacteria, diatoms or small chlorococcalean algae. The prey is ingested at the base of the flagella after being pulled in via the motion of the long flagellum, the food is incorporated into vacuoles within a few seconds. Undigested particles are extruded around the posterior end of the cell (Guiry et al., 2021).

Phacus is a genus of unicellular, flattened and pointed microalgal protists belonging to the phylum Euglenozoa (Adl, S.M. et al., 2012). It has a very conspicuous and rigid periplasm, with spiral bands, numerous green plastids and a highly visible red eyespot near the flagellar base. Movement is exerted only by the flagellum, without the surface film movements that are so visible in other euglenids (Marin, et al., 2003)

Microalgae possess a phyco-remediation capacity that consists in the removal or biotransformation of pollutants from a liquid or gaseous medium. These pollutants are captured by algal biomass and can be recovered by harvesting. Microalgae comprise a diverse polyphyletic group of microorganisms (eukaryotes and prokaryotes) that are characterized by photosynthesis (Andersen, 2013). Recently, the wide range of biomolecules they synthesize (carbohydrates, lipids, proteins and pigments) has made them commercially attractive organisms (Bux, 2013). It is estimated that around 44000 species of microalgae have been described worldwide, isolated from diverse environments such as freshwater, seawater and hydrothermal vents (Barsanti and Gualtieri, 2014). It is therefore necessary to develop new molecular techniques that allow the correct identification of these microorganisms.

4. CONCLUSION

In the present study, a high molecular diversity of microalgae was found and identified, which corresponded to the most representative morpho-genera: *Aulacoseira* sp., *Cyclotella* sp., *Chlamydomonas* sp., *Desmodesmus* sp., *Euglena* sp., *Monomastix* sp., *Mychonastes* sp., *Phacus* sp., *Paraphysomonas* sp., and *Thalassiosira* sp. Microalgae are of great interest because they are photosynthetic micro-organisms characterized by rapid growth, with cells doubling in a period of 1 to 10 days, high lipid content (more than 50% by weight of dry matter in some cases), and use less surface area for cultivation. Microalgae research is important mainly because of the combination of uses they can have. They can be used for energy purposes, mainly to obtain biodiesel, although other biofuels such as bioethanol, biomethane, biohydrogen and to generate heat and electricity can also be obtained. Other commercial applications of microalgae include high value-added products with applications in human nutrition and health, aquaculture, cosmetics and biofertilisers. In addition, microalgae can help, during their growth, to reduce CO₂ emissions through biological biomitigation and intervene in wastewater treatment.

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6. AUTHOR CONTRIBUTION. Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

7. CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest

REFERENCES

1. Andersen R. The Microalgal Cell. En: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. 2 ed. Oxford: Wiley-Blackwell. 2013. p. 3-20. Doi:10.1002/9781118567166.ch1.
2. Adl, S.M. et al. (2012). [The revised classification of eukaryotes](#). Journal of Eukaryotic Microbiology, 59(5), 429-514
3. Andramunio, C, P; Caraballo G, P; Duque, S, R; Solari L, C. 2016. Cambios Sucesionales De La Comunidad Perifítica En El Sistema De Lagos De Yahuaraca, Amazonas Colombiano. Sometido a Actualidades Biológicas.
4. Arredondo, B Y Voltolina, D. 2007. Métodos y herramientas analíticas en la evaluación de la Biomasa Microalgal. Centro de Investigaciones Biológicas Del Noroeste, SC La Paz, BCS, ISBN 968.
5. Barsanti L, Gualtieri P. Algae: anatomy, biochemistry, and biotechnology. 2 ed. Boca Raton: CRC Press/Taylor and Francis Group; 2014. p. 325. Doi:10.1201/b16544.
6. Bux F. Biotechnological Applications of Microalgae. 1 ed. Boca Raton: CRC Press/Taylor and Francis Group; 2013. p. 201.
7. Cha, Kh; Kang, Sw; Kim, Cy; Um, Bh; Na, Yr y Pan, CH. 2010. Effect of pressurized liquids on extraction of antioxidants from chlorella vulgaris. Journal of Agricultural and Food Chemistry 58(8): 4756–4761.
8. Chlamydomonas Collection, 2010. Chlamydomonas Connection, About Chlamydomonas. Consultado el 25 enero 2023. Disponible en: <http://www.chlamycollection.org/info.html>.
9. Dreux Chappell, P., Whitney, L. A. P., Haddock, T. L., Menden-Deuer, S., Roy, E. G., Wells, M. L., & Jenkins, B. D. (2013). Thalassiosira spp. community composition shifts in response to chemical and physical forcing in the northeast Pacific Ocean. Frontiers in Microbiology, 4(SEP), 273. <https://doi.org/10.3389/fmicb.2013.00273>.
10. Gómez, A; Lopez, J; Rodriguez, A; Fortiz, J; Martinez, L; Apolinar, A, Enriquez, L. 2016. Producción de compuestos fenólicos por cuatro especies de microalgas marinas sometidas a diferentes condiciones de iluminación. Latin American Journal of Aquatic Research 44(1): 137–143.
11. Guiry M.D. in Guiry, M.D. & Guiry, G.M. 11 October 2021. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <https://www.algaebase.org>; searched on 24 January 2025.
12. Guiry, M.D., Guiry, G.M. (2018). "Class: Mamiellophyceae taxonomy browser". AlgaeBase version 4.2 Worldwide electronic publication, National University of Ireland, Galway. Retrieved 2018-01-09.
13. Fučíková, Karolina; Lewis, Paul O.; Lewis, Louise A. (2014). "Putting incertae sedis taxa in their place: A proposal for ten new families and three new genera in Sphaeropleales (Chlorophyceae, Chlorophyta)". Journal of Phycology. 50 (1): 14–25. Bibcode:2014JPCgy..50...14F. doi:10.1111/jpy.12118. PMID 26988005. S2CID 24770288.
14. Harris, EH. 2001. Chlamydomonas as a model Organism. Annu. Rev. Plant Physiol. Plant Mol. Biol 52: 363–406.

15. Harris, A., Medlin, L., Lewis, J., Jones, K., D Harris, A. S., Medlin, L. K., & Jones, K. J. (1995). *Thalassiosira* species (Bacillariophyceae) from a Scottish sea-loch. *European Journal of Phycology*, 30(2), 117–148. <https://doi.org/10.1080/09670269500650881>.
16. Hegewald, Eberhard; Braband, Anke (2017). "A taxonomic revision of *Desmodesmus* serie *Desmodesmus* (Sphaeropleales, Scenedesmaceae)". *Fottea*. 17 (2): 191–208. doi:10.5507/fot.2017.001
17. Hu, Cc; Lin, Jt; Lu, Fj; Chou, Fp y Yang, DJ. 2008. Determination of carotenoids in *Dunaliella salina* cultivated in Taiwan and antioxidant capacity of the algal carotenoid extract. *Food Chemistry* 109(2): 439–446.
18. Jacob-Lopes, E; Queiroz, Z; Ragagnin De Menezes, C y Ramírez- 49 Mérida, G. 2015. Microalgas: potencial para la producción de compuestos bioactivos nanoencapsulados. *Ciencia Natura* 37: 7–17.
19. Johnson, Joni L.; Fawley, Marvin W.; Fawley, Karen P. (2007). "The diversity of *Scenedesmus* and *Desmodesmus* (Chlorophyceae) in Itasca State Park, Minnesota, USA". *Phycologia*. 46 (2): 214–229. doi:10.2216/05-69.1. S2CID 84448925.
20. Kim, J. I., Linton, E. W., & Shin, W. (2015). Taxon-rich multigene phylogeny of photosynthetic euglenoids (Euglenophyceae). *Frontiers in Ecology and Evolution*, 3, 98.
21. Krienitz, Lothar; Bock, Christina; Dadheech, Pawan; Pröschold, Thomas (2011). "Taxonomic reassessment of the genus *Mychonastes* (Chlorophyceae, Chlorophyta) including the description of eight new species". *Phycologia*. 50 (1): 89–106. Bibcode:2011Phyco..50...89K. doi:10.2216/10-15.1. S2CID 86110297.
22. Kociolek, J.P.; Balasubramanian, K.; Blanco, S.; Coste, M.; Ector, L.; Liu, Y.; Kulikovskiy, M.; Lundholm, N.; Ludwig, T.; Potapova, M.; Rimet, F.; Sabbe, K.; Sala, S.; Sar, E.; Taylor, J.; Van de Vijver, B.; Wetzel, C.E.; Williams, D.M.; Witkowski, A.; Witkowski, J. (2020). "*Cyclotella* (F.T. Kützing) A. de Brébisson, 1838". WoRMS. World Register of Marine Species. Retrieved 11 May 2020.
23. Liu, Changqing; Shi, Xiaoli; Wu, Fan; Ren, Mingdong; Gao, Guang; Wu, Qinglong (2020). "Genome analyses provide insights into the evolution and adaptation of the eukaryotic Picophytoplankton *Mychonastes homosphaera*". *BMC Genomics*. 21. doi:10.1186/s12864-020-06891-6. PMC 7354681.
24. Marin, B., Palm, A., Klingberg, M., & Melkonian, M. (2003). Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist*, 154(1), 99-145.
25. Pérez Tovar María Fernanda, Causado Olivera Roxana Paola. 2017. Análisis de las aguas del Arroyo Grande de Corozal, Sucre, Colombia, para la toma de una medida efectiva que pueda ser ordenada por los administradores de justicia municipales en pro del mejoramiento de las mismas, año 2017. [Trabajo de grado en Ingeniería Industrial y derecho]. Corporación Universitaria del Caribe – CECAR Facultad de Ciencias Básicas, Ingenierías y Arquitectura Programa de Ingeniería Industrial Facultad de Derecho y Ciencias Políticas, 2017, 44p. <https://repositorio.cecar.edu.co/server/api/core/bitstreams/38dc50ff-7060-43f2-a6f6-891a65e74451/content>.
26. Plaza Del Moral, M. 2010. Búsqueda de nuevos ingredientes funcionales 51 naturales procedentes de algas. Tesis Doc. Universidad Autónoma de Madrid. España. 371p.

27. Sánchez-Estudillo, L. 2011. Alimento nutritivo, colorido y en movimiento: Los cultivos de apoyo en Acuicultura. *Ciencia y Mar* 43(1): 55–60.
28. Saros, J.E., Anderson, N.J. (2015). The ecology of the planktonic diatom *Cyclotella* and its implications for global environmental change studies. *Biol Rev Camb Philos Soc.* 90(2). 522-41.
29. See the NCBI webpage on *Desmodesmus*. Data extracted from the "NCBI taxonomy resources". National Center for Biotechnology Information. Retrieved 2007-03-19.
30. Shubert, Elliot; Gärtner, Georg (2014). "Chapter 7. Nonmotile Coccoid and Colonial Green Algae". In Wehr, John D.; Sheath, Robert G.; Kociolek, J. Patrick (eds.). *Freshwater Algae of North America: Ecology and Classification* (2 ed.). Elsevier Inc. ISBN 978-0-12-385876-4.
31. Singh, A, K; Gaur J. P. 1989. Algal epilithon and water quality of a stream receiving oil refinery effluent *Hydrobiologia*.184(3):193-199.
32. Szczepocka, E., & Szulc, B. (2009). The use of benthic diatoms in estimating water quality of variously polluted rivers. *Oceanological and Hydrobiological Studies*, 38(1), 17- 26.
33. Tanaka, Hiroyuki (2007). Taxonomic Studies of the Genera *Cyclotella* (Kützinger) Brébisson, *Discostella* Houk Et Klee, and *Puncticulata* Håkansson in the Family *Sephanodiscaceae* Glezer Et Makarova (Bacillariophyta) in Japan. J. Cramer. ISBN 978-3-443-57044-6.
34. Trifonova, Y. S. & N. N. Davydova. 1983. Diatoms in the plankton and sediments of two lakes of different trophic type. *Hidrobiologia* 103. 265-268.
35. Wang, L; Pan, B; Sheng, J; Xu, J; y Hu, Q. 2007. Antioxidant activity of *Spirulina platensis* extracts by supercritical carbon dioxide extraction. *Food Chemistry* 105(1): 36–41.
36. Westblade, Lars F.; Ranganath, Sangeetha; Dunne, William Michael; Burnham, Carey-Ann D.; Fader, Robert; Ford, Bradley A. (2015-03-05). "Infection with a Chlorophyllic Eukaryote after a Traumatic Freshwater Injury". *New England Journal of Medicine*. 372 (10): 982–984. doi:10.1056/NEJMc1401816. ISSN 0028-4793. PMID 25738686.