A new species of marine algae from the Mexican Atlantic based on morphology and molecular data: Gelidium rodrigueziae sp. nov. (Gelidiaceae, Rhodophyta)

1 Introduction

The genus Gelidium J.V. Lamouroux is a complex group containing 144 species in the Gelidiaceae of Rhodophyta (Guiry and Guiry 2023) and is characterized by plants with pinnate or simple branches, brush-like haptera, rhizoidal filaments in the cortex and/or medulla, bilocular cystocarps with an ostiole on each side, and apical tetrasporangial sori (Boo et al. 2022b; Rodriguez et al. 2008). The genus comprises perennial red seaweeds with ecological and economic value that occur on rocks in lower intertidal areas around the world (Rodriguez et al. 2008; Santelices 1990).

Gelidium is a genus with a cosmopolitan distribution, together with its high morphological plasticity, has presented a significant challenge for taxonomic determination using only morphological characters (Boo et al. 2013, 2016a,b,c, 2022b; Freshwater and Rueness 1994; Kim et al. 2012a,b; Quiroz-González et al. 2020a). Molecular tools used by other disciplines were adopted and used in phycological studies (Freshwater and Rueness 1994). These analyses, in conjunction with morphological studies, increased the ability to differentiate Gelidium species and resulted in the description of new taxa, as well as the extension of their distributions or important nomenclatural changes (Boo et al. 2013, 2016a,b,c, 2022b; Freshwater and Rueness 1994; Kim et al. 2012a,b; Quiroz-González et al. 2020a).

The algae of the genus Gelidium are an essential element of the flora of the coasts of Mexico because they are abundant and contribute to ecological interactions. They have been recorded in different intertidal and subtidal environments, being an important part of the floristic inventories in the country (García-García 2020; Ortega et al. 2001; Rodríguez et al. 2008), and they are found in rocky intertidal areas, in coastal lagoons, in coral reefs, and attached to rocks or other organisms, such as invertebrates or other algae (Quiroz-González et al. 2020b). The genus is a structuring element of Mexican coast communities, and the taxonomy of its species must be adequately delimited.

Keywords: COI-5P; phylogeny; rbcl; seaweeds; taxonomy
Knowledge of these species has been developed mainly on the Mexican Pacific coasts, where 20 species have been recorded to date (Quiroz-González et al. 2020a, 2021a; Rodríguez et al. 2008), while in the Mexican Atlantic, only six species have been recorded: Gelidium americanum, G. corneum, G. crinale, G. floridanum, G. pusillum and G. spinosum (García-Garcia et al. 2020; González-González et al. 1996; Ortega et al. 2001). In the states of Veracruz and Tabasco, in particular, five of the six species recorded for the Mexican Atlantic have been found, the exception being G. spinosum (Garcia-Garcia et al. 2020).

The vast majority of the Gelidium species in the Mexican Atlantic have not yet been evaluated with morphological and molecular tools, and only G. americanum has been characterized (Quiroz-González et al. 2021b). In Mexico, this type of study has been developed in the Pacific region, with the proposal of two new species (Gelidium gonzalezii and G. nayaritense) and the taxonomic certification of three more (G. sanyaense, G. jhonstonii and G. sclerophyllum) (Boo et al. 2022b; Quiroz-González et al. 2020a, 2021a; Wang et al. 2017).

Given the value that the genus Gelidium represents and the need to evaluate its taxonomic status with molecular and morphological tools, the present study aimed to contribute to the knowledge of the genus through the characterization of specimens found as epibionts of the mollusk Stramonita rustica Lamarck 1822 in an Atlantic locality of Southern Mexico, using molecular markers and morphological data, which gives rise to the proposal of a new species G. rodrigueziae sp. nov.

2 Materials and methods

A total of 20 specimens were found growing on the shell of the mollusk Stramonita rustica that were collected in Playa Hermosa, Veracruz (18° 39′ 51″ N, 95° 7′ 48″ W) in May 2018. More recently, seven specimens were found growing on a rock in the Faro Verde locality, Veracruz (19°41′40″ N, 96°7′24″ W), and three more were found on this same substratum in the Andrés Sánchez Magallanes Breakwater, Tabasco (18°18′2″ N, 93°51′1″ W) in May 2022 (Figure 1). Specimens were fixed with 4 % formaldehyde solution in seawater for morphological study, and a part of each thallus was also placed in silica gel for molecular analysis. Images of their general morphological features were obtained with a Canon Eos Rebel T3 camera (Canon Inc., Tokyo, Japan). Transverse and longitudinal thallus sections were cut manually using a razor blade under a Nikon C-LEDS stereomicroscope (Nikon, Inc., Konan, Minato-ku, Tokyo, Japan). Anatomical observations were made with an Olympus CX23 optical microscope (Olympus America Inc., Center Valley, Pennsylvania, USA).

Total DNA was extracted from 8 specimens using the CTAB (Doyle and Doyle 1990) and Master Pure (Epicentre Illumina Inc., San Diego, California, USA) protocol. PCR reactions were set up with the Thermo-scientific Phire Plant Direct Kit with the following reagent proportions: 10 μl of 2× buffer, 1.0 μl of each forward and reverse primer at 10 pmol, 0.4 μl of Taq Polymerase, 1.0 μl of DNA and 11.6 μl of injectable water, for

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**Figure 1:** Sampling sites for Gelidium spp. in the Mexican Atlantic.
a final volume of 25 μl. The primers used for amplifying and sequencing were F-rbcL, starting with R-753 and F-577 with R-1381 for rbcL (Freshwater and Rueness 1994). COI-5P was amplified and sequenced using the GHaIF with GazR primers (Saunders 2005). The reactions were run in a Techne Flexi-gene thermal cycler (Bibby scientific, Maryland, USA), following the cycling protocol described by Freshwater and Rueness (1994). The PCR products were sequenced by Macrogen Inc. (South Korea), edited with the Bioedit (Hall 1999) or Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA) programs, and aligned with Clustal W (Thompson et al. 1994).

The rbcL and COI-5P alignments comprised 13 new and 41 GenBank sequences, and 10 new and 37 GenBank sequences, respectively (Supplementary Table S1). *Ptilophora scalaramosa, Gelidiophycus freshwateri*, and *Capreolia implexa* were used as the outgroups. MEGA 7.0 (Kumar et al. 2016) was used to calculate genetic distances. The best evolutionary model for phylogenetic analyses selected with the JModelTest 2.1.10 program (Darriba et al. 2012) was GTR + G + I for both data sets. Phylogenies of COI-5P and rbcL datasets were inferred using maximum likelihood (ML) and Bayesian inference (BI). The ML analyses were conducted using RAxML v8.0.X (Stamatakis 2014) set as follows: a rapid bootstrap analysis and search for the best scoring ML tree in one single program run with 1000 bootstrap replicates under the GTR + G + I substitution model. BI was performed for individual datasets with MrBayes v3.2.1 (Ronquist et al. 2012) using the Metropolis coupled Markov chain Monte Carlo under the GTR + G + I model. For each matrix, two million generations of two independent runs were performed with four chains and sampling trees every 100 generations. The burn-in period was identified graphically by tracking the likelihood at each generation to determine whether they reached a plateau. Twenty-five percent of the saved trees were removed, and the remaining trees were used to calculate Bayesian posterior probabilities (BPPs).

3 Results

3.1 Molecular phylogeny

Maximum Likelihood and Bayesian Inference analyses produced trees with similar topologies for both markers.

Figure 2: Maximum-likelihood (ML) tree inferred from rbcL sequences of *Gelidium*. ML bootstrap and Bayesian posterior probability values are shown at branches. Bold letters indicate newly generated sequences in this study. * indicates the holotype.

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For both markers, the specimens of this work formed a clade fully supported by high posterior probability and bootstrap values (1 and 100, respectively) (Figures 2 and 3). The clade formed by these Mexican specimens for both markers was the sister species of *G. gonzalezii* and closely related to *G. indonesianum* Kim, Gerung et Boo and *G. yangmeikengense* Wang et Wang. For *rbcL*, genetic distances among the Mexican specimens were 0.0–0.1 % at the intraspecific level, and 1.2–1.8 % for *G. gonzalezii* and 1.6–2.2 % for *G. yangmeikengense* and *G. indonesianum*. The intraspecific genetic distances among the specimens for the COI-5P marker were 0.0 %, while the interspecific distances between them and *G. gonzalezii* were 3.2–3.9 % and between them and *G. yangmeikengense* and *G. indonesianum* were 3.4–6.7 %.

### 3.2 Morphology

#### 3.2.1 *Gelidium rodrigueziae* Quiroz-González et Ponce-Márquez

**Holotype:** Playa Hermosa, Veracruz, México (18°39′51″y95°7′48″ O). Thalli was growing on the shell of gastropod *Stramonita rustica* in the intertidal zone: GM 898, vegetative, deposited in the Herbarium of Facultad de Ciencias, Universidad Nacional Autónoma de México (FCME); collected by L. G. Aguilar-Estrada on 18 May 2018. GenBank accession number OQ850753 (*rbcL*) and OR133600 (COI-5P). **Isotype:** GM 899. GenBank accession number OQ850754 (*rbcL*) and OR133601 (COI-5P). **Paratypes:** Faro Verde, Veracruz, México GM900, GM 901 deposited in the Herbarium of Facultad de

![Figure 3: Maximum-likelihood (ML) tree inferred from COI-5P sequences of *Gelidium*. ML bootstrap and Bayesian posterior probability values are shown at branches. Bold letters indicate newly generated sequences in this study. * indicates the holotype.](image-url)
Ciencias, Universidad Nacional Autónoma de México (FCME); collected by N. Quiroz-González on 13 May 2022. GenBank accession number OQ850755, OQ850756 (rbcL) and OR133602, OR133603 (COI-5P).

**Etymology:** Dedicated to Dra. Dení Rodriguez a Mexican phycologist, for her invaluable contribution to our knowledge of the Gelidiales from Mexico and the world.

**Diagnosis:** Thallus light to dark red, up to 0.7 cm high, forming a turf on *Stramonita rustica* (Figure 4A and B), with terete prostrate branches attached by brush-like holdfasts (Figure 4C and D). Erect axes cylindrical at the base and flattened above 150–350 µm in width. Branches are rare, and when branches are occasionally present, they are irregularly arranged with one order of ramification (Figure 4B–D).

**Description:** Plants a turf of purple-red thalli, with cylindrical prostrate axes attached to substratum with brush-like haptera (Figure 5A) and erect linear to clavate axes, with irregular to smooth margins, cylindrical at base, 0.3–0.7 cm high and 150–350 µm in width (Figure 4B–D). Axes in transverse section are ovoid, and elliptical. The shape of vegetative apices is acute (Figure 5B). Cortex of 1–2 layers of rounded cells, 3.75–4.5 µm in diameter (Figure 5C and D), outer cortical cells irregularly arranged in surface view. Medulla of 2–4 rows of circular or subcircular cells in transverse section, 10–12.5 µm in diameter, and with very large intercellular spaces. Internal rhizoidal filaments in the medulla (Figure 5C and D).

**Additional material examined:** Playa Hermosa, Veracruz, México GM 902-GM905 and GM911-GM922 vegetative, deposited in the Herbarium of Facultad de Ciencias, Universidad Nacional Autónoma de México (FCME); collected by L. G. Aguilar-Estrada on 18 May 2018. Faro Verde, Veracruz, México GM 906-GM 908 and GM 923-GM926 vegetative, deposited in the Herbarium of Facultad de Ciencias.
Universidad Nacional Autónoma de México (FCME); collected by N. Quiroz-González on 13 May 2022. Andrés Sánchez Magallanes Breakwater, Tabasco, México GM909-GM910, GM 927 vegetative, deposited in the Herbarium of Facultad de Ciencias, Universidad Nacional Autónoma de México (FCME); collected by L. G. Aguilar-Estrada on 16 May 2022.

4 Discussion

Our analyses of plastid \textit{rbcL} and mitochondrial COI-5P sequences, with morphological observations, clearly revealed the occurrence of a new species, \textit{Gelidium rodrigueziae}, from Mexican Atlantic. \textit{Gelidium rodrigueziae} is characterized by a combination of the following vegetative features: small size (0.3–0.7 cm), axes erect, terete at the base, flattening as they approach the apex, with simple or scarce branching, rhizines moderately abundant to abundant in medulla. Table 1 provides a comparison of \textit{G. rodrigueziae} with the other species recorded in the Mexican Atlantic and phylogenetically related species. \textit{Gelidium rodrigueziae} is notably different from the species to which it is closest phylogenetically. It has a much smaller and simpler thallus, with fewer layers of medullary and cortical cells than \textit{G. gonzalezii}, \textit{G. indonesianum} and \textit{G. yangmeikengense} (Quiroz-González et al. 2020a; Wang et al. 2017), is clearly distinguished from \textit{G. gonzalezii} by its simpler branching and fewer orders, as well as a smaller number of rows of cortical and medullary cells. There is no morphological similarity with the rest of the species registered for the Mexican Atlantic, since they have much larger and more branched thalli.

Phylogenetic analysis of both \textit{rbcL} and COI-5P sequences consistently demonstrated a marked difference between \textit{Gelidium rodrigueziae} and other species in the genus. The nucleotide divergence values established in the genus to distinguish species with \textit{rbcL} and COI-5P have variable ranges. There are proposals for new species based on very small ranges, with values of 0.3–1 % for \textit{rbcL} and 2.6–4.6 % for COI-5P, for example, between \textit{Gelidium palmatum} and \textit{G. millarianum}, between \textit{G. sanyaense} and \textit{G. sentosaense} G.H. Boo, as well as the divergence between \textit{G. linoides} and \textit{G. tenuifolium}. Thus, the genetic distance values between \textit{G. gonzalezii} and \textit{G. rodrigueziae} resemble the small values reported for other \textit{Gelidium} species (Boo et al. 2014, 2022a; Boo and Kim 2020; Brunelli et al. 2019; Jamas et al. 2017; Quiroz-González et al. 2020a, 2021a; Wang et al. 2017).

\textit{Gelidium rodrigueziae} is a sister species of \textit{Gelidium gonzalezii} recently described for the Mexican Pacific (Quiroz-González et al. 2020a), although they belong to very different ecoregions and biogeographic provinces according to the classification proposed by Spalding et al. (2007) (Figures 2 and 3). A similar close relationship between disjunct species has been recorded in other species of \textit{Gelidium}, such as \textit{Gelidium brasiliense} and \textit{G. sentosaense},
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<tr>
<td>Thallus size</td>
<td>0.5–6 cm, irregular below, alternating to subpinnate</td>
<td>3–50 cm Piñate</td>
<td>0.4–1.20 Simple to multiple irregular</td>
<td>3–6.5 cm Piñate, alternating or irregular branching</td>
<td>0.7–2.5 cm Multiple irregular</td>
<td>Up to 15 cm Simple</td>
<td>0.1–1.70 Irregular</td>
<td>0.3–0.7 cm Scarce or simple</td>
<td>1–2 cm Simple or pinnate</td>
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<tr>
<td>Branching pattern</td>
<td>2–4</td>
<td>1–3</td>
<td>1–3</td>
<td>2–3</td>
<td>1–3</td>
<td>–</td>
<td>1–3</td>
<td>1</td>
<td>1–2</td>
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<td>Apex</td>
<td>Acute</td>
<td>Acute</td>
<td>Obtuse/bilobed</td>
<td>Obtuse/bilobed</td>
<td>Lanceolate, blunt, or truncate apices</td>
<td>Acute/obtuse</td>
<td>Acute</td>
<td>–</td>
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<td>Stolon</td>
<td>Cylindrical, Abundant to scarce in the medulla</td>
<td>Cylindrical in the medulla</td>
<td>Cylindrical Abundant in the medulla</td>
<td>Cylindrical Abundant in medulla</td>
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<td>Internal rhizoidal filaments</td>
<td>3 rows, circular, 14–16.5 μm diameter</td>
<td>4–8 rows, circular or subcircular, 10–20 μm diameter</td>
<td>–</td>
<td>–</td>
<td>3–8 rows, 8–27 μm diameter</td>
<td>–</td>
<td>2–4 rows, 10–12.5 μm diameter</td>
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<tr>
<td>Medullary cells</td>
<td>1 row</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2–4 rows, elliptical to rounded</td>
<td>Rounded to ovate, 4–7 μm diameter</td>
<td>1–3 rows</td>
<td>1–2 rows, rounded</td>
<td>3 rows; rounded, 2.8–4.6 μm diameter</td>
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<tr>
<td>Cortical cells</td>
<td>1 row</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2–4 rows, rounded</td>
<td>1–3 rows</td>
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(–): no information.
which are phylogenetically close but are found in Brazil and China, respectively (Brunelli et al. 2019) or *G. sclerophyllum* and *G. floridanum* that are close but have been recorded in the Pacific from Mexico to Ecuador and Brazil. Their divergence has been attributed to the formation of the Isthmus of Panama (Boo et al. 2022b). Thus, the genetic distance values and their geographical distribution support the proposal of a new species of *Gelidium* for the Mexican Atlantic.

*Gelidium rodrigueziae* has been found growing in most abundance on the mollusk *Stramonita rustica*, which is frequent in the intertidal zone of the Gulf of Mexico. Shells provide substrata for the settlement of epibionts, provide shelter from predation, and from physical or physiological stresses (Gutiérrez et al. 2003; Levenets et al. 2010; Quiroz-González et al. 2020b). Recently, epibiont relationships have been of special interest, as they have served to indicate numerous new species among very different marine organisms, such as ciliates on nematodes, entoprocts on bryozoans and even flatworms on crustaceans (Borisanova and Krylova 2014; Chatterjee and Fernandez-Leborans 2013; Soares et al. 2021).

As Boo and Kim (2020) point out, small species of *Gelidium*, such as *G. rodrigueziae* are difficult to define using a few diagnostic characteristics, so the use of molecular tools is essential for the knowledge of this genus whose species richness is increasing substantially thanks to their use. Therefore, evaluating the rest of the algae of this genus on the coasts of Mexico is important, especially on substrata as promising as animal shells.

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**Competing interests:** The authors declare that they have no conflict of interest. All data was obtained and collected in accordance with local research protocols.

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**Data availability:** The DNA sequences are available for consultation in GenBank.

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