Molecular Assessment of Athecate Dinoflagellates of the Order Gymnodiniales (Dinophyceae) in Todos Santos Bay, Baja California México

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Abstract

The Gymnodiniales are the most important group of athecate dinoflagellates both for its abundance and distribution and for the harmful potential of several of its species. Although morphologically it is well known, phylogenetically it has been very little studied. HABs impact important coastal activities in Todos Santos Bay, so a detailed characterization of potentially toxic phytoplankton species is crucial in monitoring surveys. Therefore, the aim of the present study was to carry out a molecular assessment to the morphospecies of Gymnodiniales currently recorded in Todos Santos Bay, from which discussed their phylogenetic relationships. 120 samples were obtained from monthly samplings during 2019 to 2020. For all of them, 18 s small subunit (SSU) and 28 s large subunit (LSU) genes of rDNA were amplified and sequenced using single-cell PCR. Also, they were photographed. A phylogenetic analysis was performed on MrBayes from the combined 18 s - 28 s data set. The presence of 15 phylogenetic entities was confirmed, four of which constituted new records for the Mexican Pacific, as well as three harmful species, one toxic and two bloom-forming. Our molecular results evidenced the need to re-evaluate the current taxonomic system of athecate dinoflagellates at all hierarchical levels, integrating them with morphological evidence without preponderance of this criterion over taxonomic decisions.

Keywords

Diversity, HABs, New Records, Paraphyletic Groups, Athecate Dinoflagellates

1. Introduction

Dinoflagellates are the second most diverse group within phytoplankton and the most abundant group in tropical marine environments. They are mainly characterized by being solitary, mobile and unicellular, although sometimes they form chains, with variable size from 5 μm to 2 mm [1] [2]. Currently, an estimated 2000 living species are known, divided into six classes, 16 orders, 65 families and 259 genera [3] [4] [5]. [6] classified them, according to the characteristics of their cell wall in Thecales and Athecales; the latter characterized by presenting a very fragile cell wall (amphysm) composed of very fine flattened vesicles [2], and two conventional flagella (one longitudinal housed in the *sulcus* and another transversal housed in the *cingulum*). For the discrimination of species within Athecates, different morphological characters are considered to discriminate them by size, position and displacement of flagella, presence or absence of an apical *sulcus*, chloroplasts and pyrenoids, position and size of the nucleus, folds or stretchmarks, as well as ornamentation and extensions on the wall [1]. Currently, they are not considered a natural group since they comprise various orders, being Syndiniales Loeblich III, Suessiales Fensome *et al.*, and Noctilucales Haeckel the most ancestral phylogenetically, which suggests that the amphysm, its main morphological character, is equally ancestral [7].

Gymnodiniales Apstein has been the order most studied in the group of athecates, both for the number of species and ecological importance [8] [9]. It comprises an approximate of 638 species, divided into 11 families and 71 genera of which 29 are planktonic; *Gymnodinium* Stein, *Gyrodinium* Kofoed & Swezy, *Cochlodinium* Schütt and *Warnowia* Lindemann group are the largest number of species [5] [10]. Although they are morphologically well known, their phylogenetic relationships have been little studied. However, from the few studies that have integrated morphology with molecular tools, some important changes have been made at all hierarchical levels. For example, [11] analyzed sequences of the 28 s rDNA gene in species of the genera *Gymnodinium* and *Gyrodinium*, erecting to *Akashiwo* Hansen & Moestrup, *Karenia* Hansen & Moestrup and *Karodinium* Larsen. [12] succeeded in redefining *Margalefidinium fulvescens* (= *Cochlodinium fulvescens*) and [13] to *Torquentidium flavescens* (= *Gyrodinium flavescens*); [14] and [15], have also described new diversity at the specific and generic level.

On the coasts of the Mexican Pacific, even though several species have been identified as forming blooms, the diversity of Gymnodiniales is known exclusively on the morphological basis and is scarce [2] [16] [17] [18]. In the coastal zone of Baja California, particularly in the Todos Santos Bay (TSB), Harmful Algae Blooms (HABs) impact important coastal activities [19], so a robust characterization of the floristic composition with harmful potential is crucial in monitoring programs to detect potentially toxic phytoplankton. However, the only study of specific composition in the area is that of [18], also on a morphological basis, since this group is not known phylogenetically. Additionally, expe-

rience and the instrumentation of microscopic observation limit the correct identification of harmful algae, especially athecate dinoflagellates. Given the above, the aim of the present study was to carry out a molecular assessment to the morphospecies of Gymnodiniales currently recorded in Todos Santos Bay, from which discussed their phylogenetic relationships.

2. Material and Methods

2.1. Collection, Observation, and Isolation

Monthly samplings were carried out from August 2019 to April 2020, aboard one smaller vessel, of which a total of 120 samples were obtained. The samples were collected from two different sampling campaigns, with twelve and eight different points, respectively (**Figure 1**). The biological material was obtained with a phytoplankton network of 20 μm mesh size, by vertical dragging at each sampling point. The samples obtained were stored in plastic drums (covered

Figure 1. Study area with the sampling stations of the monitoring carried out inside and outside the Todos Santos Bay. The monthly collection stations are indicated by the letter “S” (S1 - S12) and the SEMAR collection stations are indicated by the letter “E” (E1 - E8).

with tape to protect the collected material from sunlight) of 250 ml capacity. To avoid deformation of the biological material, the samples were stored in containers with ice for later analysis in the laboratory. Live samples were filtered with a sieve with a mesh size of 130 μm , to eliminate zooplankton. For the observation of the collected material, a 15 ml aliquot of each sample was taken and deposited in each well of a culture plate (Plate with 6 wells). Each well was viewed under a Leica DM IRB (Germany) inverted light microscope, with brightfield illumination at 10 $\times$ magnification. Athecate cells were isolated by means of a reduced micropipette technique and rinsed several times with sterile filtered seawater. Single cells were placed in 0.2 mL PCR tubes and stored at -80°C for subsequent molecular analysis. Those isolated cells that did not undergo deformation during the rinses were photographed with a Canon EOS-Rebel T6 camera (Taiwan).

2.2. Single Cell-PCR and Sequencing

The 18 s small subunit (SSU) and 28 s large subunit (LSU) genes of rDNA were amplified. Single cell PCR was performed directly following the protocols described by [20] and [21] with minor modifications described in the PCR conditions. The primers pairs combination for SSU [20] were SR1-F/SR12-R, SR1-F/SR7-R, SR1-F/SR9-R, SR4-F/SR7-R and SR4-F/SR9-R [20], for LSU D1R-F/D2C-R and D1R-F/D3B-R [22] [23]. The amplification conditions were, for the 18 s gene: initial denaturation at 98°C for 3 min, 35 cycles at 95°C for 1 min, 56°C for 1 min and 72°C for 3 min, with a final extension at 72°C for 10 min; for the 28 s gene: initial denaturation at 98°C for 3 min, 35 cycles at 95°C for 30 s, 52°C for 30 s and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplification were confirmed through electrophoresis in 1.5% agarose gels for 20 min at 90 V, stained with Ethidium Bromide and visualized in an UV light gel documentation system. PCR products were stored at -20°C and shipped to Retrogen Inc (San Diego, CA, USA) for purification and sequencing. DNA sequences were determined only for one strand using the corresponding forward/reverse primers.

2.3. Phylogenetic Analysis

The sequences obtained (Table 1), were aligned in BioEdit v. 7.0.5 [24] with those of a selected group of athecate species from the GenBank (Table S1). *Synedinium turbo* and *Hematodinium perezii* were used as outgroup because they have been used in other analysis with dinoflagellates. We analysed SSU-LSU gene-codon partitioned sequence data sets both separately and concatenated. For the combined 18 s - 28 s data set, taxa for which 18 s or 28 s sequence data were unavailable were treated as missing data. Bayesian phylogenetic analyses were performed in MrBayes v3.2.2 [25] using a GTR + I + G (general time reversible + invariable sites + gamma distribution), evolutionary model, as suggested by the ML ratio test implemented by TOPALi v2 software [26]. Four parallel runs of the Markov Chain Monte Carlo (MCMC) searches were performed

Table 1. Data for the isolated and sequenced athecate dinoflagellates (18 s small subunit (SSU) and 28 s large subunit (LSU)) in this study. The GenBank access number, collection station, and figure for each morphospecies are included. Dash (-) indicates that there was no photographic record of the morphospecies.

Taxa	GenBank accession number	Collection station	Figure
<i>Akashiwo sanguinea</i> (Hirasaka) Hansen & Moestrup	OL439705	1	
<i>A. sanguinea</i>	OL439706	2	
<i>A. sanguinea</i>	OL439707	3	3(A)
<i>A. sanguinea</i>	OL439688		
<i>A. sanguinea</i>	OL439689	4	
<i>Balechina pachydermata</i> (Kofoid & Swezy) Loeblich Jr., Loeblich III	OL439708	6	3(B)
<i>Gymnodinium catenatum</i> Graham	OL439693	11	-
<i>G. sp. 1</i>	OL439690	12	-
<i>G. sp. 2</i>	OL439691		
<i>G. sp. 3</i>	OL439692	11	3(C)
<i>Gyrodinium heterostriatum</i> (Kofoid & Swezy) Gómez, Artigas & Gast	OL439710	10	3(D)
<i>Gyrodinium sp.</i>	OL439709	9	3(E)
<i>Levanderina fissa</i> (Levander) Moestrup, Hakanen, Hansen, Daugbjerg & Ellegaard	OL439711	4	-
<i>Lepidodinium viride</i> Watanabe, Suda, Inouye, Sawaguchi & Chihara	OL439694	5	3(G)
<i>Margalefidinium fulvescens</i> (Iwataki, Kawami & Matsuoka) Gómez, Richlen & Anderson	OL439712	1	
<i>M. fulvescens</i>	OL439713	2	
<i>M. fulvescens</i>	OL439695	8	3(H)
<i>M. fulvescens</i>	OL439696	10	
<i>M. fulvescens</i>	OL439697	12	
<i>Pseliodinium fusus</i> (Schütt) Gómez	OL439714		
<i>P. fusus</i>	OL439698	8	3(I)
<i>Takayama acrotrocha</i> (Larsen) Salas, Bolch & Hallegraeff	OL439715	11	-
<i>T. tasmanica</i> Salas, Bolch & Hallegraeff	OL439700		
<i>T. tasmanica</i>	OL439701	12	3(F)
<i>T. sp.</i>	OL439699	5	-

Continued

<i>Torquentidium flavescens</i> (Kofoid & Swezy) Shin, Li, Lee & Matsuoka	OL439702		
<i>T. flavescens</i>	OL439703	6	3(I)
<i>T. flavescens</i>	OL439704		

starting with a random tree, sampling the data every 100 generations for 5×10^6 generations, and discarding the first 25% of trees as burn-in. Pairwise distance values (p-distances) were calculated using Mega v5 [27].

3. Results

A total of 80 cells corresponding to athecate dinoflagellates were isolated for TSB, where only 60 amplified. Of the total of sequences obtained, only 28 corresponded to dinoflagellates of the order Gymnodiniales. Sequence analyses of athecate dinoflagellates from TSB SSU-LSU obtained through single-cell PCR, confirmed the presence of 15 phylogenetic entities, four of which constituted new records for the Mexican Pacific (*Gyrodinium heterostriatum*, *Lepidodinium viride*, *Torquentidium flavescens* and *Takayama acrotrocha*). The Gymnodinaceae family was the largest represented with seven species, followed by Kareniaceae, Ceratoperidiniaceae and Gyrodiniaceae, each with two species and two other species in an uncertain position.

The alignment with SSU consisted of 56 sequences, 11 newly ones generated in this study, with a length of 1229 base pairs (bp), while the alignment with 28 s large subunit (LSU) consisted of 75 sequences, 16 newly generated in this study, with a length 976 bp. In the combined 18 s - 28 s analysis (Figure 2) and in all single analyses, BI trees did not differ, but there were slight differences among markers due to sampling or in poorly supported branches; so, we only describe the results from the combined analysis SSU-LSU (Figure 2).

Phylogenetic reconstruction based on the concatenated data set showed seven large clades (Figure 2). Clade I, with low phylogenetic support (BI = 0.65), showed two sister subclades (A and B). Subclade A, with high phylogenetic support (BI = 0.99), sequences from the Genbank and sequences generated in this study for the genus *Margalefidinium* were grouped (Figure 3(H)). Subclade B, with good phylogenetic support (BI = 0.85), grouped GenBank sequences and generated in this study for the sister genera *Levanderina* and *Akashiwo* (Figure 3(A)). Clade II, belonging to the Kareniaceae family, with low phylogenetic support (BI = 0.60), was made up of GenBank sequences for the sister genera *Karlodinium* and *Takayama* (Figure 3(F)), which in turn were resolved in a larger clade as sisters of *Karenia*. Of our sequences only *Takayama* was represented in this clade. Clade III, belonging to the Ceratoperidiniaceae family, with the maximum phylogenetic support (BI = 1), was made up of sequences generated in this study and from GenBank, for the sister genera *Torquentidium* and *Psellodinium* (Figure 3(I) and Figure 3(J)), which in turn were resolved into

Figure 2. Bayesian topology based on the combined data set SSU-LSU rDNA for Gymnodiniales species. The numbers at the nodes are the Bayesian posterior probability (PPB) values. The main clades are indicated by Roman numerals (I, II, III, IV, V, VI, VII). Sub-clades are indicated by letters ((A), (B), (C), (D)). The sequences generated in this study are in bold. The bar under the tree indicates substitutions by site.

a larger clade such as sister of *Kirithra* and *Ceratoperidinium*. Clade IV, with low phylogenetic support (BI = 0.63), was made up of GenBank sequences and generated in this study for the genus *Balechina* (Figure 3(B)). Clade V, belonging to the Gyrodiniaceae family, with high phylogenetic support (BI = 0.97), was made up of GenBank sequences and sequences generated in this study for the genus *Gyrodinium* (Figure 3(D) and Figure 3(E)). Clade VI, with the maximum phylogenetic support (BI = 1), was in turn divided into two sister

Figure 3. (A)-(J). A. *Akashiwo sanguinea*. (B). *Balechina pachydermata*. (C). *Gymnodinium* sp. 2 - sp. 3. (D). *Gyrodinium heterostriatum*. (E). *Gyrodinium* sp. 1. (F). *Takayama tasmanica*. (G). *Lepidodinium viride*. (H). *Margalefidinium fulvescens*. (I). *Torquentidium flavescens*. J. *Pselodinium fusus*. The letter “N” indicates the position of the nucleus. Scale bar: (A), (C)-(J) = 20 μ m; (B) = 30 μ m.

subclades (C and D). Subclade C, with high phylogenetic support (BI = 0.93), was made up of GenBank sequences of the genus *Polykrikos*. Subclade D, with low phylogenetic support (BI = 0.56), was made up of the GenBank genera and generated in this study for the genus *Lepidodinium* (Figure 3(G)), which in turn was resolved into a larger clade as a sister of *Gymnodinium* and *Barrufeta*, in turn, this clade formed a larger group with GenBank sequences generated in this study for the paraphyletic genus *Gymnodinium* (Figure 3(C)). Clade VII, with high phylogenetic support (BI = 0.93), was made up of sequences generated in this study in an uncertain position.

Interspecific distances values with 18 s small subunit (SSU) ranged between a minimum of 3.2% for *Akashiwo sanguinea* - *Takayama acrotrocha* and a maximum of >100% for *Margalefidinium fulvescens* - *Gymnodinium impudicum*. For 28 s large subunit (LSU) values ranged between a minimum of 19% for *A. sanguinea*-*Karlodinium armiger* and a maximum of >100% for *M. polykrikoides*-*Polykrikos kofoidii*. Interspecific distance values are not included due to the large amount of data obtained.

4. Discussion

Although our main aim was the molecular assessment of the species athecate dinoflagellates present in Todos Santos Bay, it was not possible in all cases, since the resulting phylogeny did not confirm its phylogenetic position. But it did show an important taxonomic problem in the current classification of the group at all hierarchical levels, which is discussed below.

The genus *Margalefidinium*, traditionally classified within the Gymnodiniaceae [28], appears in our analysis in a basal position, without any relationship with the species of this family. The interspecific distance values with *Margalefidinium polykrikoides* and *M. fulvescens* were even greater than 100%, suggesting a total structure that seems to correspond to a taxonomic position above the family level. However, although with our present morphological and molecular data it is not possible to establish its taxonomic position, we can confirm phylogenetically the presence of *M. fulvescens* in the Bay. On the other hand, the genus *Akashiwo*, included within the Gymnodiniaceae family, showed a close relationship with the monospecific genus *Levanderina*, currently in an uncertain position [29], but not with the species of its current family. Previously, [10] had already suggested that this genus should be reclassified within a new family; our results support that *Akashiwo* does not belong to the Gymnodiniaceae family, and its phylogenetic position suggests that, together with *Levanderina*, they could comprise a new family supported by genetic distance values between them. In Bahía Todos Santos, we found representatives for both genera, those sequences close to *Levanderina* showed an intraspecific divergence of 3.3%, which suggests a second species not yet described for this genus; in the case of the close sequences with *Akashiwo sanguinea*, they showed a similarity of 100%, which confirms their presence in the bay, but not their taxonomic position.

The Kareniaceae grouped four of our sequences within the genus *Takayama*, two of which showed 100% similarity with *T. tasmanica* and a third 100% similarity with *T. acrotrocha*, confirming its presence in the bay and a new record for the Mexican Pacific. A fourth sequence, which presented a 99.9% similarity with *Takayama* sp., reported by [9], suggests a new phylogenetic entity not yet described. However, this work does not provide morphological data to complement our current data to describe them as a new species. The Ceratoperidiniaceae family, grouped five of our sequences, two of which showed 100% similarity with *Pseliodinium fusus* and three 99.5% with *Torquentidium flavescens* without percentage of genetic divergence between them, which confirmed their presence in the Bay and a new record for the Mexican Pacific (Figure 3(I)). Likewise, the sequences of *T. convolutum*, *T. pirum* and *T. cf. helix*, formed a monophyletic clade, without divergence between them, which suggests a possible misidentification of these sequences as distinct species. On the other hand, one of our sequences showed a 99% similarity with *Balechina pachydermata*, the only species known for the genus [30], the sequences analyzed for this species did not show genetic divergence between them. Although the presence of this species in the

bay is confirmed, its taxonomic position remains uncertain, however, it is suggested that a new family must be described to position this group.

For the Gyrodiniaceae, *Gyrodinium* grouped two of our sequences with *Gyr. heterostriatum*, *Gyr. helveticum* and *Gyr. rubrum*, strongly supported. Although the similarity between *Gyr. helveticum* and our sequence referred to as *Gyrodinium* sp. 1 was 99.5%, its phylogenetic position does not support that both are the same species, although the values of genetic distance between them could confirm it. However, a detailed study with more precise markers and with a higher interspecific resolution could confirm their taxonomic position, since the morphological data that have been observed now suggest that they are different species. Our second sequence showed a 99.8% similarity to *Gyr. heterostriatum*, recently described by [31], with which we confirm its presence in the bay and a new record for the Mexican Pacific (**Figure 3(D)**). The range of genetic distance between the *Gyrodinium* species varied with SSU in 6.6% - 24% and with LSU in 8.6% - 19.4%, much higher than the values of interspecific distances observed in other genera of the group, which shows the need to reevaluate the status of these species within *Gyrodinium*, including our sequences, as a part of only one genus.

For the Gimnodiniaceae, two of our sequences were grouped, showing 100% identity with *Gymnodinium catenatum* and *Lepidodinium viride*, the latter also being a new record (**Figure 3(G)**), respectively, confirming their presence in the bay. On the other hand, in our phylogeny *Gymnodinium* constitutes a paraphyletic group within the family, a clade is made up of *G. aureolum*, another *G. catenatum* and *G. nolleri* and a last clade *G. dorsalicum* and *G. impudicum*. In previously published phylogenies [11] [32] [33], paraphyletic is also shown within this genus, so we consider it necessary to carry out a taxonomic restructuring. The clade made up of *Lepidodinium* species, with maximum support, was well differentiated from *Gymnodinium*, although one of the *L. viride* sequences was in the same clade as *G. impudicum*, but with a genetic distance of 10.3%, which can be attributed to a misidentification of this sequence. Three of our sequences, identified by their morphological characteristics as species of the genus *Gymnodinium*, were grouped in a basal position without any relationship with others. The first sequence labeled *Gymnodinium* sp. 1, had a 90% similarity with *Gertia stigmatica* (a species not considered for this study) from GenBank, however, the sequences labeled *Gymnodinium* sp. 2 and sp. 3 presented the same morphotype and they had a 94% similarity with *Karlodinium gentienii*, but without any relationship with the species of the Kareniaceae family; they also presented a genetic divergence between 8.6% - 19.4% with respect to *Karlodinium*. These results suggest that the three sequences correspond to a new family, a new genus, and new species, which must be described by detailed morphology.

Regarding the detection of FANS-forming species, it is important to have a precise characterization of the flora for Bahía Todos Santos, as an important site for aquaculture, since HABs are a worldwide problem that they mainly affect coastal areas, generating great effects in the economic (tourism, aquaculture, commercial and sport fishing) and health (public health) sectors, as well as wild-

life. In toxic marine phytoplankton, dinoflagellates are the most important group in number and diversity, according to [34] and [35], 104 species of toxic dinoflagellates are listed, where 23 species correspond to athecate dinoflagellates, 19 for the order Gymnodiniales and four for the order Amphidinales, of the total of athecates dinoflagellates, only 11 generate HABs. In this study we detected three harmful species (*Akashiwo sanguinea*, *Levanderina fissa* and *Margalefidinium fulvescens*), one toxic (*Gymnodinium catenatum*) and two potentially bloom-forming species (*Lepidodinium viride* and *Takayama tasmanica*), for the rest of the species their effect is unknown. In Mexico, there have been several epizootics related to the flowering of *A. sanguinea*, the last reported event was in 2007, where this species possibly caused anoxic conditions (abundance of 4.6×10^3 cells/l), which caused the massive death of benthic species in the North of Baja California Sur [36] [37] [38]. *Levanderina fissa* has been recorded in high abundances (from 163 to 265×10^3 cells/l) in Bahía de la Paz, Mexico, although the blooms of this species were harmless for the area [39]. *M. fulvescens* has been reported in lagoons and bays in the southern Gulf of California [40], being present in abundances of 600 to 45,800 cells/l, in La Paz Bay, Mexico [41], without causing any damage. *Gymnodinium catenatum* has recurrently formed FAN (since its description in 1943 to date) in different regions of the Mexican Pacific, presenting high densities (1×10^6 cells/l) in the northern part of the Gulf of California and little abundant records in the Mexican Tropical Pacific. This species produces paralyzing toxins, which is why it has caused poisoning in humans and wildlife [42] [43] [44]. The potentially bloom-forming species, *Takayama tasmanica* and *Lepidodinium viride*, have not been reported as toxic or harmful. *T. tasmanica* has been reported in Bahía de la Paz, with low abundance (400 - 600 cells/l) [45], in [46] point out that the species has occurred in great abundance along the East Coast and Gulf of the United States, without generating any repercussions. *L. viride* represents the first record for the Mexican Pacific, so there is no information on blooms of this species, however, [9] have reported this species as harmful, since it has been related to fish deaths on the shores of the Mediterranean Sea, due to oxygen depletion.

Finally, our analyses reveal the need to carry out an in-depth evaluation of the phylogenetic relationships within the athecate dinoflagellates at all hierarchical levels. Even though molecular tools are used in different parts of the world, as a complement in the diversity analysis in this group, now there is still not a deep interpretation of the phylogeny, being the preponderant criterion morphology over molecular evidence in the taxonomic decision making, generated an inconsistency in the integration of morphological and molecular results with the consequent instability in the current classification system.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Fensome, R.A., Taylor, F.J.R., Norris, G., Sarjeant, W.A.S., Wharton, D.I. and Williams, G.L. (1993) A Classification of Fossil and Living. Micropaleontology. Special Publication No. 7, Sheridan Press, Hanover.
- [2] Licea, S., Moreno, J.L., Santoyo, H. and Figueroa, G. (1995) Dinoflagelados del Golfo de California. UABCS, SEP-FOMES y PROMARCO, México.
- [3] Taylor, F.J.R., Hoppenrath, M. and Saldarriaga, J.F. (2008) Dinoflagellate Diversity and Distribution. *Biodiversity and Conservation*, **17**, 407-418.
<https://doi.org/10.1007/s10531-007-9258-3>
- [4] Gómez, F. (2005) A List of Free-Living Dinoflagellate Species in the World's Oceans. *Acta Botanica Croatica*, **64**, 129-212.
- [5] Guiry, M.D. and Guiry, G.M. (2021) AlgaeBase. World-Wide Electronic Publication, National University of Ireland, Galway. <http://www.algaebase.org/>
- [6] Lindemann, E. (1928) Abteilung Peridineae (Dinoflagellatae). In: Engler, A. and Prantl, K., Eds., *Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen unter Mitwirkung zahlreicher hervorragender Fachgelehrten begründet vom*, Verlag von Wilhelm Engelman, Leipzig, 3-104.
- [7] Gómez, F., Moreira, D. and López-García, P. (2011) Avances en el estudio de los dinoflagelados (Dinophyceae) con la filogenia molecular. *Hidrobiológica*, **21**, 343-364.
- [8] Gómez, F., Moreira, D. and Lopez-Garcia, P. (2010) Molecular Phylogeny of Noctiluroid dinoflagellates (Noctilucales, Dinophyceae). *Protist*, **161**, 466-478.
<https://doi.org/10.1016/j.protis.2009.12.005>
- [9] Reñe, A., Camp, J. and Garcés, E. (2015) Diversity and Phylogeny of Gymnodiniales (Dinophyceae) from the NW Mediterranean Sea Revealed by a Morphological and Molecular Approach. *Protist*, **166**, 234-263.
<https://doi.org/10.1016/j.protis.2015.03.001>
- [10] Gómez, F. (2012) A Checklist and Classification of Living Dinoflagellates (Dinoflagellate, Alveolata). *CICIMAR Oceanías*, **27**, 65-140.
<https://doi.org/10.37543/oceanides.v27i1.111>
- [11] Daugbjerg, N., Hansen, G., Larsen, J. and Moestrup, Ø. (2000) Phylogeny of Some of the Major Genera of Dinoflagellates Based on Ultrastructure and Partial LSU rDNA Sequence Data, Including the Erection of Three New Genera of Unarmoured Dinoflagellates. *Phycologia*, **39**, 302-317.
<https://doi.org/10.2216/i0031-8884-39-4-302.1>
- [12] Hu, Z., Deng, Y., Li, Y. and Tang, Y.Z. (2018) The Morphological and Phylogenetic Characterization for the Dinoflagellate *Margalefidinium fulvescens* (= *Cochlodinium fulvescens*) Isolated from the Jiaozhou Bay, China. *Acta Oceanologica Sinica* **37**, 11-17.

- <https://doi.org/10.1007/s13131-018-1295-0>
- [13] Hyeon, H.S., Zhun, L. and Kazumi, M. (2020) Reclassification of *Gyrodinium flavescens* Kofoed & Swezy as *Torquentidium flavescens* comb.nov. (Ceratoperidiniaceae, Dinophyceae), Based on Morphology and Phylogeny. *Phycologia*, **5**, 133-139. <https://doi.org/10.1080/00318884.2019.1709396>
- [14] Takahashi, K., Benico, G., Mun-Lum, W. and Iwataki, M. (2019) *Gertia stigmatica* gen. et sp. nov. (Kareniaceae, Dinophyceae), a New Marine Unarmored Dinoflagellate Possessing the Peridinin-Type Chloroplast with an Eyespot. *Protist*, **170**, Article ID: 125680. <https://doi.org/10.1016/j.protis.2019.125680>
- [15] Ok, J.H., Jeong, H.J., Lee, S.Y., Park, S.A. and Noh, J.H. (2021) *Shimiella* gen. nov. and *Shimiella gracilentia* sp. nov. (Dinophyceae, Kareniaceae), a Kleptoplastidic Dinoflagellate from Korean Waters and Its Survival under Starvation. *Journal of Phycology*, **50**, 70-91. <https://doi.org/10.1111/jpy.13067>
- [16] Esqueda-Lara, K. and Hernandez-Becerril, D.U. (2010) Dinoflagelados microplanctonicos marinos del Pacifico central de México) Isla Isabel, Nayarit y costas de Jalisco y Colima. Instituto de Ciencias del Mar y Limnología. Universidad Nacional Autónoma de México, México.
- [17] Meave-del Castillo, M.E., Zamudio-Reséndiz, M.E. and Castillo-Rivera, M. (2012) Riqueza fitoplanctónica de la Bahía de Acapulco y zona costera Aledaña, Guerrero, México. *Acta Botánica Mexicana*, No. 100, 405-487. <https://doi.org/10.21829/abm100.2012.41>
- [18] Almazán-Becerril, A., Aké-Castillo, J.A., García-Mendoza, E., Sánchez-Bravo, Y.A., Escobar-Morales, S. and Valadez Cruz, F. (2016) Catálogo de microalgas de Bahía de Todos Santos, Baja California. CICESE, México.
- [19] García-Mendoza, E., Cáceres-Martínez, J., Rivas, D., Fimbres-Martínez, M., Sánchez-Bravo, Y., Vásquez-Yeomans, R. and Medina-Elizalde, J. (2018) Mass Mortality of Cultivated Northern Bluefin Tuna *Thunnus thynnus orientalis* Associated with *Chattonella* Species in Baja California, Mexico. *Frontiers in Marine Science*, **5**, Article No. 454. <https://doi.org/10.3389/fmars.2018.00454>
- [20] Matsuoka, K., Kawami, H., Fuji, R. and Iwataki, M. (2006) Further Examination of the Cyst-Theca Relationship of *Protoperidinium thulense* (Peridinales, Dinophyceae) and the Phylogenetic Significance of Round Brows Cysts. *Phycologia*, **45**, 632-641. <https://doi.org/10.2216/05-42.1>
- [21] Hernández-Rosas, A., Meave-del Castillo, M.E., Díaz-Larrea, J. and Rodríguez, F. (2018) Single-Cell PCR Amplification of Thecate Dinoflagellates: A Case Study of *Tripes* (Dinophyceae). *Journal of Applied Phycology*, **30**, 1117-1124. <https://doi.org/10.1007/s10811-017-1269-1> <https://link.springer.com/article/10.1007/s10811-017-1269-1>
- [22] Scholin, C.A., Herzog, M., Sogin, M. and Anderson, D.M. (1994) Identification of Group and Strain-Specific Genetic Markers for Globally Distributed *Alexandrium* (Dinophyceae) II. Sequence análisis of a Fragmento the Large Subunit Ribosomal RNA Gene. *Journal of Phycology*, **30**, 999-1011. <https://doi.org/10.1111/j.0022-3646.1994.00999.x>
- [23] Hansen, G., Daugbjerg, N. and Henriksen, P. (2000) Comparative Study of *Gymnodinium mikimotoi* and *Gymnodinium aureolum*, comb. Nov. (= *Gymnodinium aureolum*) based on morphology, Pigment and Composition and Molecular Data. *Journal of Phycology*, **36**, 394-410. <https://doi.org/10.1046/j.1529-8817.2000.99172.x>
- [24] Hall, T.A. (1999) BioEdit a User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*,

41, 95-98.

- [25] Ronquist, F., Teslenko, M., Van Der Mark P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Liu, L., Suchard, M.A. and Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology*, **61**, 539-542. <https://doi.org/10.1093/sysbio/sys029>
- [26] Milne, I., Lindner, D., Bayer, M., Husmeier, D., McGuire, G., Marshall, D.F. and Wright, F. (2009) TOPALi v2: A Rich Graphical Interface for Evolutionary Analyses of Multiple Alignments on HPC Clusters and Multi-Core Desktops. *Bioinformatics*, **25**, 126-127. <https://doi.org/10.1093/bioinformatics/btn575>
- [27] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, **28**, 2731-2739. <https://doi.org/10.1093/molbev/msr121>
- [28] Gómez, F., Richlen, M.L. and Anderson, D.M. (2017) Molecular Characterization and Morphology of *Cochlodinium strangulatum*, the Type Species of *Cochlodinium*, and *Margalefidinium* gen.nov. for *C. polykrikoides* and Allied Species (Gymnodiniales, Dinophyceae). *Harmful Algae*, **63**, 32-44. <https://doi.org/10.1016/j.hal.2017.01.008>
- [29] Moestrup, Ø., Hakanen, P., Hansen, G., Daugbjerg, N. and Ellegaard, M. (2014) On *Levanderina fissa* Gen. & Comb. nov. (Dinophyceae) (syn. *Gymnodinium fissum*, *Gyrodinium instriatum*, *Gyr. uncatenatum*), a Dinoflagellate with a Very Unusual sulcus. *Phycologia*, **53**, 256-292. <https://doi.org/10.2216/13-254.1>
- [30] Gómez, F., López-García, P., Takayama, H. and Moreira, D. (2015) *Balechina* and the New Genus *Cucumeridinium* gen. nov. (Dinophyceae), Unarmored Dinoflagellate with Thick Cell Coverings. *Journal of Phycology*, **51**, 1088-1105. <https://doi.org/10.1111/jpy.12346>
- [31] Gómez, F., Artigas, L.F. and Gast, R.J. (2020) Phylogeny and Synonymy of *Gyrodinium heterostriatum* Comb. nov. (Dinophyceae), a Common Unarmored Dinoflagellate in the World Oceans. *Acta Protozoologica*, **59**, 77-98. <https://doi.org/10.4467/16890027AP.20.007.12675>
- [32] De Salas, M.F., Bolch, C.J.S., Botes, L., Nash, G., Wright, S.W. and Hallegraeff, G.M. (2003) *Takayama* gen. nov. (Gymnodiniales, Dinophyceae), a New Genus of Unarmoured Dinoflagellates with Sigmoid Apical Grooves, Including the Description of Two New Species. *Journal of Phycology*, **39**, 1233-1246. <https://doi.org/10.1111/j.0022-3646.2003.03-019.x>
- [33] De Salas, M.F., Bolch, C.J. S. and Hallegraeff, G.M. (2004) *Karenia asterichroma* sp. nov. (Gymnodiniales, Dinophyceae), a New Dinoflagellate Species Associated with Finfish Aquaculture Mortalities in Tasmania, Australia. *Phycologia*, **43**, 624-631. <https://doi.org/10.2216/i0031-8884-43-5-624.1>
- [34] Moestrup, Ø., Akselmann-Cardella, R., Churro, C., Fraga, S., Hoppenrath, M., Iwataki, M., Larsen, J., Lundholm, N. and Zingone, A. (2009) IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae. <http://www.marinespecies.org/hab>
- [35] Hansen, G. (2021) Gymnodiniales. In: Lundholm, N., Churro, C., Fraga, S., Iwataki, M., Larsen, J., Mertens, K., et al., Eds., *IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae*. <http://www.marinespecies.org/hab>
- [36] Ochoa, J.L., Sanchez-Paz, A., Cruz-Villacorta, A., Núñez-Vázquez, E.J. and Sierra-Beltran, A.P. (1997) Toxic Events in the North Pacific Coastline in Mexico during 1992-1995: Origin and Impact. *Hydrobiologia*, **352**, 195-200. <https://doi.org/10.1023/A:1003015103760>

- [37] Sierra-Beltrán, A.P., Cruz, A., Núñez-Vázquez, E.J., Del Villar, L.M., Cerecero, J. and Ochoa, J.L. (1998) An Overview of the Marine Food Poisoning in Mexico. *Toxicon*, **36**, 1493-1502. [https://doi.org/10.1016/S0041-0101\(98\)00139-1](https://doi.org/10.1016/S0041-0101(98)00139-1)
- [38] Gárate-Lizárraga, I., Pérez-Cruz, B., Díaz-Ortiz, J. and Band-Schmidt, C.J. (2008) Microalgas y biotoxinas marinas en las costas mexicanas. *Conversus*, **9**, 22-26.
- [39] Gárate-Lizárraga, I. (2014) Proliferation of *Levanderina fissa* and *Polykrikos hartmannii* (Dinophyceae: Gymnodiniales) in Bahía De La Paz, Gulf of California, México. *CICIMAR Oceanides*, **29**, 25-35. <https://doi.org/10.37543/oceanides.v29i2.137>
- [40] Morquecho-Escamilla, M.L. and Alonso-Rodríguez, R. (2008) First Record of *Cochlodinium fulvescens* in Mexican Pacific. *Harmful Algae News*, **37**, 5-6.
- [41] Gárate-Lizárraga, I. (2014) Ocurrance of *Cochlodinium fulvescens* (Gymnodiniales: Dinophyceae) in the Southwestern Gulf of California. *Revista de Biología Marina y Oceanografía*, **49**, 123-127. <https://doi.org/10.4067/S0718-19572014000100013>
- [42] Mee, L.D., Espinosa, M. and Díaz, G. (1986) Paralytic Shellfish Poisoning with a *Gymnodinium catenatum* Red Tide on the Pacific Coast of Mexico. *Marine Environmental Research*, **19**, 77-92. [https://doi.org/10.1016/0141-1136\(86\)90040-1](https://doi.org/10.1016/0141-1136(86)90040-1)
- [43] Cortés-Altamirano, R. and Sierra-Beltrán A.P. (2008) Biotoxins from Freshwater and Marine Harmful Algal Blooms Occurring in Mexico. *Toxin Reviews*, **27**, 27-77. <https://doi.org/10.1080/15569540701883437>
- [44] Band-Schmidt, C.J., Bustillos-Guzmán, J.J., López-Cortes, D.J., Gárate-Lizárraga, I., Núñez-Vázquez, E.J. and Hernández-Sandoval, F.E. (2010) Ecological and Physiological Studies of *Gymnodinium catenatum* in the Mexican Pacific. *Marine Drugs*, **8**, 1935-1961. <https://doi.org/10.3390/md8061935>
- [45] Garate-Lizarraga, I. (2014) Unarmored Dinoflagellates Present during a Bloom of *Cetatosperidinium falcatum* in Bahia de la Paz, Gulf of California. *Revista de Biología Marina y Oceanografía*, **49**, 577-587. <https://doi.org/10.4067/S0718-19572014000300014>
- [46] Anderson, D.M., Fensin, E., Glover, C.J., Hoeglund, A.E., Hubbard, K.A., Kulis, D.M., Landsberg, J.H., Lefebvre, K.A., Provoost, P., Richlen, M.L., Smith, J.L., Solow, A.R. and Trainer, V.L. (2021) Marine Harmful Algal Blooms (HAB,s) in the United States: History, Current Status and Futures Trends. *Harmful Algae*, **102**, Article ID: 101975. <https://doi.org/10.1016/j.hal.2021.101975>

Supplement

Table S1. GenBank 18 s small subunit (SSU) and 28 s large subunit (LSU) sequence data used for phylogenetic analysis. The dash (-) indicates that there is no collection record.

Taxa	Distribution data	GenBank accession number
<i>Akashiwo sanguine</i> (Hirasaka) Hansen & Moestrup	Tolo Harbor (Hong Kong)	MG890411
<i>A. sanguinea</i>	Soko Islands (Hong Kong)	MG890410
<i>A. sanguinea</i>	Gruissan, Ayrolle Lagoon (France)	MT373696
<i>A. sanguinea</i>	Soko Islands (Hong Kong)	MG914054
<i>A. sanguinea</i>	Jangmok Bay (Korea)	MK571208
<i>A. sanguinea</i>	Jangmok Bay (Korea)	MK571209
<i>Balechina pachydermata</i> (Kofoid & Swezy) Loeblich Jr., Loeblich III	Port of Valencia (Spain)	KR139789
<i>B. pachydermata</i>	Sao Sebastiao Channel (Brazil)	KR139790
<i>B. pachydermata</i>	Sao Sebastiao Channel (Brazil)	KR139792
<i>Barrufeta resplendens</i> Sampedro & Fraga	Gulf of Mexico	KT203382
<i>Bar. bravensis</i> (Hulburt) Gu, Lu & Mertens	Northwest Mediterranean Sea (Spain)	FN647673
<i>Ceratoperidinium</i> <i>margalefii</i> Loeblich III	Catalonia, La Muga river mouth (Spain)	KF245456
<i>Gymnodinium aureolum</i> (Hulburt) Hansen	Benguela Current (Namibia)	AY999082
<i>G. catenatum</i> Graham	Manila Bay (Philippines)	AB265962
<i>G. catenatum</i>	Southern Coast (Korea)	MK215823
<i>G. dorsalisulcum</i> (Hulbert, McLaughlin & Zahl) Shauna, Salas & Hallegraeff	Perhentian Island (Malaysia)	MH732684
<i>G. impudicum</i> (Fraga & Bravo) Hansen & Moestrup	Hase (South Korea)	DQ779993
<i>G. impudicum</i>	Corsica, Mediterranean Sea (France)	MH732686
<i>G. nolleri</i> Ellegaard & Moestrup	-	AF200673
<i>Gyrodinium</i> cf. <i>britannicum</i> Kofoid & Swezy	Catalan Coast	KP790191
<i>Gyr. corallinum</i> Kofoid & Swezy	Catalan Coast	KP790192
<i>Gyr. dominans</i> Hulbert	Masan (South Korea)	FN669510

Continued

<i>Gyr. fusiforme</i> Kofoid & Swezy	Otaru Bay (Japan)	AB120002
<i>Gyr. helveticum</i> (Penard) Takano & Horiguchi	Lake Shikotsu (Japan)	AB120004
<i>Gyr. helveticum</i>	Lake Baikal (Russia)	FJ024299
<i>Gyr. helveticum</i>	Lake Baikal (Russia)	MG493227
<i>Gyr. heterogrammun</i> Larsen	Catalan Coast	KP790158
<i>Gyr. heterogrammun</i>	Catalan Coast	KP790196
<i>Gyr. heterostriatum</i> (Kofoid & Swezy) Gómez, Artigas & Gast	North Sea (Netherlands)	MT677911
<i>Gyr. moestrupii</i> Yoon, Kang & Jeong	Western Korea	HE611580
<i>Gyr. rubrum</i> (Kofoid & Swezy) Takano & Horiguchi	Otaru Bay (Japan)	AB120003
<i>Gyr. rubrum</i>	-	AY571369
<i>Gyr. spirale</i> (Bergh) Kofoid & Swezy	Otaru Bay (Japan)	AB120001
<i>Gyr. spirale</i>	Otaru Bay (Japan)	AY571371
<i>Gyr. viridescens</i> Kofoid & Swezy	Catalan Coast	KP790208
<i>Hematodinium perezii</i> Chatton & Poisson	-	EF0655717
<i>Karenia brevis</i> (Davis) Hansen & Moestrup	Gulf of Mexico	AF352818
<i>K. brevis</i>	Corpus Christi, Texas (USA)	AY355455
<i>K. brevisulcata</i> (Chang) Hansen & Moestrup	Concarneau Bay (France)	KJ508359
<i>K. cristata</i> Botes, Sym & Pitcher	South African Coast	AY243963
<i>K. mikimotoi</i> (Miyake & Kominami ex Oda) Hansen & Moestrup	-	EF492505
<i>K. mikimotoi</i>	New Caledonia	KJ508365
<i>K. selliformis</i> Haywood & Steidinger & MacKenzie	New Zealand	HM067007
<i>Karlodinium armiger</i> Bergholtz, Daugbjerg & Moestrup	Catalan Coast	KP790218
<i>Kar. ballantini</i> Salas	Manila Bay (Philippines)	LC521284
<i>Kar. gentienii</i> Nézan, Chomérat & Siano	Brest Bay (France)	KJ508378
<i>Kar. jujuense</i> Li & Shin	Northern East China Sea	KX519410
<i>Kar. zhouanum</i> Luo & Gu	-	MK848615

Continued

<i>Kirithra asteri</i> Boutrup, Tillmann, Daugbjerg & Moestrup	Argentina	MF666674
<i>Kir. sigma</i> Hu, Zhun, Shin & Tang	China	MN380260
<i>Levanderina fissa</i> (Levander) Moestrup, Hakanen, Hansen, Daugbjerg & Ellegaard	New River estuary (USA)	AY721981
<i>L. fissa</i>	Korean coasts	AY421786
<i>L. fissa</i>	Long Island Sound	DQ388457
<i>L. fissa</i>	Catalan Coast	KP790163
<i>Lepidodinium chlorophorum</i> (Elbrächter & Schnepf) Hansen, Botes & Salas	-	AB367942
<i>Lep. chlorophorum</i>	Brest Bay (France)	KJ508396
<i>Lep. chlorophorum</i>	-	AM184122
<i>Lep. chlorophorum</i>	-	AB367942
<i>Lep. viride</i> Watanabe, Suda, Inouye, Sawaguchi & Chihara	South Africa	DQ499645
<i>Lep. viride</i>	Catalan Coast	KP790222
<i>Lep. viride</i>	Gulf of Naples (Italy)	FJ024698
<i>Margalefidinium polykrikoides</i> (Margalef) Gómez, Richlen, Anderson	Korean coasts	AY421781
<i>M. polykrikoides</i>	Lampung Bay (Indonesia)	LC438753
<i>M. fulvenscens</i> (Iwataki, Kawami & Matsuoka) Gómez, Richlen & Anderson	Hurum Bay (Indonesia)	AB288380
<i>M. fulvenscens</i>	Santa Monica Bay (USA)	HQ896315
<i>M. fulvenscens</i>	Jiaozhou Bay (China)	MN829244
<i>M. fulvenscens</i>	California (USA)	AB295051
<i>Pselodinium fusus</i> (Schütt) Gómez	Catalan Coast	KP790150
<i>P. fusus</i>	Douarnenez Bay (France)	KJ508394
<i>Polykrikos</i> cf. <i>schwartzii</i> Bütschli	-	AY526522
<i>Pol. Herdmaniae</i> Hoppenrath & Leander	-	DQ822481
<i>Pol. herdmaniae</i>	Catalan Coast	KP790225
<i>Pol. kofoidii</i> Chatton	-	DQ371291
<i>Pol. kofoidii</i>	-	EF613367
<i>Pol. lebouriae</i> Herdman	-	DQ975472

Continued

<i>Pol. lebourae</i>	-	FJ947044
<i>Pol. tanit</i> Reñé	Arenys Harbour, Catalonia (Spain)	KF806598
<i>Syndinium turbo</i> Chatton	-	DQ146404
<i>Takayama</i> cf. <i>pulchellum</i> (Larsen) Salas, Bolch & Hallegraeff	Xiamen Harbor (China)	AY800130
<i>T. acrotrocha</i> (Larsen) Salas, Bolch & Hallegraeff	Italy	HM067010
<i>T. acrotrocha</i>	Singapore	DQ656117
<i>T. helix</i> Salas, Bolch, Botes & Hallegraeff	North West Bay (Australia)	AY284950
<i>T</i> sp.	Quiberon Bay (France)	KJ508388
<i>T. tasmanica</i> Salas, Bolch & Hallegraeff	Tuggerah Lakes (Australia)	AY284949
<i>T. tasmanica</i>	East China Sea	KC485077
<i>T. tasmanica</i>	Catalan Coast	KP790230
<i>T. tuberculata</i> Salas	Southern Ocean (Australian)	EF469230
<i>Torquentidium</i> cf. <i>helix</i> (Pouchet) Shin, Li, Lee & Matsu	Tasmania, Nubeena (Australia)	KF245459
<i>Tor. convolutum</i> (Kofoed & Swezy) Shin, Li, Lee & Matsu	-	MF948385
<i>Tor. convolutum</i>	East China Sea	MF948386
<i>Tor. flavescens</i> (Kofoed & Swezy) Shin, Li, Lee & Matsuoka	-	MK733283
<i>Tor. flavescens</i>	-	MK733280
<i>Tor. pirum</i> (Schütt) Shin, Li, Lee & Matsuoka	Jiaozhou Bay (China)	MH469535