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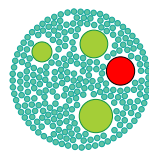
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Genetic diversity and population structure of *Karenia selliformis* (Dinophyceae): a global approach based on the 28S gene

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ABSTRACT

Karenia selliformis is a toxin-producing athecate dinoflagellate found worldwide. This species can form extensive harmful algal blooms (HABs) that have been associated with the mass mortality of marine fauna. On 24 August 2020, a dinoflagellate bloom was recorded in Bahía Todos Santos (BTS), Baja California, Mexico. During this high-biomass phytoplankton event, in which *K. selliformis* was detected and confirmed morphologically and by molecular methods, temperature 23.68°C, chlorophyll *a* > 10 mg m⁻³, and cell densities of 200–900 cells l⁻¹ were recorded. A total of 44 sequences (620 bp) of the ribosomal large subunit (LSU) and 37 sequences (570 bp) of the internal transcribed spacer (ITS1 + 5.8S + ITS2) region of rDNA, obtained from GenBank and generated in this study, were analysed. In total, 15 sites of the LSU were informative while 605 sites were invariant. Fifteen polymorphisms and eight ribotypes were identified, with a haplotypic diversity (*Hd*) of 0.647 and a nucleotide diversity (π) of 0.00558. In addition, 21 sites of the ITS were informative while 549 sites were invariant. For these 21 polymorphic sites, five ribotypes were identified, with an *Hd* value of 0.611 and π value of 0.01330. Our results demonstrate distinct ribotypes within *K. selliformis* and provide molecular evidence for a cryptic species complex across biogeographic provinces. This study highlights the need to reassess species boundaries within *Karenia* using integrative approaches.

HIGHLIGHTS

- Global genetic structure supports a *Karenia selliformis* cryptic complex.
- First bloom record in the Mexican Pacific under warm-water conditions.
- High genetic diversity contrasts with low morphological differentiation.

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KEYWORDS Cryptic complex; dinoflagellates; genetic variation; harmful algal bloom; ITS; *Karenia*; LSU; Pacific Ocean

Introduction

Harmful algal blooms (HABs) caused by dinoflagellates have traditionally been defined by high cell concentrations and the presence of toxins (Berdalet *et al.*, 2016; Oh *et al.*, 2023). However, certain HAB-forming species can adversely affect marine ecosystems and human health at low cell densities, which makes the identification of potentially toxic species crucial for monitoring water quality and safety (Fleming *et al.*, 2011; Lim *et al.*, 2023). Dinoflagellates, along with other unicellular phytoplankton, can exhibit limited expression and morphological differentiation, thereby making identifying and monitoring HAB-forming species challenging (Murray *et al.*, 2012; Lin, 2024). Thus, studies that evaluate the morphology and genetic variation of dinoflagellates are necessary to elucidate their evolutionary history and taxonomic relationships (Janouškovec *et al.*, 2017; Ott *et al.*, 2022).

The family Kareniaceae Bergholtz, Daugbjerg, Moestrup & Fernández-Tejedor comprises six genera of athecate dinoflagellates: *Asterodinium* Sournia; *Gertia* Takahashi, Benico, Lum & Iwataki; *Shimiella* Ok, Jeong, Lee & Noh; *Karlodinium* Larsen; *Karenia* Hansen & Moestrup; and *Takayama* Salas, Bolch, Botes & Hallegraeff (Guiry & Guiry, 2025). Among the genera of Kareniaceae, *Karenia* contains the highest number of harmful species (9 of 11 species are harmful), whereas there are fewer potentially harmful species of *Karlodinium* (6 of 16 species) and *Takayama* (1 of 7 species) (Hansen, 2021; Escarcega-Bata *et al.*, 2023; Cen *et al.*, 2024). Indeed, *Karenia* species produce various toxins, including brevetoxins, gymnocins (A and B), gymnodimins, haemolysins, polyunsaturated fatty acids and sterols (Seki *et al.*, 1995; Miles *et al.*, 2003; Chang *et al.*, 2006; Steidinger *et al.*, 2008; Brand *et al.*, 2012; Fowler *et al.*, 2015). Given that they have been reported to form HABs worldwide (Heil *et al.*, 2001; Guillou *et al.*, 2002; Munday *et al.*, 2004; Haywood *et al.*,

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2004, 2007; Iwataki *et al.*, 2022; Orlova *et al.*, 2022; Boudriga *et al.*, 2023; Wolny *et al.*, 2024), properly identifying *Karenia* species is of utmost importance.

Morphologically, *Karenia* species are characterized by the presence of a straight apical groove, which extends from the ventral to the dorsal portion of the epicone. They also have numerous chloroplasts that contain fucoxanthin and fucoxanthin derivatives (Daugbjerg *et al.*, 2000). *Karenia selliformis* Haywood, Steidinger & MacKenzie differs morphologically from other members of the genus in its elongated shape and the horizontal position of the nucleus, which is located in the hypocone. However, the recently described *Karenia hui* Lu, Cen, Wang & Moestrup also exhibits this character (Cen *et al.*, 2024), along with numerous chloroplasts (46–105) and a sulcal intrusion in the epicone, which is open in *K. selliformis*, *Karenia asterichroma* Salas, Bolch & Hallegraeff, *Karenia brevis* Gert Hansen & Moestrup, *Karenia papilionacea* Haywood & Steidinger and *K. hui* (Haywood *et al.*, 2004; Iwataki *et al.*, 2022; Cen *et al.*, 2024). While these morphological characters can be used to identify bloom-forming species (Iwataki *et al.*, 2022), underscoring their importance for HAB monitoring, they are insufficient when dealing with cryptic species.

The identification of cryptic species has been made possible by research into the genetic variation and structure of marine dinoflagellate populations. Nonetheless, the morphological characters exhibited by *Karenia* make identification difficult; thus, in many cases, genetic identification employing different molecular markers (e.g. 18S, 28S and internal transcribed spacer [ITS] regions), which collectively provide intra- and interspecific resolution, is required. Similarly, integrative taxonomy has produced accurate and coherent species classifications that have elucidated the evolution of these microorganisms through speciation processes and adaptive radiation (Bachvaroff *et al.*, 2014; Efimova *et al.*, 2014; Ott *et al.*, 2022).

While HABs of *K. selliformis* have been reported around the world in locations such as New Zealand (Haywood *et al.*, 2004), Mexico (Haywood *et al.*, 2007), Tunisia (Munday *et al.*, 2004; Boudriga *et al.*, 2023), Kuwait (Heil *et al.*, 2001), Chile (Guillou *et al.*, 2002), Japan (Iwataki *et al.*, 2022), Russia (Orlova *et al.*, 2022) and the USA (Wolny *et al.*, 2024), reports of this species on the Mexican Pacific coast are rare. Indeed, only one bloom of *K. longicanalis* has been reported for the region, which occurred in Acapulco Bay in 2019 (Escarcega-Bata *et al.*, 2024). No other blooms of potentially harmful *Karenia* species have been reported on the Mexican coast, although the presence of *K. selliformis* has been recorded several

times along the Mexican Pacific (Meave Del Castillo *et al.*, 2012; Escobar-Morales & Hernández-Becerril, 2015; Maciel-Baltazar, 2015; Almazán-Becerril *et al.*, 2016; Escarcega-Bata *et al.*, 2023). Despite the ecological importance of this species and its potential impact on human health and economic activities, molecular information, abundance estimates, and detailed morphological descriptions are lacking in the Mexican Pacific. In this context, the objective of the present study was to evaluate the global genetic variation of *K. selliformis* by analysing the 28S gene.

Materials and methods

Site description and sample collection

Bahía Todos Santos (BTS) is located on the northwest coast of the Baja California peninsula, Mexico (31°–40°–56'N, 116°36'–50'W; Fig. 1). The semi-enclosed BTS spans an area of ~180 km², exhibits an average depth of less than 50 m, and contains a submarine canyon (400 m depth) in the southwest area.

On 24 August 2020, phytoplankton sampling was carried out in BTS under conditions of an elevated chlorophyll *a* (Chl *a*) concentration and relatively high *K. selliformis* abundance (all data presented in this study are from this single sampling event). These environmental conditions enabled both the collection of morphologically and genetically representative cells of the species and a robust analysis of its intraspecific diversity at two sites (Fig. 1): Station 1 (31° 48'21.9"N, 116°36'58.9"W) and Station 2 (31° 50'28.9"N, 116°37'3"W). Biological material was collected via vertical casts with a phytoplankton net (20 µm mesh) at each sampling station. The samples were concentrated, placed in 250 ml plastic containers, and stored in a cooler for subsequent analysis in the laboratory. Additionally, samples were collected at depths of 1 m and 20 m in Niskin bottles (5 l) and transferred to amber bottles (250 ml). The Niskin samples were fixed *in situ* with 1% Lugol's acid and analysed under an inverted microscope to determine absolute abundance. Sampling was not conducted to characterize bloom dynamics or ecological impacts.

Microscopy

Prior to morphological analysis, each sample was filtered through a sieve (130 µm mesh) to remove zooplankton and other organisms. A 500 µl aliquot of each sample was observed under a brightfield optical microscope (DM750, Leica Microsystems GmbH, Wetzlar, Germany) at low magnification (100×). An epifluorescence microscope (DMLB, Leica Microsystems GmbH, Wetzlar, Germany) was also used (excitation: 480–490 nm;

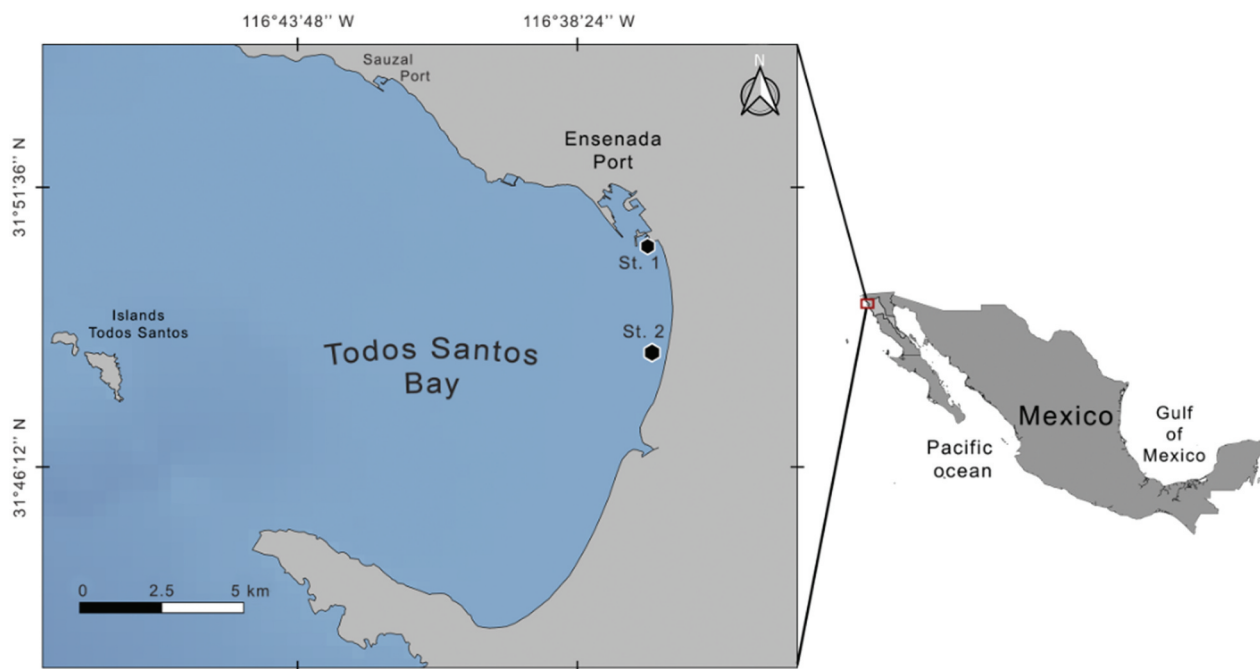


Fig. 1. Study area with sampling sites (indicated with Arabic numbers) in Bahía Todos Santos, Mexico.

emission: 520–540 nm). Cells of interest were isolated using the reduced micropipette technique described by Escarcega-Bata *et al.* (2021). Micrographs were taken with an EOS Rebel T6 camera (Canon Inc., Tokyo, Japan). Multiple cell planes were observed to identify morphological characters, including the shape and size of the epicone and hypocone, position and displacement of the cingulum and sulcus, shape and size of the apical groove, number of chloroplasts, and position and size of the nucleus. Cell abundance was assessed with the Utermöhl method (Lund *et al.*, 1958) using a 50 ml column and a brightfield inverted microscope (DM IRB, Leica Microsystems GmbH, Wetzlar, Germany).

Single-cell PCR and sequencing

The single-cell PCR protocol described by Hernández-Rosas *et al.* (2018) and Escarcega-Bata *et al.* (2021) was followed for the amplification of the large ribosomal subunit (LSU). The amplification conditions were: initial denaturation at 98°C for 3 min, 35 cycles at 95°C for 30s, 52°C for 30s and 72°C for 1 min, with a final extension at 72°C for 10 min. The LSU was amplified using the primer pairs D1R-F (5' -ACCCGCTGAATTTAAGCATA -3') and D3B-R (5' -TCGGAGGGAACCAGCTACTA -3') (Scholin *et al.*, 1994; Hansen *et al.*, 2000). PCR amplification was confirmed by electrophoresis using a 1.5% agarose gel (20 min at 90 V). Staining was performed with ethidium bromide. Gels were visualized using a UV light documentation system (FluorShot PRO, Shanghai Bio-Tech Co, Shanghai, China). PCR products were stored at –80°C and sent to Retrogen Inc.

(San Diego, California, USA) for purification and sequencing. The LSU sequences for both chains were determined using the referenced primers. No ITS sequences were obtained in the present study; however, analyses based on this marker were conducted using sequences retrieved from GenBank.

Sequence alignments and phylogenetic analyses

Sequences were aligned in BioEdit v. 7.0.5 (Hall, 1999) with those obtained from GenBank of the National Center for Biotechnology Information for a selected group of *Karenia* species. *Syndinium turbo* Chatton and *Hematodinium perezii* Chatton & Poisson formed the outgroup. Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference (BI) using the LSU and ITS markers. The ML analysis was performed in RAXML (Stamatakis, 2006). Support for each branch was determined from 1000 bootstrap replicates. The BI analysis was performed in MrBayes v. 3.2.2 (Ronquist *et al.*, 2012). The evolutionary model GTR + G (general time-reversible + gamma distribution) was selected based on the ML ratio test using the jModelTest software (Posada, 2008). Four Markov chain Monte Carlo chains were used, starting with a random tree and sampling every 1000 iterations for 1×10^8 generations; the first 25% of trees were discarded as chain warm-up. Pairwise distance values (p-distances), Jukes-Cantor + Gamma-corrected (LSU) and Kimura 2-corrected (ITS) distances were calculated in MEGA v. 11 (Tamura *et al.*, 2011).

Genetic variation, genetic diversity, structure and differentiation

The measures of genetic variation and diversity were estimated, and polymorphic sites were identified from a genetic matrix that included the complete set of LSU and ITS (ITS1 + 5.8S + ITS2) sequences using DnaSP v. 6.12.01 (Rozas et al. 2017). To evaluate the genetic structure of *K. selliformis*, gene flow measurements were obtained from estimates of the number of migrants (Nm) under different models and the fixation index (F_{ST}), and both parameters were obtained using DnaSP v. 6.12.01 (Rozas et al. 2017).

Ribotype analysis

An analysis of the sequences grouped by ribotype was performed in DnaSP v. 6.12.01 (Rozas et al. 2017). To evaluate the relationships of the LSU ribotypes and ITS (ITS1 + 5.8S + ITS2), a statistical parsimony network was constructed using PopArt v. 4.8.4 (Leigh & Bryant, 2015).

Processing of satellite-derived imagery

In situ measurements of hydrographic parameters (temperature, salinity and fluorescence) and satellite-derived Chl *a* concentration were used to complement the environmental context of the sampling effort and to support the interpretation of regional patterns in relation to population structure. To analyse sea surface temperature (SST) and the Chl *a* concentration, a dataset of satellite-derived images (1 km spatial resolution) was constructed for the west coast of North America (16–44°N, 140–100°W). The data were available in daily, 5-day, 15-day and

monthly composites. To enhance coverage and reliability, the daily products were merged from multiple satellite sensors. The Chl *a* data were obtained from the following sensors: CZCS, OCTS, SeaWiFS, MODIS-Terra/Aqua, MERIS, VIIRS-SNPP, OLCI-A/B, VIIRS-JPSS1 and SGLI. The SST data were merged from the MODIS-Terra/Aqua and VIIRS sensors. The dataset (HDF4 format) contained numerical values, and the full-resolution images employed Lambert equal-area projection mapping. The SST values were linearly scaled, while the Chl-*a* values were logarithmically scaled. To collect oceanographic data during each sampling date, an RBR CTD, which recorded temperature, conductivity, photosynthetically active radiation (PAR) (spectrometer, Ocean Optics, Orlando, Florida, USA) and fluorescence (fluorescence sensor, Turner Designs, San Jose, California, USA), was deployed. Finally, based on geographic data obtained from the literature, we created a distribution map to visualize the known global distribution of *K. selliformis* in Ocean Data View v. 5.1.7 (Schlitzer, 2019).

Results

Morphology

Karenia selliformis Haywood, Steidinger & MacKenzie (Haywood et al., 2004) (Fig. 2A–F). (Al-Kandari et al., 2009 (p. 157, plate 7A–D); Al-Kandari et al., 2025 (Fig. 3A–L), Al-Yamani & Saburova, 2019 (p. 113, plate 61); Cen et al., 2024 (Figs 1B1–B3, 2B1–B2); Haywood et al., 2004 (p. 172, Fig. 4A–J); Iwataki et al., 2022 (Figs 2A–P; 3A–C); Lassus et al., 2016 (p. 101, plate 32C); Mardones et al., 2020 (Fig. 3A–G); Orlova et al., 2022 (Fig. 8A–O)).

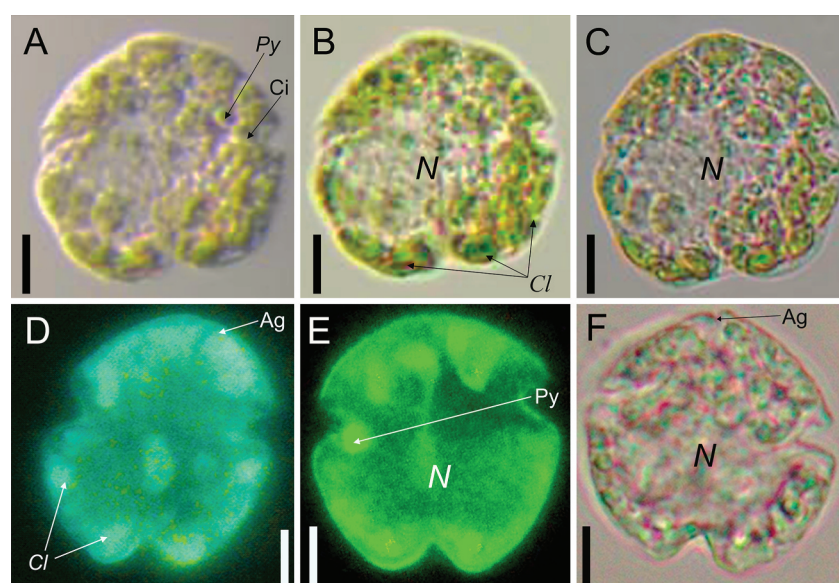


Fig. 2. Light and fluorescence micrographs of *Karenia selliformis* during bloom in 2020 in Bahía Todos Santos, Mexico. Cells in dorsal (A, C, E–F) ventral (B) and lateral (D) views. Apical groove (Ag), Cingulum (Ci), Chloroplast (Cl), Nucleus (N), Pyrenoids (Py). Scale bar: 10 μ m.

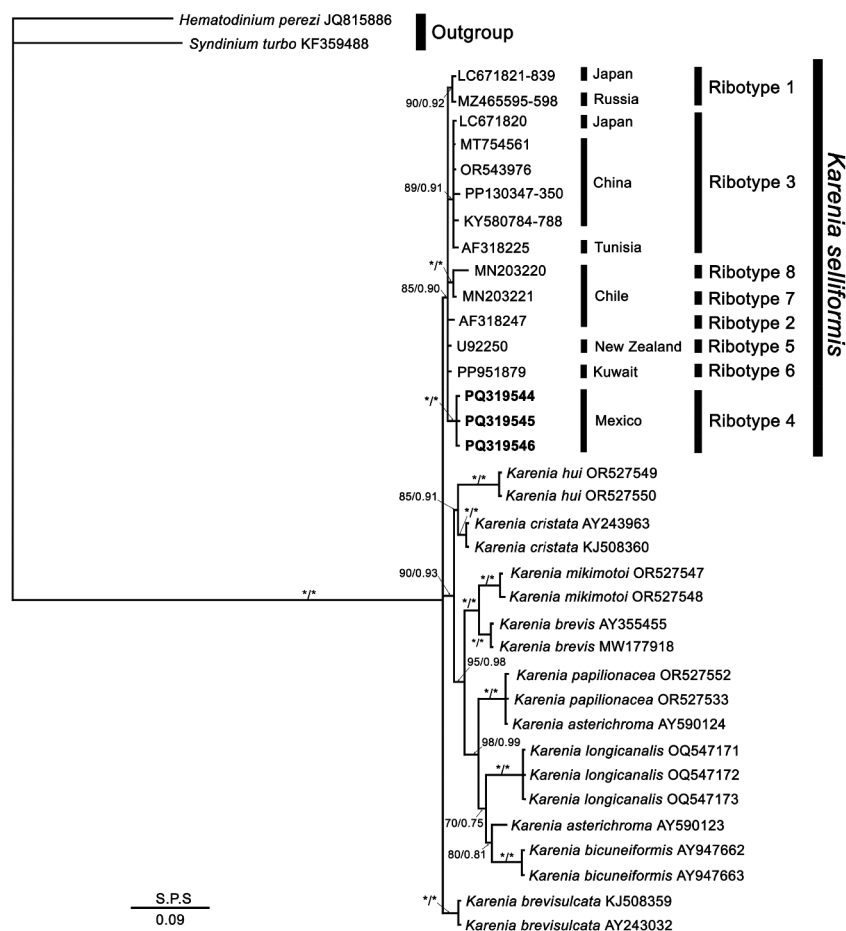


Fig. 3. Bayesian inference topology and maximum likelihood based on LSU rDNA (620 pb) sequences data from species of genus *Karenia* (Dinophyceae). BI (right) followed by ML bootstrap values (left) on branches. Asterisks indicate full support (ML/BI = 100/1). Sequences generated in this study are in bold. S.P.S. = substitutions per site.

DESCRIPTION: Cells are solitary and athecate, discoid, and dorsoventrally flattened (Fig. 2A–C), measuring 34–40 μm (mean = 36.7 ± 2.11 , $n = 30$) in length and 30–39 μm (mean = 34.3 ± 2.8 , $n = 45$) in width. Epicone is roundish and smaller than the hypocone (Fig. 2A–B, E). The hypocone is excavated in the centre and bilobed (Fig. 2D–F); the right lobe extends further than the left. The cingulum is slightly pre-median, narrow and deep (Fig. 2E–F). The apical groove is straight, narrow and deep and descends 1/5 of the cell length. The chloroplasts are numerous, peripheral and green to yellow in colour (Fig. 2A–B), with pyrenoids (Fig. 2E). The nucleus is large and spherical and is located in the central portion of the hypocone; it is clearly visible in dorsal and ventral views (Fig. 2B–C, E–F).

Phylogeny

In this study, three partial LSU rDNA sequences were obtained for *K. selliformis*. The final LSU alignment (620 bp) included 65 sequences. Phylogenetic reconstruction from the LSU dataset (with maximum phylogenetic support; ML/BI = 100/1) was performed using

sequences of *Karenia* species (Fig. 3). In the resulting topology, sequences of *K. selliformis* formed a monophyletic clade with good phylogenetic support (ML/BI = 85/0.90). In turn, seven subclades were recognized within this clade. The first subclade, with high phylogenetic support (ML/BI = 90/0.92), grouped sequences from Japan (LC671821–LC671839) and Russia (MZ465595–MZ465598). The second subclade, with high phylogenetic support (ML/BI = 89/0.91), grouped sequences from Japan (LC671820), China (MT754561, OR543976, PP130347–PP130350 and KY580784–KY580788) and Tunisia (AF318225). The third subclade, with maximum phylogenetic support (ML/BI = 100/1), grouped two sequences from Chile (MN203220 and MN203221). Subclades four, five and six were made up of sequences from Chile (AF218247), New Zealand (U92250) and Kuwait (PP951879), respectively. Finally, subclade seven consisted of sequences from Mexico, which were generated in this study (PQ319544–PQ319546).

The uncorrected genetic distances (p distances) obtained in the present study for LSU ranged from 0.16 to 2.10%, and the corrected distances (from the best model) ranged from 0.17 to 2.14% (Table 1). The uncorrected genetic distances (p distances) obtained

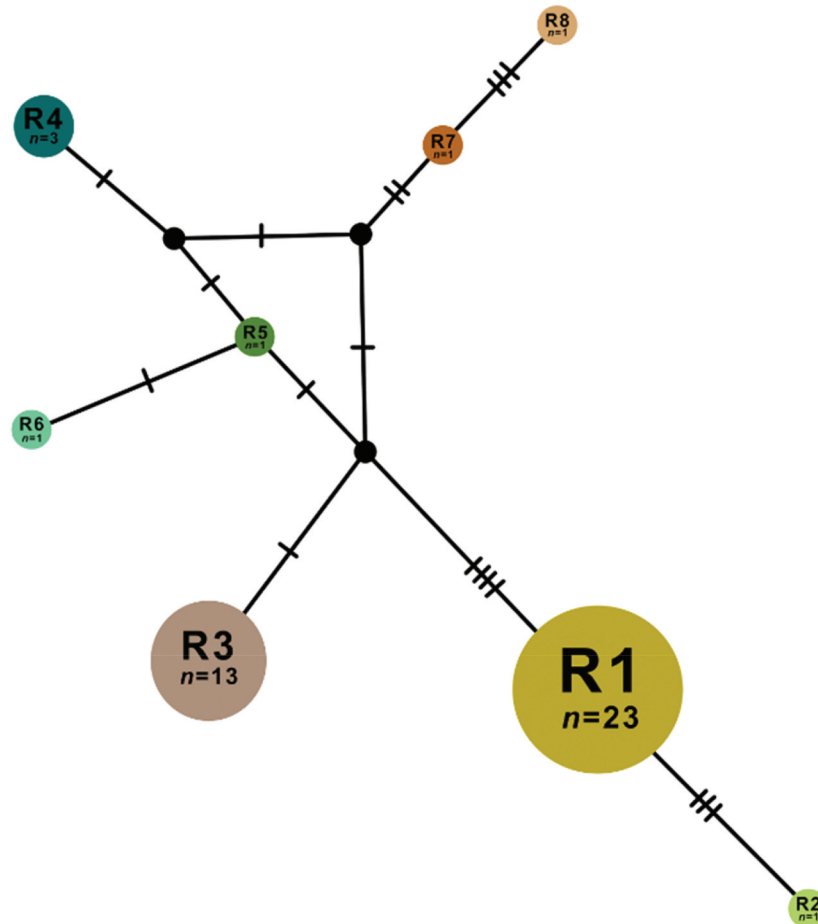


Fig. 4. Statistical parsimony network of the *Karenia selliformis* LSU. Lines represent parsimonious connections between haplotypes with probability >95%. Crossed bars represent the number of mutational steps between haplotypes. Each circle represents a ribotype and its size corresponds to its frequency. Filled circles represent a missing or unsampled ribotype; n = number of sequences belonging to each ribotype.

in the present study for ITS (ITS1 + 5.8S + ITS2) ranged from 0.52–2.80%, and the corrected distances (from the best model) ranged from 0.53–2.87% (Supplementary table S1).

Genetic variation and diversity

The LSU dataset of *K. selliformis* consisted of 44 sequences (620 bp). Fifteen sites were informative (Table 2), and 605 sites were invariant, representing

2.42% of the variation for that gene. Eight ribotypes (R1–R8; Table 3) were defined from the 15 polymorphic sites. The genetic diversity of the LSU in *K. selliformis* indicated 15 segregating sites (S), with a haplotypic diversity (*Hd*) of 0.647 and nucleotide diversity (π) of 0.00558.

Ribotype analysis

The statistical parsimony network for LSU revealed the presence of eight interconnected ribotypes: R1–

Table 1. Genetic divergence (percentage) between *Karenia selliformis* sequences of LSU.

	1	2	3	4	5	6	7	8
1. <i>Karenia selliformis</i> (R1)	–	0.49	0.81	1.14	0.81	0.97	1.14	1.62
2. <i>K. selliformis</i> (R2)	0.48	–	1.30	1.30	0.97	1.14	1.64	2.14
3. <i>K. selliformis</i> (R3)	0.80	1.29	–	0.65	0.32	0.48	0.65	0.81
4. <i>K. selliformis</i> (R4)	1.13	1.29	0.64	–	0.32	0.48	0.65	1.14
5. <i>K. selliformis</i> (R5)	0.80	0.96	0.32	0.32	–	0.17	0.65	1.14
6. <i>K. selliformis</i> (R6)	0.96	1.13	0.48	0.48	0.16	–	0.81	1.30
7. <i>K. selliformis</i> (R7)	1.13	1.61	0.64	0.64	0.64	0.80	–	0.50
8. <i>K. selliformis</i> (R8)	1.61	2.10	0.80	1.13	1.13	1.29	0.48	–

Uncorrected distances are shown below the diagonal, and Jukes–Cantor + Gamma distance values are located above the diagonal. Ribotypes are shown in parentheses. Letters highlighted in bold indicate data generated in this study.

Table 2. Proportion of variable sites in *Karenia selliformis* rDNA sequences for LSU haplotypes.

Ribotype	11	129	267	448	477	523	531	541	548	554	562	584	597	610	616
R1	T	T	C	G	C	G	T	T	C	T	T	C	T	G	A
R2	.	.	.	.	.	.	.	.	T	C	C	.	.	.	.
R3	.	C	.	.	T	A	C	.	.	.	.	.	.	.	G
R4	.	C	.	.	T	.	C	.	.	.	C	T	C	.	G
R5	.	C	.	.	T	.	C	.	.	.	C	.	.	.	G
R6	.	C	.	.	T	.	C	.	.	.	C	.	.	T	G
R7	C	C	.	.	T	.	C	C	.	.	.	T	.	.	G
R8	C	C	T	A	T	A	C	C	.	.	.	T	C	.	G

The dot indicates the same base as in the R1 ribotype sequence. The ribotype in bold indicates the one generated in this study.

Table 3. GenBank accession numbers associated with each of the resulting ribotypes for the 28S gene.

Ribotype	GenBank accession number
R1	LC671821-839, MZ465595-598
R2	AF318247
R3	MT754561, OR543976, PP130347-350, AF318225, KY580784-88, LC671820
R4	PQ319544-546
R5	U92250
R6	PP951879
R7	MN203221
R8	MN203220

R8 (Fig. 4). The ribotype that grouped the largest number of sequences was R1 ($n = 23$), followed by R3 ($n = 13$) and R4 ($n = 3$). The remaining ribotypes (R2, R5, R6, R7 and R8) were unique, each with only one sequence. The statistical parsimony network for ITS (ITS1 + 5.8S + ITS2) revealed the presence of four interconnected ribotypes: R1–R5 (Fig. 6). The ribotype that grouped the largest number of sequences was R1 ($n = 21$), followed by R3 ($n = 10$), R2 ($n = 3$), R4 ($n = 2$) and R5 ($n = 1$).

The ribotype network for LSU revealed a structure composed of different levels of mutational divergence. The lowest divergence was identified between ribotypes R5 and R6, which were separated by one mutational step. In contrast, the greatest differentiation was observed between ribotypes R2 and R8, with a separation of 13 mutational steps. In this sense, ribotypes R1 and R2, as well as R7 and R8, presented moderate differentiation, with three mutational steps between each pair of ribotypes. Between ribotypes R4 and R5, two mutational steps were identified, with the presence of an unsampled or absent intermediate ribotype acting as a transition. Similarly, between R3 and R5, a distance of two mutational steps was present, with another unsampled or absent ribotype acting as a transition between R3 and R5 and also between R1 and R5. In total, the network suggests the existence of three unsampled or absent ribotypes, the third connecting to the R7 ribotype.

Structure and differentiation

Using the LSU haplotypes and the model of Nei (1973), we obtained a migration rate (N_m) of 0.06 and a genetic differentiation (G_{ST}) value of 0.79595.

Based on the model of Nei (1982), the migration rates calculated from nucleotide sequence data were as follows: $N_m = 0.06$, $\Delta St = 0.00424$ and $\Gamma St = 0.80026$. Based on the model of Lynch & Crease (1990), with the Jukes and Cantor correction, the N_m value was 0.13, and the population subdivision (N_{ST}) value was 0.65348. Based on the model of Hudson *et al.* (1992), the N_m value was 0.13 and the fixation index (F_{ST}) value was 0.65496.

Sea surface temperature (SST) and the surface chlorophylla (Chl *a*) concentration

To provide environmental context to the genetic and morphological data, satellite-derived estimates of sea surface temperature (SST) and surface Chl *a* concentrations were examined for the period surrounding the sampling event (August 2020; Fig. 5) and compared across months. During August, SST values reached an average of 23.68°C, the highest recorded in the seasonal time series. This value contrasts with those of other months, suggesting potential thermal conditions associated with the presence of *K. selliformis*. The Chl *a* concentrations at the surface reached $>10 \text{ mg m}^{-3}$ near Station 2, coinciding with *K. selliformis* densities of 290 cells l^{-1} . These values support the sampling event as being representative of a high-biomass episode and, thus, useful for assessing intraspecific variability.

To provide environmental context for the morpho-molecular characterization of *K. selliformis*, satellite-derived SST and surface Chl *a* estimates were examined for the period surrounding the biological sampling event conducted in August 2020. These environmental data were used exclusively to characterize the regional background conditions during the sampling and were not intended to represent bloom dynamics or population-level temporal variability. Monthly satellite-derived composites from June to November 2020 and March 2021 were examined as independent temporal windows to contextualize the environmental conditions associated with the August 2020 sampling event. These datasets do not correspond to additional biological sampling periods but rather provide a broader environmental framework for interpreting the conditions under which the

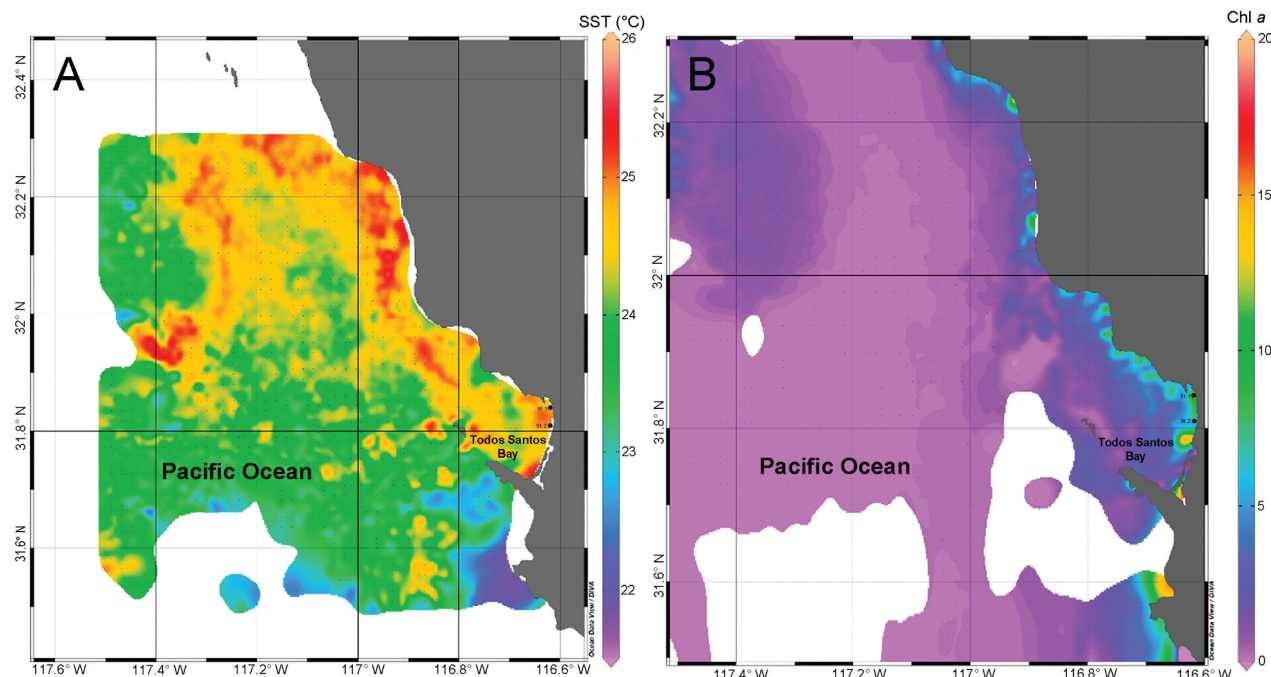


Fig. 5. Satellite-derived images of sea surface temperature (°C) (A) and chlorophyll *a* (mg m^{-3}) (B) at Bahía Todos Santos, Mexico in August 2020.

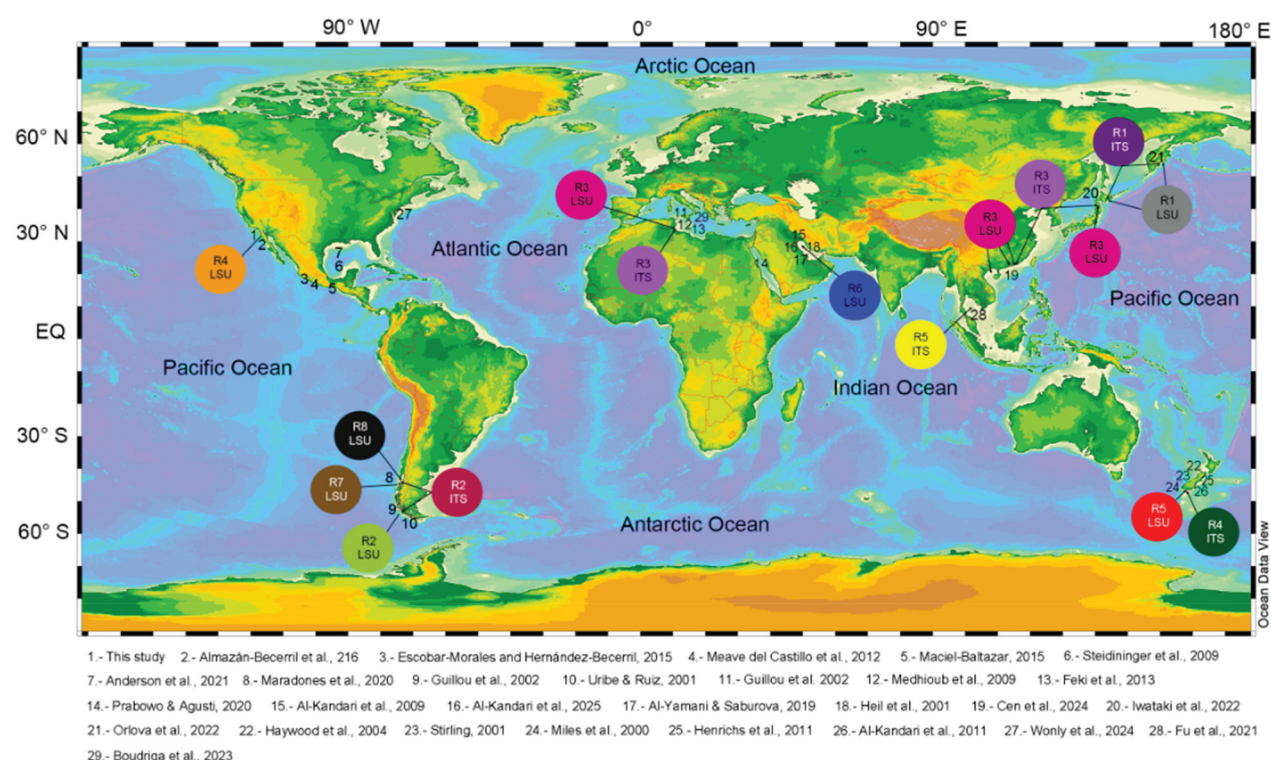


Fig. 6. Known geographic distribution of *Karenia selliformis* in coastal waters around the world. The circles indicate the ribotypes for LSU and ITS found. The citations below the map correspond to each record of the species.

samples were collected. Averages were calculated for both SST and Chl *a*. A one-way analysis of variance (ANOVA) was conducted for each variable, and significant differences were found between sampling dates. In particular, SST was significantly different across the analysed periods ($p < 0.05$). The Bonferroni multiple comparison test confirmed

statistically significant differences in all pairwise comparisons ($p < 2e^{-16}$ in most cases), indicating that SST varied significantly between periods. These findings were supported by the mean plot with confidence intervals, which shows the notable temporal variation in SST. The results suggest that SST underwent significant changes during the evaluated periods.

Importantly, August stood out among the study periods, given that it exhibited an average SST of 23.68°C (Fig. 5), which was significantly higher than those of the other periods.

The ANOVA revealed significant differences in Chl *a* between sampling dates, and the Bonferroni test showed that almost all group comparisons were statistically significant. Specifically, the mean Chl *a* concentration in March was much higher than that of the other periods (12.94 mg m⁻³). In contrast, the mean Chl *a* concentration in June showed the least difference from that of the other periods and was most similar to the mean Chl *a* concentration in August (3.26 mg m⁻³). The mean Chl *a* concentration in August was significantly different from that of July (5.54 mg m⁻³) when a dense *Lingulaulax polyedra* (Stein) Head, Mertens & Fensome bloom was present in BTS. Additionally, *Karenia* abundance values in August were 200 cells l⁻¹ and 900 cells l⁻¹ at 2 m and 15 m, respectively. Surface chlorophyll *a* concentrations exceeding 10 mg m⁻³ were detected near Station 2, indicating elevated total phytoplankton biomass. In contrast, *K. selliformis* was present at moderate cell densities (approximately 290 cells l⁻¹ at the surface). Cell abundances ranged from approximately 200 cells l⁻¹ at the surface to a maximum of ~900 cells l⁻¹ at 15 m depth during the sampling event. This pattern is consistent with previous descriptions of phytoplankton community structure in Bahía Todos Santos, where summer high-biomass events are typically characterized by multispecies assemblages rather than exhibiting strict monospecific dominance (González-Dávalos *et al.*, 2025).

Discussion

Morphology

Although the *Karenia* specimens collected in this study from BTS presented some of the morphological variations that have been documented in previous studies (Iwataki *et al.*, 2022), their shape and size coincided with those of *K. selliformis*. In our specimens, the epicone was rounded and small relative to the hypcone, which is consistent with previous descriptions of the species (Haywood *et al.*, 2004; Escobar-Morales & Hernández-Becerril, 2015; Iwataki *et al.*, 2022; Orlova *et al.*, 2022; Cen *et al.*, 2024; Wolny *et al.*, 2024). The specimens collected from BTS were larger (34–40 µm) compared with those reported by Haywood *et al.* (2004) in the original description (20–30 µm; New Zealand) and those reported by Escobar-Morales & Hernández-Becerril (2015) in the corresponding record from the Mexican Pacific (22–24 µm; coast of Colima). However, the cell dimensions of the specimens in the present study coincide with those reported by Iwataki *et al.* (2022) (35.3–43.6 µm; Hokkaido,

Japan). The variation in cell dimensions observed within the same species can be interpreted as an expression of phenotypic plasticity associated with the specific environmental conditions of each biogeographic province. In the Mexican Pacific, differences in cell length and width between the morphotype reported for the coast of Colima (Escobar-Morales & Hernández-Becerril, 2015) and that documented in this study are probably due to contrasts in climatic and oceanographic conditions for this portion of the eastern Pacific Ocean rather than taxonomic divergence. These results are consistent with previous evidence showing that the morphology of marine dinoflagellates can vary significantly in response to environmental gradients, which reinforces the need to interpret morphological characters within an ecological and biogeographic context (Anderson *et al.*, 2012; Röder *et al.*, 2012; Pranoy & Jagadish, 2024; Biswas & Saha, 2025).

Another relevant character was the presence of numerous small chloroplasts, which are a distinctive feature of *K. selliformis* (Haywood *et al.*, 2004; Mardones *et al.*, 2020; Iwataki *et al.*, 2022). It should be noted that the specimens collected from BTS showed numerous parietal chloroplasts containing pyrenoids, similar to those described by Orlova *et al.* (2022) and Cen *et al.* (2024). The presence or absence of chloroplasts is a useful criterion for differentiating species during blooms, despite the considerable variability in shape and number, given the reports of 18–105 chloroplasts per cell and even reports of cells without chloroplasts (Iwataki *et al.*, 2022). Nonetheless, a detailed analysis of chloroplast shape and size is needed to accurately compare this trait across species.

The nucleus of *K. selliformis* is known to be variable in shape, with reports of reniform, oval, elliptical, oblong and elongated morphologies (Haywood *et al.*, 2004; Escobar-Morales & Hernández-Becerril, 2015; Mardones *et al.*, 2020; Iwataki *et al.*, 2022; Orlova *et al.*, 2022; Cen *et al.*, 2024; Wolny *et al.*, 2024). In the present study, the nucleus was slightly elongated and spherical, but always located in the hypcone. The morphological differences between the previous reports of *K. selliformis* from the coast of Colima and BTS may be due to differences in environmental conditions between sites and the influence of the fixatives used to prepare the specimens, which can result in morphological changes.

Phylogeny

The dataset of partial LSU sequences analysed revealed different molecular entities (i.e. ribotypes; Table 3) distributed among different localities worldwide. It is important to note that each of these entities exhibited divergence at the intraspecific level

(Table 1), which is consistent with the data presented by Mardones *et al.* (2020). In addition, the topologies reported by Iwataki *et al.* (2022) and Orlova *et al.* (2022) were consistent with the one generated in this study; however, not all available sequences were considered in either study. Thus, the results presented by these authors do not reflect the current genetic variability of *K. selliformis*. Cen *et al.* (2024) provided *K. selliformis* sequences from the coast of China; however, the data used to construct the LSU topology were limited, as the clades obtained in that study were considered groups (two in total), similar to the topology presented by Iwataki *et al.* (2022) (two in total). Additionally, in both studies, sequences were excluded from these groups, which underestimated the genetic variability of this species. Recently, in the study by Al-Kandari *et al.* (2025), a single *K. selliformis* sequence was presented for the waters of Kuwait (PP951879); however, this corresponded to a new ribotype, which was closely related to the New Zealand ribotype (Figs 3, 4). A better approximation of this genetic variability was presented by Mardones *et al.* (2020). These authors pointed out the presence of at least three different phylotypes, although they suggested that greater efforts were needed to detect additional isolates from other places, including Kuwait and Mexico. A greater number of sequences from different regions of the world (i.e. China, Chile, Japan, Kuwait, Mexico [this study], New Zealand, Russia and Tunisia) were included in the present study, allowing us to detect at least eight ribotypes.

In the phylogenetic reconstruction based on the ITS dataset (ITS1 + 5.8S + ITS2; final alignment of 570 bp), the *Karenia selliformis* sequences formed a well-defined clade (Supplementary fig. S1). The resulting topology showed two main clades. Clade I, with maximum phylogenetic support (ML/Bi = 100/1), grouped sequences from Russia and Japan. Clade II, with good phylogenetic support (ML/Bi = 82/0.85), was subdivided into two subclades that included sequences from Chile, Thailand, New Zealand, Tunisia, Japan and China. Additionally, the dataset containing partial ITS sequences (ITS1 + 5.8S + ITS2) also revealed different ribotypes (Supplementary fig. S1; Supplementary tables S2–S3). It is important to note that each of these entities presented greater divergence at the intraspecific level (Supplementary table S1) than LSU. Mardones *et al.* (2020) indicated that this marker presented at least three phylotypes; however, these authors only used sequences from Chile, New Zealand and Tunisia, with the study revealing low genetic variability. Orlova *et al.* (2022) reported two phylotypes based on a dataset containing additional sequences from Russia; however, the arrangement of the phylotypes was not adequate, as the authors also considered sequences from Chile (AF318247 and MT808180)

within phylotype I, despite this being an independent phylotype. In the topology presented by Iwataki *et al.* (2022) and Cen *et al.* (2024), the phylotypes were treated as groups (two in total), however, no detailed discussion of this grouping was provided, and the variability observed in this topology was not considered. Although the present study did not generate sequences for ITS, the sequence data of previous studies (obtained from GenBank) support the existence of a cryptic complex within *K. selliformis*, suggesting the presence of a greater number of genetic entities worldwide (Supplementary fig. S1). Nonetheless, isolates from other sampling sites are needed to explore this possibility, given that current reports have been based on morphological descriptions.

Genetic variation and diversity

The genetic diversity analysis employing both the LSU and ITS markers supported the presence of genetically independent entities within *K. selliformis*. Although our analysis only included 44 LSU and 37 ITS sequences, it was possible to detect high interpopulation genetic variation within the species. This variation was observed through the formation of several genetic groups in the statistical parsimony network. With LSU, the eight ribotypes obtained formed at least four ribogroups, with ribotypes R1, R2 and R8 being the most notable in terms of the number of connections and unsampled ribotypes separating them, as well as the evident genetic distance between them (0.48% and 1.61%, respectively). On the other hand, with ITS, the five resulting ribotypes revealed at least three ribogroups (Supplementary fig. S2), of which the most representative were R1, R4 and R5, which reflected the large genetic distance between them (0.52% and 2.63%, respectively). In addition, the total coefficient of interpopulation genetic differentiation obtained with the two calculated indices (LSU: $G_{ST} = 0.79595$ and $F_{ST} = 0.65496$; ITS: $G_{ST} = 0.91751$ and $F_{ST} = 0.97650$) revealed a value close to 1 for both markers between ribogroups, which supports the existence of total structure between them (Excoffier & Lischer, 2010). The lack of genetic connection between populations was also supported by the number of migrants calculated for each index under different models (LSU: $N_m = 0.06–0.13$; ITS: $N_m = 0.01–0.03$). These results were also consistent with those of our phylogenetic analysis. The ribogroups defined in the resulting ribotype networks also formed an independent phylogenetic group, with good phylogenetic support.

Mardones *et al.* (2020) suggested that *K. selliformis*, as it is currently understood, could constitute a cryptic complex that requires further study. The authors based this conclusion on the

analysis of *K. selliformis* sequences from Chile, as well as sequences from China, New Zealand and Tunisia obtained from GenBank. The evidence provided by our genetic diversity analyses using two markers, which included sequences from Mexico (only with the LSU molecular marker), supports the idea that *K. selliformis*, which is currently understood to exhibit a worldwide distribution, is actually composed of a set of differentiated genetic entities that are morphologically indistinguishable based on the current evidence. This conclusion agrees with that of Mardones *et al.* (2020). Thus, *K. selliformis* constitutes an authentic complex of cryptic species. Moreover, although an analysis of genetic diversity is lacking, high interspecific differentiation was observed consistently in its phylogeny.

With both markers, the interspecific genetic distance values were higher between some ribogroups, such as ribotypes R1 (which grouped sequences from Japan and Russia) and R3 (which grouped sequences from China, Japan and Tunisia). With LSU, the relationships among the remaining ribotypes and major ribogroups suggest that the R4 ribogroup, which currently corresponds to Mexico and is supported by the calculated interspecific genetic distance values (0.32–1.30%), actually corresponds to an independent species that should be described as such. Unfortunately, in the present study, we do not have sufficient evidence to be able to definitively support this idea. Despite having conducted the necessary morphological characterization, we have not yet identified a set of characters that allows us to establish that the genetic group from Mexico is distinct from those of Chile, China, Japan, Kuwait, New Zealand, Russia and Tunisia (Guillou *et al.*, 2002; Haywood *et al.*, 2004; Mardones *et al.*, 2020; Iwataki *et al.*, 2022; Orlova *et al.*, 2022; Cen *et al.*, 2024; Al-Kandari *et al.*, 2025).

The ecological or biogeographic factors that may have resulted in the observed genetic isolation between different *K. selliformis* populations must be evaluated in detail in future studies. At present, the only apparent barrier between these populations is ecological, with one group distributed in temperate zones and another group distributed in tropical zones. However, among the *K. selliformis* populations of Chile, it is possible to detect three genetically independent groups, which correspond to the same region (with LSU). Thus, the mechanisms of genetic isolation cannot be established from the current evidence. Nonetheless, the high number of variable sites in LSU (620 bp analysed) and ITS (570 bp analysed) and the high number of sampled and unsampled ribotypes indicate that the process that gave rise to the genetic separation between the groups was not geologically recent. The high number of ribotypes and variable sites are indicators of stable populations

that underwent divergence many millions of years ago (Rogers & Harpending, 1992; Grant & Bowen, 1998).

Thus far, the evidence suggests that species discrimination within this complex should be carried out using all approaches, including geometric and numerical morphometry, and geographic and ecological data. Based on the results of this study and those of Mardones *et al.* (2020), cryptic genetic variants are responsible for the worldwide distribution of *K. selliformis*. Thus, detailed studies of populations are needed that focus on the toxic potential and environmental temperatures associated with this species.

Harmful algal blooms (HABs) of Karenia selliformis in the Pacific Ocean

Karenia selliformis has been reported in multiple regions of the Atlantic, Indian and Pacific Oceans (Fig. 6; Table 4); however, there are few records have documented its involvement in HAB events (Mardones *et al.*, 2020; Iwataki *et al.*, 2022; Orlova *et al.*, 2022). In the Pacific Ocean, extensive blooms of *K. selliformis* have been reported, associated with the mortality of invertebrates and vertebrates, particularly in temperate and subpolar systems, including the coast of Japan, Russia and southern Chile (Uribe & Ruiz, 2001; Mardones *et al.*, 2020; Iwataki *et al.*, 2022; Orlova *et al.*, 2022). These events highlight the ecological relevance of this species despite its comparatively infrequent bloom reports.

In this study, ‘*Karenia* bloom’ is used in an ecological and functional sense rather than as a strictly abundance-based definition. For *Karenia* species and other harmful dinoflagellates, ecological relevance and potential harmful effects are not necessarily associated with high cell densities. Accordingly, the August 2020 event is referred to as a *Karenia selliformis* bloom because it represents a high-biomass phytoplankton episode under favourable environmental conditions during which *K. selliformis* was consistently detected and confirmed morphologically and molecularly, despite not dominating total phytoplankton biomass. Along the western Pacific coast, a large-scale bloom was documented off Kamchatka, Russia, during September and October 2020 under SST values of ~9–13°C, with high cell densities and elevated biomass (Orlova *et al.*, 2022). Similarly, an extensive bloom along the coast of Hokkaido, Japan, occurred in September 2021 under relatively low to moderate SST conditions (8.1–19°C), reaching high cell abundances (Iwataki *et al.*, 2022). These events were associated with temperate to cold regional conditions and demonstrate that *K. selliformis* can proliferate under a broad thermal range. The ability of

Table 4. References for each *Karenia selliformis* record in coastal waters worldwide. GenBank accession numbers are included and water temperature for each site.

Country	Locality	Reference	Temperature (°C)	GenBank accession number	
				LSU	ITS
Mexico	Bahía Todos Santos, Baja California	This study	23.68	PQ319544-46	–
	Bahía de Acapulco, Guerrero	Almazán-Becerril <i>et al.</i> (2016)	–	–	–
	Costa de Chiapas	Meave Del Castillo <i>et al.</i> (2012)	19–31	–	–
	Costa de Colima	Maciel-Baltazar (2015)	–	–	–
		Escobar-Morales & Hernández-Becerril (2015)	–	–	–
	Gulf of Mexico	Anderson <i>et al.</i> (2021)	–	–	–
		Steidinger <i>et al.</i> (2009)	–	–	–
Japan	Aomori	Iwataki <i>et al.</i> (2022)	23	LC671820	LC671820
	Hokkaido		9.8–17.6	LC671821–839	LC671821–839
Russia	Kamchatka	Orlova <i>et al.</i> (2022)	9–13	MZ465595–98	MZ485599–602
Chile	Magellanic Fjords, Southern Chile	Uribe & Ruiz (2001)	13.5	–	–
	Canal Costa	Guillou <i>et al.</i> (2002)	–	AF318247	AF318247
	Patagonian Fjords	Mardones <i>et al.</i> (2020)	15*	MN203220–21	MT808180, MT804573
China	Tolo Harbour	Cen <i>et al.</i> (2024)	20*	MT754561, OR543976	OR527560–565
	Baguang Bay			PP130350	OR543987
	Beibu Gulf			PP130347–349	PP126483
New Zealand	Foveaux Strait, South Island	Al-Kandari <i>et al.</i> (2011)	19*	–	HM807324
		Haywood <i>et al.</i> (2004)	19*	U92250	–
		Henrichs <i>et al.</i> (2011)	19*	–	HM067008
		Stirling (2001)	–	–	–
		Miles <i>et al.</i> (2000)	–	–	–
Kuwait	Kuwait Bay	Al-Kandari <i>et al.</i> (2009)	–	–	–
		Al-Kandari <i>et al.</i> (2025)	23.5*	PP951879	–
		Al-Yamani and Saburova (2019)	–	–	–
		Heil <i>et al.</i> (2001)	26.9–28.6	–	–
Tunisia	Bay of Gabes	Guillou <i>et al.</i> (2002)	20*	AF318225	AF318225
	Sfax, Gulf of Gabes	Medhioub <i>et al.</i> (2009)	17–21*	–	–
	Gulf of Gabes	Feki <i>et al.</i> (2013)	–	–	–
	Harbour of Sfax	Boudriga <i>et al.</i> (2023)	27–30	–	–
Saudi Arabia	Thuwal, Central Red Sea	Prabowo and Agusti (2020)	23.06–32.60	–	–
USA	Delmarva Peninsula	Wolny <i>et al.</i> (2024)	15.5–20.8	–	–
Thailand	Gulf of Thailand	Fu <i>et al.</i> (2021)	29.6–30.8	–	MT160343

Note: *Growth temperature in culture.

this species to persist and disperse across distant regions has been linked to its life cycle characteristics, including the formation of resistant stages (Mardones *et al.*, 2020).

In the eastern Pacific, blooms of *K. selliformis* have been most frequently reported along the coast of Chile, particularly in fjord systems of southern Patagonia (Uribe & Ruiz, 2001; Mardones *et al.*, 2020). A bloom in 1999 was associated with SST values of ~13.5°C and resulted in notable marine fauna mortality (Uribe & Ruiz, 2001). Subsequent events, including those documented during the austral summer of 2018, also resulted in ecological impacts, although toxin analyses revealed the production of compounds distinct from classical gymnodimines (Brand *et al.*, 2012; Mardones *et al.*, 2020). Experimental studies have shown optimal growth of Chilean strains at moderate temperatures, emphasizing the physiological plasticity of the species (Mardones *et al.*, 2020). Genetic analyses identified at least three ribotypes based on the LSU marker (R2, R7–R8) and a single ribotype (R2) using the ITS region along the Chilean coast (Table 3, Supplementary table S3). Taken together, these findings highlight the Chilean region as an area of

particular ecological and evolutionary interest for future studies on *K. selliformis*, given its capacity to form blooms and produce bioactive compounds.

In contrast, records of *K. selliformis* blooms in the Mexican Pacific are scarce, despite the wide distribution of this species from the southern coast of the Gulf of Tehuantepec to the northwestern coast of the Baja California peninsula (Maciel-Baltazar, 2015; Almazán-Becerril *et al.*, 2016; Escarcega-Bata *et al.*, 2023). In Bahía Todos Santos (BTS), only a single record of *K. selliformis* had been previously published (Almazán-Becerril *et al.*, 2016), with no prior reports of blooms attributed to this species either in BTS or elsewhere in the Mexican Pacific. Therefore, the August 2020 event analysed in this study represents the first documented bloom of *K. selliformis* in BTS, with cell densities ranging from 200–900 cells l⁻¹. This bloom occurred under relatively warm surface waters (23.68°C) and elevated chlorophyll *a* concentrations (>10 mg m⁻³; Fig. 5). Compared to the typical seasonal SST range of the southern California Current system, this event represents the first *K. selliformis* bloom in the Pacific Ocean associated with relatively warm surface conditions.

Although blooms of *K. selliformis* in the Pacific have been more commonly associated with temperate

to cold waters, warm-water blooms have been documented elsewhere. For example, a massive bloom in Kuwait Bay (Arabian Sea) in September 1999 reached densities of up to 6×10^6 cells l^{-1} and was associated with SST ranging from 26.9–28.6°C, demonstrating that *K. selliformis* is capable of proliferating under warm conditions (Heil *et al.*, 2001). These observations support the view that temperature is a key, but not exclusive, driver of HAB development, as bloom formation is ultimately controlled by a combination of biological, chemical and physical processes (Smayd & Reynolds, 2001).

Notably, no apparent fish, bird or marine mammal mortalities were reported during the August 2020 event in BTS. However, the absence of visible impacts does not preclude potential harmful effects. The term ‘harmful’ is used here following current HAB definitions, which encompass toxic potential, sublethal effects and ecosystem-level risks, even at relatively low cell densities. Although harmful algal blooms (HABs) have traditionally been defined based on high cell concentrations and the presence of toxins, multiple studies have demonstrated that certain species can induce substantial ecological and health impacts even at relatively low abundances. For example, species of *Dinophysis* have been documented to cause shellfish toxicity events at densities as low as 100–200 cells l^{-1} (Panton & Purdie, 2022; Velasco-Senovilla *et al.*, 2023), frequently leading to harvest closures even in the absence of large-scale blooms (García-Mendoza *et al.*, 2014; Mardones *et al.*, 2020). These observations highlight that harmful effects are not necessarily linked to high biomass accumulation. Similarly, in the present study, *K. selliformis* did not reach high cell densities; however, its persistent presence and marked genetic diversity suggest that it may still represent a species of concern. Unlike other HAB-forming taxa, the effects of *K. selliformis* appear to be mediated by compounds that have not yet been fully characterized, including long-chain polyunsaturated fatty acids (PUFAs) and other cytotoxic substances (Chang *et al.*, 2006; Brand *et al.*, 2012). This mode of action complicates the identification of harmful effects based solely on cell abundance or the detection of well-known phycotoxins. In addition, field monitoring during and after the bloom was limited due to COVID-19 restrictions, preventing a comprehensive assessment of delayed or sublethal impacts.

The detection of *K. selliformis* under elevated Chl *a* conditions in BTS, together with its broad biogeographic distribution and substantial genetic divergence, reinforces the need to monitor this species as a persistent component of coastal phytoplankton communities. Although cell concentrations were moderate during August 2020, elevated Chl *a* concentrations during this period were largely associated with the presence of other phytoplankton taxa in the bay, including a concurrent *Lingulaulax*

polyedra bloom reported for July 2020, rather than being driven solely by *K. selliformis*. Nonetheless, the coexistence of this species under such conditions highlights its capacity to persist across a range of environmental scenarios. Furthermore, several HAB events worldwide have demonstrated that relatively low cell abundances can still result in harmful outcomes, particularly for species whose impacts are mediated by compounds other than well-characterized phycotoxins (Reguera *et al.*, 2014). In coastal regions with active aquaculture operations, the absence of publicly reported mortality events should therefore be interpreted with caution, as operational losses or sublethal effects are not always formally documented or disseminated. These considerations underscore the need for precautionary interpretations when evaluating the harmful potential of bloom-forming dinoflagellates (Basti *et al.*, 2021; Hallegraeff *et al.*, 2021; Díaz & Álvarez, 2023).

In recent years, research on ichthyotoxic and potentially harmful dinoflagellates has increased substantially, especially for species whose mechanisms of impact remain poorly understood. Within this context, the information presented here provides a valuable baseline documenting the occurrence of *K. selliformis* under specific environmental conditions in the southern California Current region. Such baseline data are essential for incorporating this species into early warning and monitoring frameworks, particularly in systems where multiple harmful taxa may co-occur. Accurate identification and molecular characterization of harmful or potentially harmful dinoflagellates play a central role in improving HAB surveillance and risk assessment. The morpho-molecular framework presented in this study contributes to the inclusion of *K. selliformis* in regional and national lists of potentially harmful species, enabling both academic institutions and aquaculture stakeholders to recognize this taxon when environmental conditions become favourable for its proliferation. Early molecular recognition facilitates timely evaluation of toxic potential during future bloom events, particularly given that the compounds associated with *K. selliformis* remain emergent and insufficiently characterized. Toxicity was not directly evaluated in this study; interpretations are therefore based on species identity, morphological and molecular confirmation, and environmental context. This study provides a reference framework for the accurate identification of cryptic taxa and supports future efforts to link genetic lineages with toxin production, physiological traits and ecological impacts.

The presence of pronounced genetic structure in *K. selliformis*, even in the absence of bloom conditions or observable ecological impacts, underscores the importance of integrating molecular tools into routine phytoplankton monitoring programs. Such approaches improve our understanding of cryptic speciation processes and the distribution

of genetically distinct populations within morphologically conserved taxa. Increasing evidence suggests that widely distributed dinoflagellate species with limited morphological differentiation may represent complexes of independent evolutionary lineages, emphasizing the taxonomic and ecological relevance of continued phylogeographic research.

Very few studies have evaluated the genetic variation of phytoplankton species. Given that phytoplankton are unicellular organisms in which morphological differentiation does not always reflect the underlying genetic diversity, and where conserved and convergent morphologies may occur, it is necessary to conduct studies that evaluate genetic variation to understand their evolutionary history, the processes involved in their origin, and their phylogenetic relationships. The results of this study support the phylogenetic independence of the different cryptic entities of the *K. selliformis* complex. Given that there are many taxonomic problems associated with classifying phytoplankton species, analyses are needed (i.e. phylogenetic, species delimitation, ecological, biogeographic and ultrastructural) to elucidate these important relationships. Following the study of Zamudio-Resendiz *et al.* (2022), the present study is the second to contribute an intrapopulation analysis based on morphological and molecular data for a phytoplankton species in Mexican waters.

Additionally, the persistent presence of *K. selliformis* and the genetic diversity revealed in this study highlight the relevance of monitoring this species, even in the absence of observable ecological impacts or toxin measurements. While this work does not assess ecological or toxicological traits, it lays a foundation for future investigations into the functional roles of divergent ribotypes. Given their bloom-forming potential and their evolutionary and ecological importance within coastal phytoplankton communities, monitoring and genetically characterizing *K. selliformis* and similar taxa should be prioritized in future research.

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Author contributions

A. Escarcega-Bata: Conceptualization, data curation, investigation, methodology, supervision, writing – review and

editing, writing – original draft; M. C. Ruiz-de la Torre: Funding acquisition, investigation, methodology, validation, writing – review and editing; E. C. Sánchez: Data curation, methodology, writing – review and editing; J. Arana-García: Validation, writing – review and editing; M. L. Núñez Resendiz: Investigation, writing – review and editing; B. Martín-Atienza: Data curation, investigation, methodology; A. Senties: Visualization, Writing – review and editing.

Disclosure statement

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Supplementary material

The following supplementary material is accessible via the Supplementary Content tab on the article's online page at <https://doi.org/10.1080/09670262.2026.2658160>

Supplementary table S1. Genetic divergence (percentage) between *Karenia selliformis* sequences of Internal Transcriber Spacer (ITS1 + 5.8S + ITS2). Uncorrected distances are shown below the diagonal, and Kimura 2 distance values are located above the diagonal. Ribotypes are shown in parentheses.

Supplementary table S2. Proportion of variable sites in *Karenia selliformis* rDNA sequences for Internal Transcriber Spacer (ITS1 + 5.8S + ITS2) haplotypes. The dot indicates the same base as in the R1 ribotype sequence.

Supplementary table S3. GenBank accession numbers associated with each of the resulting ribotypes for Internal Transcriber Spacer (ITS1 + 5.8S + ITS2).

Supplementary fig S1. Bayesian inference topology and maximum likelihood based on Internal Transcriber Spacer (ITS1 + 5.8S + ITS2) (570 pb) sequences data from *Karenia selliformis* (Dinophyceae). BI (right) followed by ML bootstrap values (left) on branches. Asterisks indicate full support (ML/BI = 100/1). S.P.S. = substitutions per site.

Supplementary fig S2. Statistical parsimony network of the *Karenia selliformis* Internal Transcriber Spacer (ITS1 + 5.8S + ITS2). Lines represent parsimonious connections between haplotypes as a probability greater than 95%. Crossed bars represent the number of mutational steps between haplotypes. Each circle represents a ribotype and its size corresponds to its frequency. Filled circles represent a missing or unsampled ribotype; *n* = number of sequences belonging to each ribotype.

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