Ciencias Marinas (2025), 51:e3498 **Research Article**

Morphological and molecular characterization of the free-living *Symbiodinium natans*-clade A (Dinophyceae) from Bahía de La Paz, Gulf of California

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Abstract. The genus *Symbiodinium* is a group of mostly endosymbiotic dinoflagellates, commonly known as zooxanthellae, which live in association with marine invertebrates and protists. The objective of the present study was the morphological and molecular identification of 2 strains of Symbiodiniales (SNCETMAR-1 and SNCETMAR-2) isolated from the anemone *Actinostella* sp. from Bahía de La Paz, Gulf of California. Culture identification was conducted via photomicroscopy, including epifluorescence and scanning electron microscopy. A sequence of the SNCETMAR-2 strain was obtained from the 28S rDNA region. The combination of methods allowed the strains to be identified as *Symbiodinium natans*-clade A. This taxon is a group of endosymbiotic and free-living organisms, which facilitated the cultivation of the strains in the laboratory. The life cycle of this group presented 2 phases, a motile and a non-motile phase in the coccoid state, the last stage where both sexual and asexual division occur. This study extends the reports of clade A of the family Symbiodiniaceae in the Gulf of California and provides valuable information for its culture, identification, and phylogenetic analysis.

Key words: *Actinostella*, endosymbiont, Gulf of California, life stages, *Symbiodinium*, zooxanthellae.

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Introduction

Symbiodinium Gert Hansen & Daugbjerg is a photosynthetic genus with opportunistic and free-living clades and subclades, which is distributed in tropical and subtropical areas (Takabayashi et al. 2012, Granados-Cifuentes et al. 2015, Hoppenrath et al. 2023). The genus name is considered neutral and means "living together" and "whirling" (LaJeunesse et al. 2018). These organisms have also been called *Zooxanthella* K. Brandt (Guiry and Andersen 2018). This refers to the mutualistic symbiosis shown by the alga, which in this case is the symbiosis of the dinoflagellate with invertebrates such as cnidarians, clams, copepods, flatworms, sponges, and some protozoans, such as foraminiferans, radiolarians, and ciliates (Baker 2003, Hirose et al. 2008, LaJeunesse et al. 2018). In addition, they are classified as cytobionts or intracellular symbionts (Taylor and Harrison 1983).

Species of the Symbiodiniaceae family (e.g., *Symbiodinium microadriaticum* LaJeunesse and *Symbiodinium pilosum* Trench & R.J. Blank ex La Jeunesse) show different photosynthetic responses under identical laboratory conditions. These photosynthetic differences explain the presence of this family in the diverse niches they can occupy (Iglesias-Prieto and Trench 1994). Host–symbiont specificity demonstrates the ability of a symbiont to be specialized for a certain host that inhabits a specific region; in addition, variations in said

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specificity are shown over wide geographic ranges. Physical (temperature and irradiance) and biological (host diversity and abundance and symbiont diversity and abundance) variables modulate symbiont–host specificity (Iglesias-Prieto et al. 1992, 2004; Iglesias-Prieto and Trench 1994, 1997). In addition to their role as symbionts, extracts of strains of the Symbiodiniaceae family isolated from the anemone *Stichodactyla haddoni* (Saville-Kent) have been reported to be cytotoxic to the crustacean *Artemia salina* (Linnaeus) (Bigham-Soostani et al. 2021).

The Symbiodiniaceae family is a diverse group comprising several generic and subgeneric clades, each consisting of an unknown number of subspecies or subclades (Iglesias-Prieto et al. 2004, LaJeunesse et al. 2018). Phylogenetic reconstructions with ribosomal (28S and 23S) and chloroplast (psbA) genes have revealed 9 clades (A to I) (Hirose et al. 2008, Hansen and Daugbjerg 2009, Pochon and Gates 2010, Yamashita and Koike 2013, LaJeunesse et al. 2018). New genera have been proposed with respect to phylogenetic groupings: *Symbiodinium* Gert Hansen & Daugbjerg (clade A), *Breviolum* J.E. Parkinson & LaJeunesse (clade B), *Cladocopium* (clade C), *Durusdinium* LaJeunesse (clade D), *Effrenium* LaJeunesse & H.J. Jeong (clade E), *Fugacium* LaJeunesse (clade F), and *Gerakladium* LaJeunesse (clade G) (LaJeunesse et al. 2018). Few clades show apparent morphological differences: clade B has cell sizes of 6 to 12 µm, and clade C has an apical groove called acrobase (LaJeunesse et al. 2018).

The strains analyzed in this study belong to clade A, corresponding to the oldest lineage of the Symbiodiniaceae family, which is made up of *S. microadriaticum*, *Symbiodinium necroappetens* LaJeunesse, S. Y. Lee, Knowlton & H. J. Jeong, *Symbiodinium tridacnidarum* S. Y. Lee, H. J. Jeong, N. S. Kang & LaJeunesse, *Symbiodinium natans* Gert Hansen & Daugbjerg, and *Symbiodinium linucheae* (Hansen and Daugbjerg 2009, LaJeunesse et al. 2018). Clade A includes free-living species, such as *S. pilosum* and *S. natans* (Yamashita and Koike 2013, LaJeunesse et al. 2015), in addition to groups with transient, opportunistic, free-living, and symbiotic forms that can be found associated with invertebrate hosts or protists (LaJeunesse et al. 2018). It can also inhabit different substrates, such as sand (Carlos et al. 1999, Hoppenrath et al. 2023), and form symbioses with soft corals (e.g., *Stereonephthya cundabiluensis* Verseveldt), stony corals, or hard corals, such as *Orbicella faveolata* (Ellis & Solander) (Hirose et al. 2008, Kemp et al. 2014).

MATERIALS AND METHODS

Isolation and growing conditions

Cells were isolated from the column of the solitary anemone *Actinostella* sp. (Hexacorallia: Actinaria: Actiniidae; Fig. 1) that was collected by Ana E Ramos-Santiago on August 9, 2018, at the CETMAR beach (4°08′39.2″ N, 110°20′41.0″ W; Fig. 2), Bahía de La Paz, in the southwestern Gulf of California, Mexico. Non-motile cells were isolated on an AXIO Vert.A1 inverted microscope (Carl Zeiss, Oberkochen, Germany), using capillaries with reduced tips. A progressive escalation was carried out until we obtained 25-mL cultures in flat 50-mL tubes. The SNCETMAR-1 and SNCETMAR-2 strains were kept in GSe medium with vermicompost extract (Bustillos-Guzmán et al. 2015) and K medium (Keller et al. 1987) modified with vermicompost extract at 34 salinity, 24 °C ± 1 °C temperature, continuous 150-µmol $E \cdot m^{-2} \cdot s^{-1}$ illumination, and a 12 h light:12 h dark cycle.

Morphological identification

Strain identification was done with the help of specialized literature (LaJeunesse et al. 2015, 2018; Hoppenrath et al. 2023). We evaluated morphometry and obtained micrographs of live cells on the Axio Vert.A1 inverted photonic microscope (Carl Zeiss) in bright field. In addition, we used the Axio Scope.A1 epifluorescence microscope (Carl Zeiss) with a 6-megapixel Axiocam 506 color digital camera to observe cells stained with the fluorescent marker DAPI (4',6-diamidino-2-phenylindole; Sigma) and visualize the position and size of the nucleus. To process samples for scanning electron microscopy (SEM), we followed the protocol used by Ramos-Santiago (2023) for naked dinoflagellates. Broadly, the methodology consisted of a prefixation process with 4% glutaraldehyde, postfixation with 2% osmium tetroxide (OsO_4), in-between washes to eliminate fixative residues, dehydration with an ethanol gradient (EtOH) at 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and twice at 99%, and a drying process of the samples with hexamethyldisilazane (HMDS). The samples were sent to the Academic Service of Scanning Electron Microscopy (SAMEB) of the *Instituto de Ciencias del Mar y Limnología* (ICMyL), *Universidad Nacional Autónoma de México* (UNAM), Mexico City, Mexico.

Molecular identification

DNA extraction and amplification

DNA extraction from the SNCETMAR-1 and SNCETMAR-2 strains was performed using the Quick-DNA Miniprep Plus Universal kit (Zymo Research, USA). For amplification, a mixture of 6.25 µL of DreamTaq Green PCR 2X (Thermo Scientific, USA), 2 µL of milli-Q H_2O, 1 µL of each primer (F and R), and 1 µL of DNA was used. Primers for 28S rDNA were used (Hosoi-Tanabe et al. 2006). Amplification conditions consisted of a denaturation step at 95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, at an annealing temperature of 52 °C for 1 min, 72 °C for 2 min, and, finally, an extension at 72 °C for 7 min. The PCR samples were verified by 1.5% agarose gel electrophoresis with 1X TBE Buffer. The image was digitized under the Bio-Rad ChemiDoc XRS+ system. The PCR products were sent for purification and sequencing to the company Macrogen (Seoul, South Korea). It is important to note that DNA extraction was successful in both strains; however, amplification was only achieved with the SNCETMAR-2 strain.

Phylogenetic analysis

The sequence was edited with the Sequencher program v. 4.1.4. Sequences from the GenBank database were used to perform a BLAST analysis of the consensus sequence (Forward + Reverse). Sequences from the different clades of the Symbiodiniaceae family from the 28S rDNA region

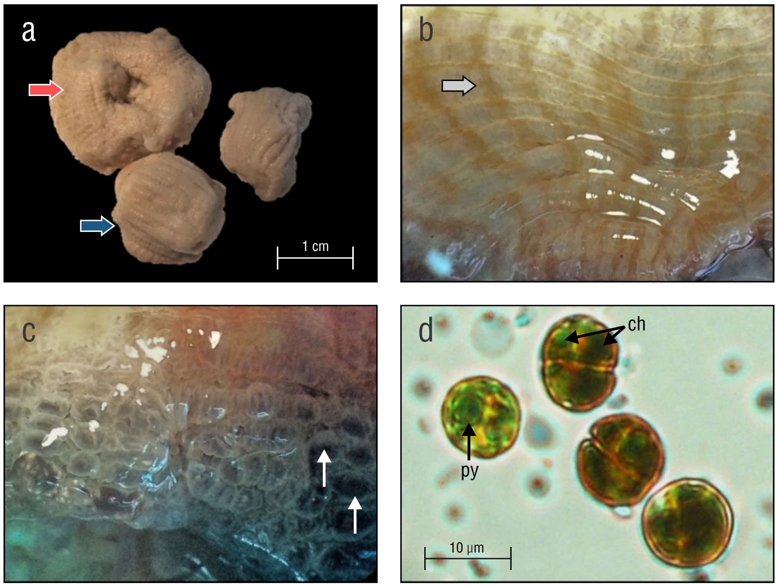

Figure 1. *Symbiodinium*-clade A and the host anemone *Actinostella* sp. Specimen of the anemone *Actinostella* sp. preserved in 4% formalin, the top view of the collar and oral disc with retracted tentacles is observed (red arrow); lateral view of the spine (blue arrow) (**a**). Details of live *Actinostella* sp., coloration is observed, lower detail of the collar with small aligned warts (gray arrow) (**b**). Lateral view of the spine with large and numerous warts (white arrows) (**c**). Freshly isolated cells of *Symbiodinium* sp. (SNCETMAR-2 strain), with green–yellow chloroplasts (ch) and pyrenoid (py) (black arrows) (**d**).

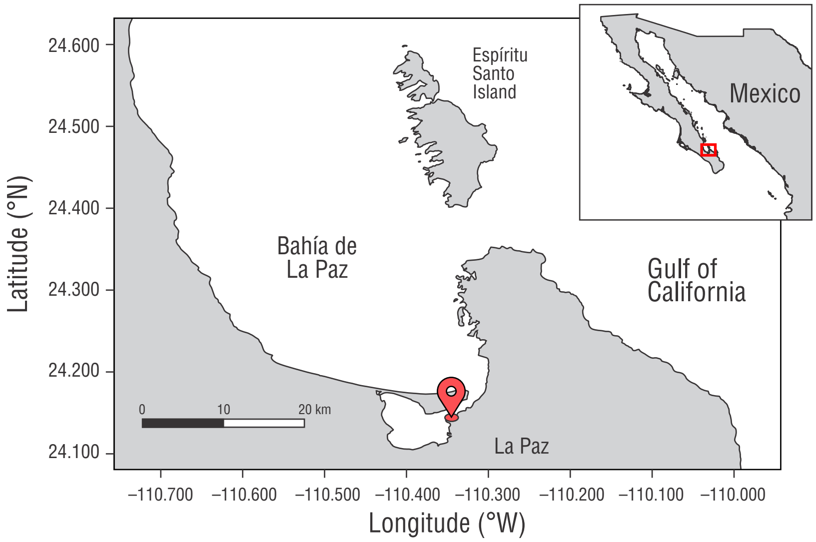

Figure 2. Location of Bahía de La Paz, Baja California Sur, Mexico. The red marker indicates the location of the CETMAR beach sampling site (24°08′39.2″ N, 110°20′41.0″ W).

were selected for the reconstruction of the phylogenetic trees. Sequence alignment was performed with the MEGA v. 10.0.5 program, with the MUSCLE alignment algorithm. The model that best described the nucleotide substitution rates of the sequences was selected with the JModel test v. 2.1.10, using the general time reversible (GTR) model for the phylogenetic analysis, which was done with the maximum likelihood (ML) and maximum parsimony (MP) algorithms in the MEGA v. 10.0.5 program and Bayesian inference (BI) with the MrBayes program v. 3.2.7a. Trees were constructed with a bootstrap of 1,000 replicates for ML and 3 × 106 generations for BI. The trees were edited with Photoshop CS6 v. 13.1.2.

Results

Symbiodinium-clade A morphology

Solitary cells, reddish in color, with rotating movement, which are distributed at the bottom and on the walls of the culture tube (Fig. 3a, b). Reticulated, brownish, and peripheral chloroplasts (Fig. 3c). The pyrenoid is located in the central area of the cell and has a diameter of 2.65 ± 0.52 µm (Fig. 3c–e). The mastigote (motile) cells are mushroom-shaped, laterally compressed, with the epicone slightly larger than the hypocone (Fig. 3c–i). Motile cells measure 10.93 ± 0.98 µm long and 7.79 ± 1.06 µm wide (mean ± SD; *n* = 30) in strain SNCETMAR-1 (Table S1), and 11.78 ± 1.05 µm long and 8.02 ± 1.38 µm wide (mean ± SD; *n* = 30) in the SNCETMAR-2 strain (Table S1). The nucleus is located in the anterior part of the cell (Fig. 3h, l). Another morphotype observed were coccoid non-motile cells, with a diameter of 12.01 ± 0.83 µm (mean ± SD; *n* = 30; Fig. 3j–l). The non-motile morphotype lacks morphological characteristics typical of motile cells.

We documented 2 life forms in both strains of *Symbiodinium*, motile cells and non-motile cells in the coccoid state, the latter being the dominant phase. Non-motile cells were observed in pre-division (Fig. 4a–c). Different phases of the division processes were documented: dyads with duplicated pyrenoids and the presence of one accumulation body and, on the other hand, cells with 2 accumulation bodies, which could indicate the fusion of 2 gametes (Fig. 4c–f). Cells forming clusters in triads and tetrads (Fig. 3g–j) and the presence of zygotes formed by fusion (syngamy) of gametes (Fig. 4k, l) were also observed. In coccoid cells in the process of reproduction, red-orange accumulation bodies were observed; these are probably lipids used as reserve substances or an eyespot (Fig. 5).

With SEM, the morphology of motile cells and the aforementioned reproductive stages were corroborated, as well as the cingulum, which is descending and displaced approximately one width of the cingulum (Fig. 6). We were unable to observe the arrangement of amphiesmal plates with this technique.

Molecular identification

We obtained a 514 bp sequence of the 28S rDNA region of strain SNCETMAR-2 (PP563703). In BLAST analyses performed in this study, the sequence showed 100% identity with the OQ449283 sequence, identified as *S. natans*. However, efforts were made to ensure that the selected sequences came from published studies or subsequent research in which their molecular identification has been corroborated. For the phylogenetic reconstruction of this taxonomic group, we included sequences from the 9 clades accepted for the Symbiodiniaceae family (Table 1). The sequence from this study clustered within clade A, with bootstrap support values of 91 and 97 for MP and ML, and posterior probability of 0.98 with BI (Fig. 7). The SNCETMAR-2 strain sequence formed a subclade with *S. natans* sequences with bootstrap support values between 70 and 89 (Fig. 7). We compared 2 *S. natans* sequences (AB704055 and AB704058) with the SNCETMAR-2 sequence; the AB704055 sequence showed a transition (C to T) at position 416, whereas in the AB704058 sequence there is a transition (A to G) at position 461. The analysis of the pairwise genetic divergences between the sequences identified as clade A revealed low values, ranging between 0.025 and 0.032. In contrast, divergences with sequences from other clades were greater than 0.141 (Table 2).

Discussion

The size of motile *S. natans*-clade A cells can range from 9.5 to 11.5 µm in length and 7.4 to 9 µm in width (Hansen and Daugbjerg 2009, LaJeunesse et al. 2015, Lee et al. 2015, Guiry and Guiry 2024), which agrees with what was reported in this study. The subclades *S. necroappetens* and *S. microadriaticum* show similar morphologies; nonetheless, these have cell sizes ranging from 9 to 12 µm (larger than *S. natans*) and from 7 to 10 µm (smaller than *S. natans*), respectively (LaJeunesse et al. 2015).

Currently, morphological and molecular identification within the Symbiodiniaceae family is complex. The number, shape, and position of the amphiesmal plates are used as descriptive morphological characteristics for the group; however, these are not sufficient for specific identification, as they may be similar or different within and between the groups that make up the clades (Lee et al. 2015, LaJeunesse et al. 2018). On the other hand, the morphological information of the amphiesmal plates in the motile stage (mastigote) of distantly related clades can yield different morphological information, as in clades A and E (Lee et al. 2015). The shape and size of the pyrenoid, chloroplasts, and nucleus have been used as morphological characters; however, in the Symbiodiniaceae family these characters are shared and cannot be used to differentiate clades or subclades (Lee et al. 2015). There is only

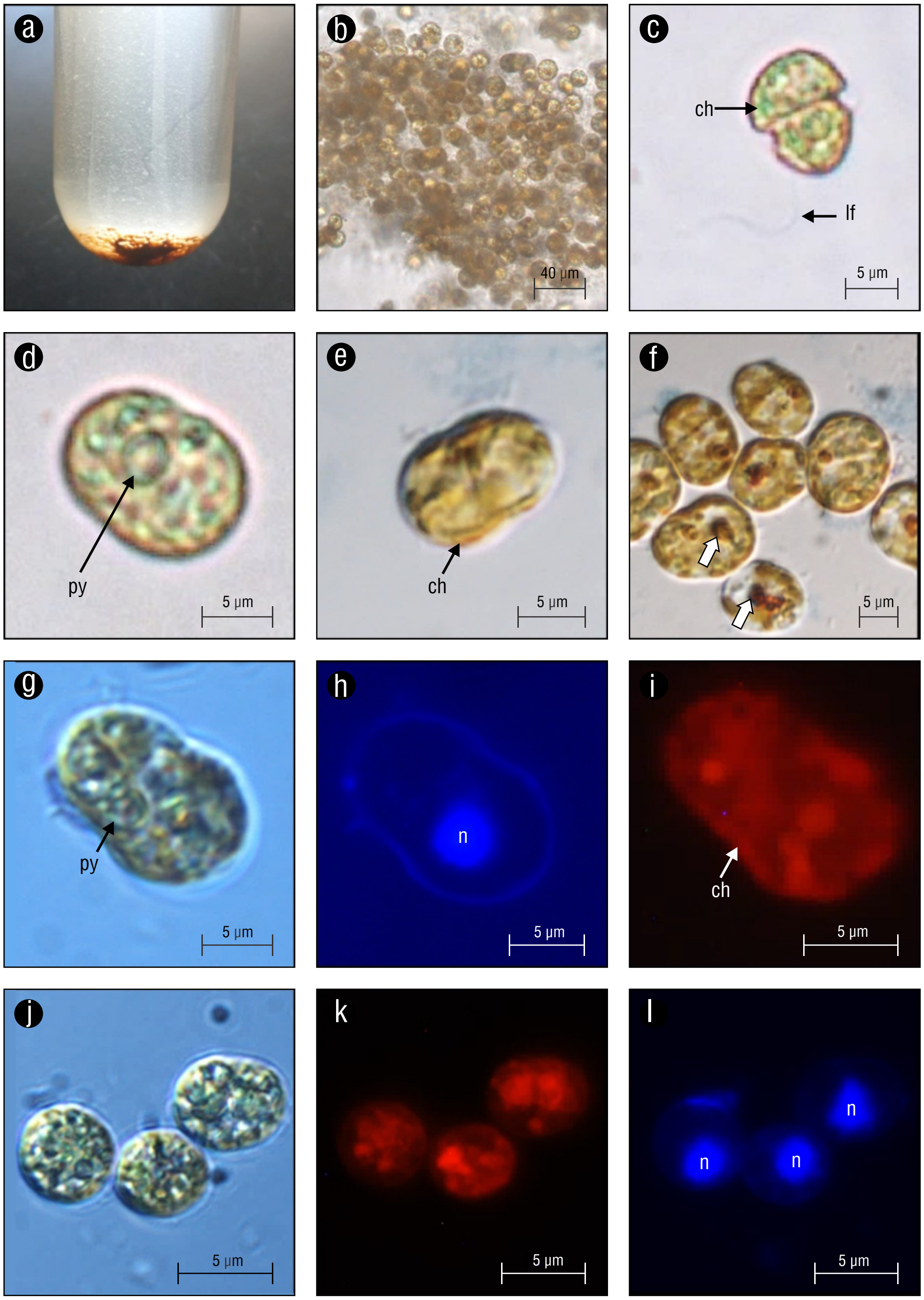

Figure 3. Subclade *Symbiodinium natans*-clade A from Bahía de La Paz. SNCETMAR-2 strain in GSe medium (**a**). SNCETMAR-1 strain (**c**–**f**). SNCETMAR-2 strain (**b**, **g**–**l**). Coccoid cells, non-motile, grouped together by mucilage (**b**). Ventral view of motile cells with the typical mushroom shape (**c**–**e**). Rounded and elongated reproductive cysts, they show reserve substances or an eye spot (white arrows) (**f**). Non-motile mushroom-shaped cells and coccoid state (**g**, **j**). DAPI staining shows the position and shape of the nucleus (n) in the 2 phases of the life cycle of *Symbiodinium* sp. (**h**, **l**). Epifluorescence images of chloroplasts (**i**, **k**). ch = chloroplasts, py = pyrenoid, lf = longitudinal flagellum.

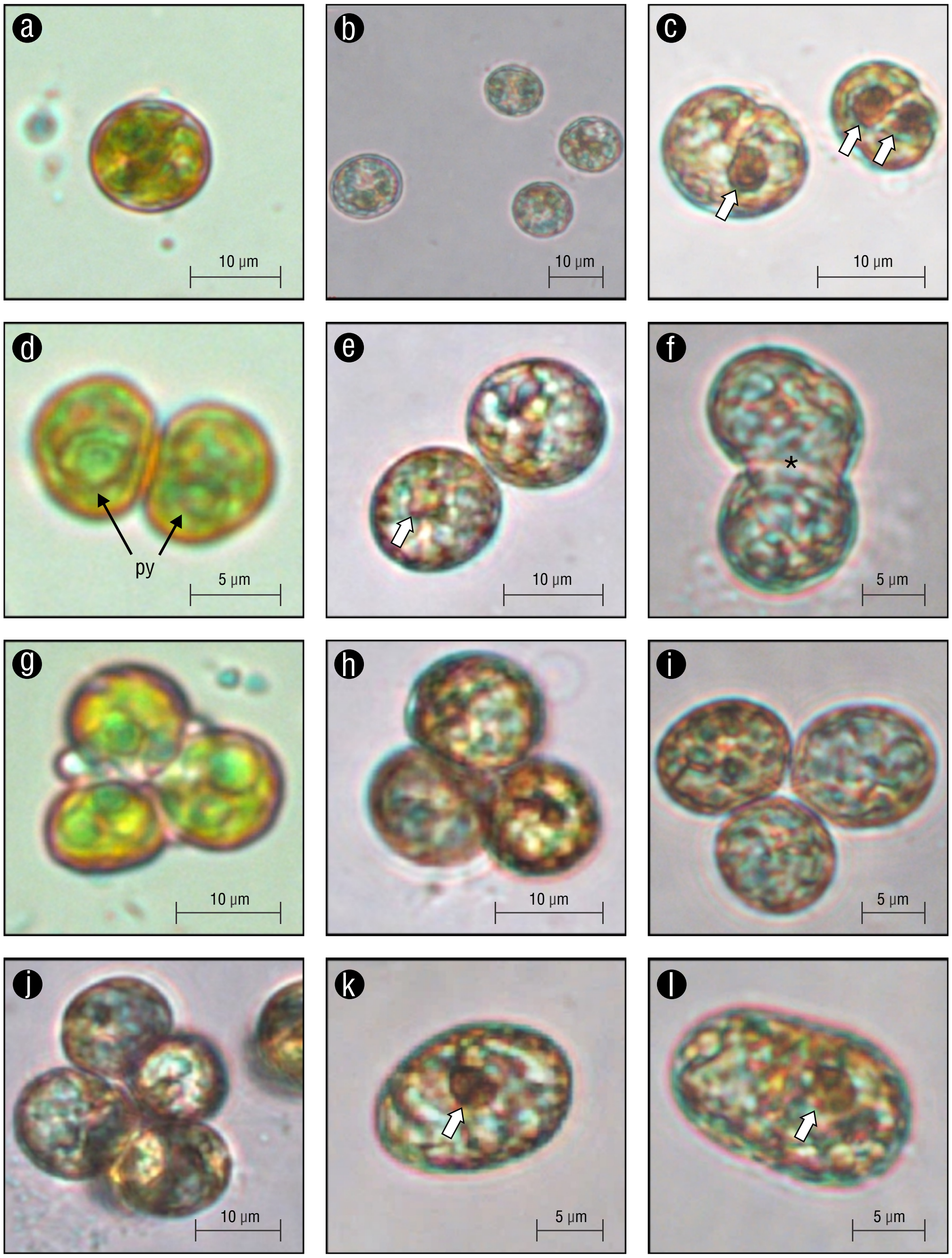

Figure 4. *Symbiodinium natans*-clade A cysts from Bahía de La Paz. SNCETMAR-1 strain (**e**, **h**, **j**–**l**). SNCETMAR-2 strain (**a**, **b**, **c**, **d**, **f**, **g**, **i**). Non-motile predivision cyst or coccoid stage (**a**–**c**). Bicellular division cyst (dyad phase) (**d**–**f**). Meiosis I, triad phase (**g**–**i**). Meiosis II, tetrad phase, where 4 haploid cells are formed (**j**). Zygote (diploid) (**k**, **l**). White arrow with margin = reserve substances (lipids) or an eyespot, asterisk (*) = binary fission process, py = pyrenoid.

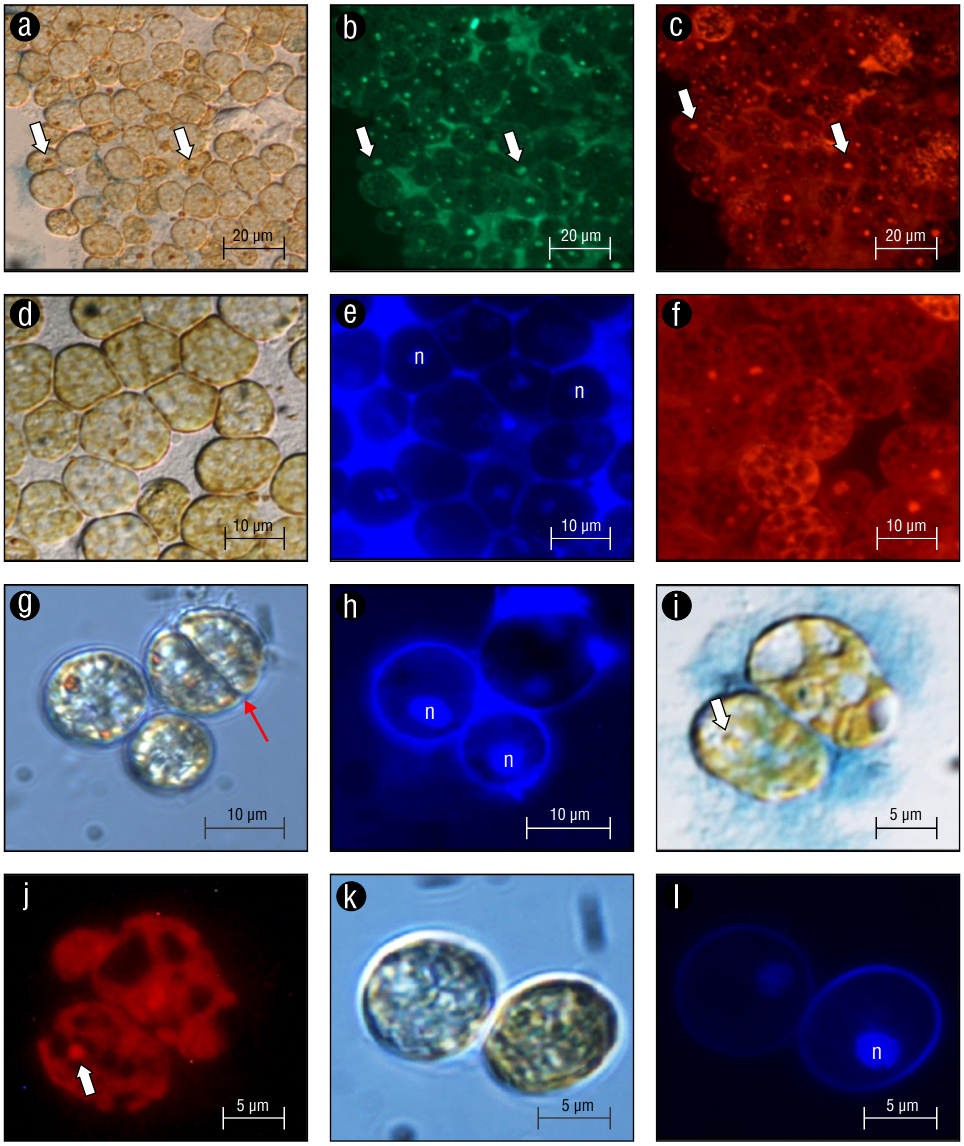

Figure 5. *Symbiodinium*-clade A cells (SNCETMAR-2 strain) in epifluorescence microscope. Optical micrographs of non-motile cysts (**a**, **d**, **g**, **i**, **k**). Chloroplast autofluorescence in ventral view (**b**, **c**, **f**, **j**). DAPI staining of the nucleus (n) (**e**, **h**, **l**). White arrow with margin = lipids as storage products or eyespot, red arrow = division plane.

one autapomorphy character, which is the reduction of a pronounced elongated apical vesicle (acrobase or apical groove), a characteristic observed in clade C (LaJeunesse et al. 2018). Nevertheless, members belonging to clade A lack this character, so this structure is not relevant in the strains analyzed in this study.

The morphology of the group can vary depending on the phase, with an observable coccoid phase, typical of asexual reproduction, and ellipsoidal to mushroom shapes (motile phase). Coccoid cells can measure 8–10 µm in diameter (LaJeunesse et al. 2018), whereas motile cells can average 6–12 µm in length (Hansen and Daugbjerg 2009, LaJeunesse et al. 2018). In culture, cells were observed forming groups in triads and tetrads, corresponding to meiosis I and late meiosis II of sexual reproduction, respectively. Figueroa et al. (2021) reported these reproductive phases in a *Symbiodinium* strain of clade C (*Cladocopium latusorum* Turnham, Sampayo & LaJeunesse) from Moorea in French Polynesia, South Pacific Ocean.

Phylogenetic reconstructions with ribosomal (28S and 23S) and chloroplast (psbA) genes reveal 9 evolutionarily divergent clades (A to I) (*see* Table 2, Pochon and Gates 2010, LaJeunesse et al. 2018). The high and low pairwise genetic divergence values in this study were very similar to those of Pochon and Gates (2010) because we included some long subunit sequences identified by these authors as clade E (AF060899), clade F3 (AJ830916, AJ291525), clade G (AJ291539), clade H (AJ291513), and clade I

(FN561562). The different clades of the Symbiodiniaceae family have different genetic, physiological, and ecological attributes; therefore, these clades can be subdivided into an unknown number of phylospecies (Hirose et al. 2008, De Palmas et al. 2015, LaJeunesse et al. 2018). The 9 clades accepted for the group were obtained with the analysis of the 28S region. The sequence from this study had greater genetic affinity with sequences from clade A; specifically, with sequences from the free-living species *S. natans* from Japan and Spain (Yamashita and Koike 2013, LaJeunesse et al. 2015). Although this clade shows high genetic diversity (around 15 subclades), the 28S marker helped us identify clade A and the presence of 4 subclades within it; however, a highly variable marker such as the ITS set has been observed to help identify specificities with respect to the environments where these microorganisms develop (pelagic and benthic) and their symbiotic associations to understand the divergence within the same clade or group (Mordret et al. 2016).

Clade A is widely distributed in the Atlantic, Pacific, and Indian oceans and in the Red Sea. Nevertheless, some subclades may be limited to certain ocean basins, for example, the Caribbean Sea, which has the highest number of reports (LaJeunesse et al. 2015). The clade has been reported in Callao Salvaje, Tenerife, in the Canary Islands (Hansen and Daugbjerg 2009, Guiry and Guiry 2024); Japan and Hawaii (Carlos et al. 1999, Hirose et al. 2008, Yamashita and Koike 2013); Florida Keys in the USA (Lee et al. 2015); and Puerto Morelos in the Mexican Caribbean (Kemp et al. 2014).

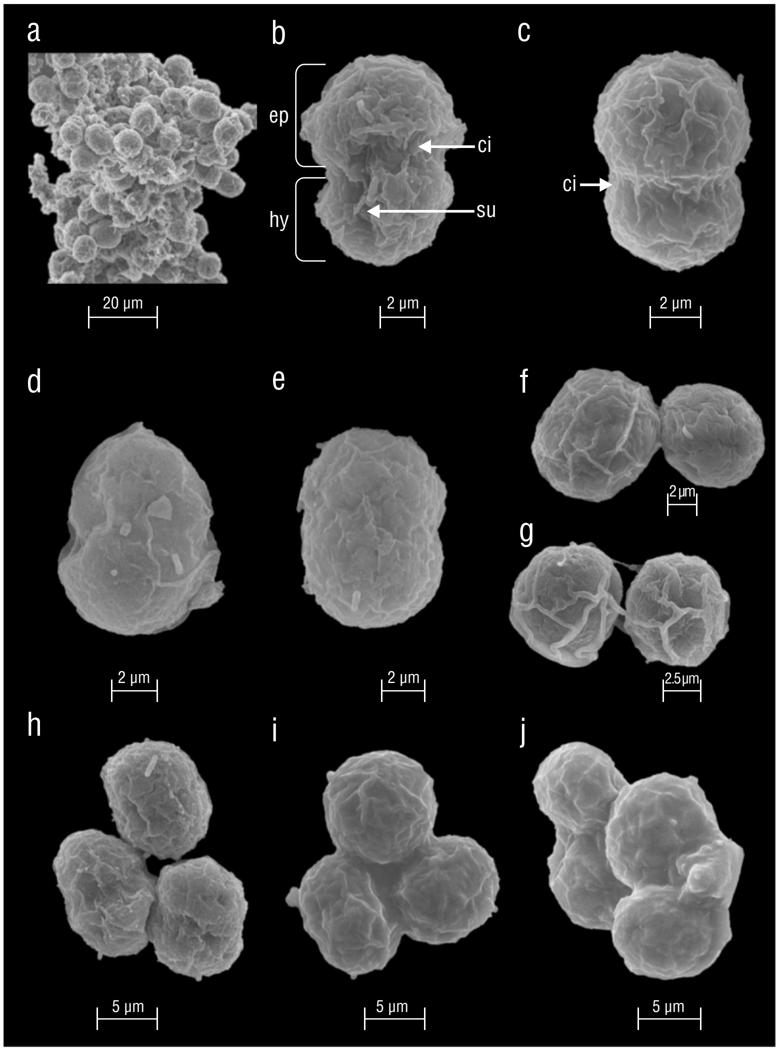

Figure 6. Vegetative cells of *Symbiodinium natans*-clade A observed under a scanning electron microscope. SNCETMAR-1 strain (**b**, **c**, **g**, **h**). SNCETMAR-2 strain (**a**, **d**, **e**, **f**, **i**, **j**). Vegetative cysts, the dominant phase of *Symbiodinium* sp. (**a**). Ventral view (**b**). Dorsal view (**c**, **e**). Zoosporangium with cingulum housing the transverse flagellum (**d**). Binary fission process (**f**, **g**). Meiosis I (**h**, **i**). Meiosis II (**j**). ci = cingulum, su = sulcus, ep = epicone, hy = hypocone.

Table 1. Sequences of the Symbiodiniaceae family included in the phylogenetic analysis of the 28S rDNA region and *Gymnodinium catenatum*, which was used as an outgroup.

GenBank ID	Taxon	Strain	Isolation origin	Site	Reference
PP563703	*Symbiodinium natans*	SNCETMAR-2	*Actinostella* sp.	Bahía de La Paz, Mexico	This study
AB704058	*Symbiodinium natans*	FGS-D6-Sy	Sand	Gahi Island, Okinawa, Japan	Yamashita and Koike (2013)
AB704055	*Symbiodinium natans*	GTP-A6-Sy	Tide pool	Gahi Island, Okinawa, Japan	Yamashita and Koike (2013)
EU315917	*Symbiodinium natans*	CAT2393	ND	Tenerife, Spain	Hansen and Daugbjerg (2009)
KT634312	*Symbiodinium* sp.	zs12xd	*Zoanthus* sp.	Tavernier, Florida Keys, USA	Graham et al. (2015)
LK934674	*Symbiodinium* sp.	CCMP2456	*Alveopora japonica* Eguchi, 1965	Jeju, South Korea	Not published
AB778578	*Symbiodinium* sp.	KMG004-a-02	*Pteraeolidia ianthina* (Angas, 1864)	Chiba, Japan	Yorifuji et al. (2015)
KF740671	*Symbiodinium pilosum*	rt-185	*Zoanthus sociatus* (Ellis, 1768)	Jamaica	Jeong et al. (2014)
ON263282	*Symbiodinium necroappetens*	A13	ND	ND	Not published
MK692538	*Symbiodinium linucheae*	SSA01	ND	ND	Not published
KM972549	*Symbiodinium microadriaticum*	rt-061	*Cassiopea xamachana* Bigelow, 1892	Florida, USA	Lee et al. (2015)
LK934669	*Symbiodinium microadriaticum*	CCMP2467	*Alveopora japonica*	Jeju, South Korea	Not published
KT149349	*Symbiodinium minutum*	Mf1.05b	ND	Caribbean	Parkinson et al. (2015)
LK934670	*Symbiodinium minutum*	CCMP830	*Alveopora japonica*	Jeju, South Korea	Not published
KT149351	*Symbiodinium psygmophilum*	PurPFlex	ND	Caribbean	Parkinson et al. (2015)
KF364606	*Symbiodinium* sp.	RCC 1521	Surface net trawl	Blanes, Mediterranean Sea	Jeong et al. (2014)
AF060899	*Gymnodinium varians*	CCMP 421	ND	ND	Wilcox (1998)
AJ291539	*Symbiodinium* sp.	1584	*Amphisorus* sp.	Guam	Pawlowski et al. (2001)

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Table 1 (*continued*)

GenBank ID	Taxon	Strain	Isolation origin	Site	Reference
AJ291536	*Symbiodinium* sp.	1643	*Marginopora*	Luminao, Guam	Pawlowski et al. (2001)
KF740689	*Symbiodinium* sp.	MTB4	*Orbicella faveolata* (Ellis & Solander, 1786)	USA	Jeong et al. (2014)
KF740686	*Symbiodinium* sp.	Tha09-57	*Oulastrea crispata* (Lamarck, 1816)	Thailand	Jeong et al. (2014)
FN561562	*Symbiodinium* sp.	nr-i4	Foraminífera subfamily Soritinae Ehrenberg	Oahu, Hawaii, USA	Pochon and Gates (2010)
AJ291513	*Symbiodinium* sp.	751	*Sorites* sp.	Florida, USA	Pawlowski et al. (2001)
KF740682	*Symbiodinium* sp.	Zam03-3m-83	*Millepora* Linnaeus, 1758	Japan	Jeong et al. (2014)
FJ529530	*Symbiodinium* sp.	C3nt	*Seriatopora hystrix* Dana, 1846	Australia	Sampayo et al. (2009)
AJ830916	*Symbiodinium* sp.	MS26_5244x	*Amphisorus hemprichii* Ehrenberg, 1965	Guam Island	Not published
AJ291525	*Symbiodinium* sp.	1635	*Marginopora* sp.	Piti, Guam	Pawlowski et al. (2001)
KU359161	*Symbiodinium kawagutii*	symka	ND	ND	Not published
AF360577	*Symbiodinium kawagutii*	Clade C	*Montipora verrucosa* (Lamarck, 1816)	Hawaii, USA	Santos et al. (2001)
SD	*Gymnodinium catenatum*	BAPAZ 16	ND	Bahía de La Paz, BCS, Mexico	Not published

+ND: No data.

In the southern Gulf of California, studies report clade C (abundant and widely distributed) associated with *Pavona gigantea* (Verrill) and clade D (extremophiles, its distribution centered in the Indo-West Pacific) with *Pocillopora verrucosa* (Ellis & Solander) (Iglesias-Prieto et al. 2004, LaJeunesse et al. 2018, Méndez-Méndez 2020). This study expands the list of *Symbiodinium* clades in the Gulf of California and describes the presence of clade A associated with the anemone *Actinostella* sp.

Usually, *Symbiodinium* clade A is associated with shallow water corals in the Caribbean and tidal pools, which generated the hypothesis that this clade is adapted to shallow areas (less than 1 m) due to the presence of photoacclimation and photoprotection pathways that counteract the high irradiance and high temperatures that can occur in these environments (Iglesias-Prieto and Trench 1997, Hirose et al. 2008, Takahashi et al. 2009, Yamashita and Koike 2013, Kemp et al. 2014). The results of the present study agree with this hypothesis, since the dinoflagellate was isolated in Bahía de La Paz, at a depth of less than 1 m (benthic habitat), in an area with high irradiance and high temperatures, which can reach 27 to 32 °C in the summer (Sea temperature 2024).

Clade A has been shown to be one of the easiest to culture due to its physiological and ecological characteristics and can occur in non-symbiotic, free-living form. This study provides information on the laboratory culture of symbiotic dinoflagellates from clade A from Bahía de La Paz; these strains have been maintained since 2018 to the present and can be cultured in specialized media (e.g., ASP-8A) and conventional media used for planktonic and benthic dinoflagellates, such as IMK, L1, modified GSe, and modified K media (this study, Hirose et al. 2008, LaJeunesse et al. 2015, Lee et al. 2015).

CONCLUSIONS

The results of the morphometric analyses combined with the phylogenetic analysis are conclusive for the *Symbiodinium*-clade A taxon. The phylogenetic analysis of the 28S region showed 9 clades currently accepted for the Symbiodiniaceae family. Clade A was divided into different subclades of phylospecies, showing that the PP563703 sequence of strain SNCETMAR-2 has phylogenetic affinity with sequences from *S. natans* (currently accepted taxon). This study reported the first detailed description of *S. natans*-clade A for Bahía de La Paz, Gulf of California; this dinoflagellate alternates between 2 life phases, a free-living (planktonic) phase and a non-obligate symbiotic phase (benthic) that showed an association with the sea anemone *Actinostella* sp.

English translation by Claudia Michel-Villalobos.

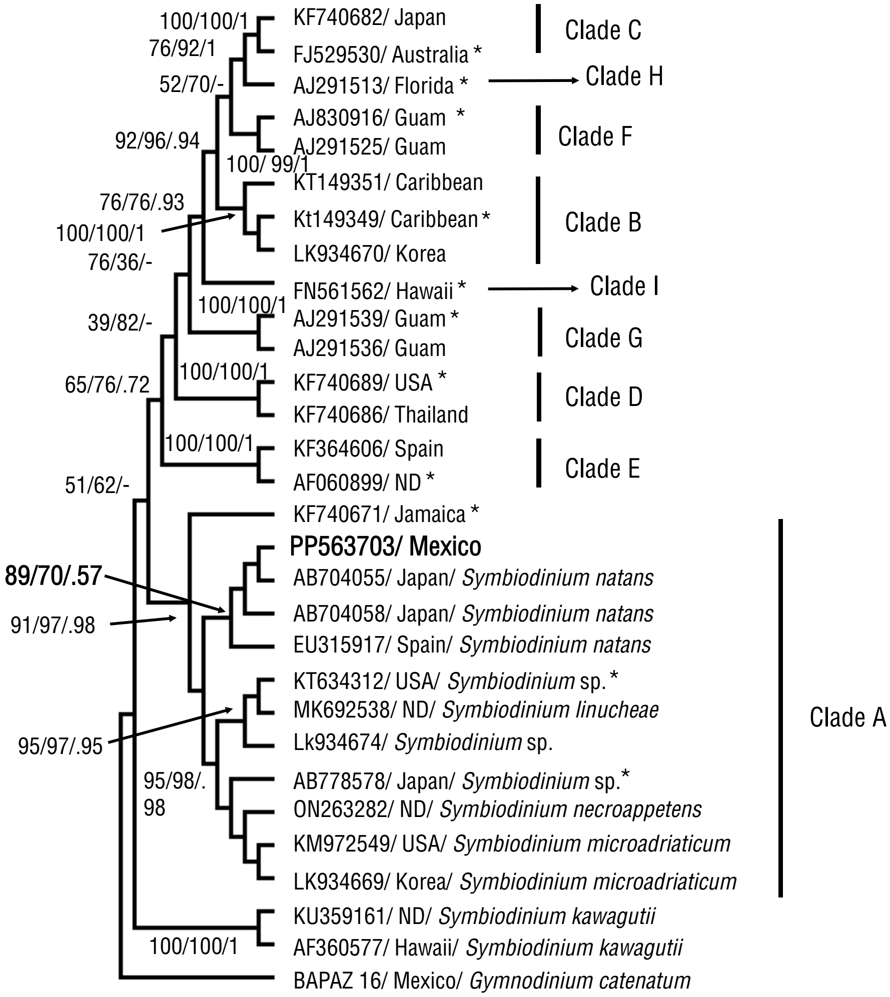

Figure 7. *Symbiodinium* phylogenetic tree of the 28S rDNA region. The sequence of the SNCETMAR-2 strain (PP563703) from this study is shown in bold. The analysis was deduced using the method of maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). The percentage of bootstrap support values and posterior probability of the clades and subclades are shown in the nodes. The analysis included 30 sequences and analyzed partial sequences of 558 bp. Sequences selected for genetic divergence analysis in Table 2 are indicated with asterisks. ND = no data.

Table 2. Pairwise genetic distances based on 13 Symbiodiniaceae family sequences selected from the 28S rDNA region. The genetic divergences observed between the sequence of this study (*) belonging to clade A and the other clades are shown in bold. The letters A–I indicate the clades of the family Symbiodiniaceae.

		A				B	C	D	E	F	G	H	I	*Symbiodinium kawagutii*
		KF740671	**PP563703***	KT634312	AB778578	KT149349	FJ529530	KF740689	AF060899	AJ830916	AJ291539	AJ291513	FN561562	AF360577
A	KF740671													
	PP563703*	0.025												
	KT634312	0.028	**0.014**											
	AB778578	0.032	**0.018**	0.004										
B	KT149349	0.237	**0.240**	0.240	0.244									
C	FJ529530	0.208	**0.208**	0.212	0.216	0.134								
D	KF740689	0.198	**0.219**	0.219	0.216	0.208	0.170							
E	AF060899	0.155	**0.141**	0.155	0.159	0.216	0.187	0.194						
F	AJ830916	0.212	**0.212**	0.216	0.219	0.120	0.092	0.198	0.201					
G	AJ291539	0.265	**0.265**	0.265	0.261	0.226	0.184	0.177	0.233	0.216				
H	AJ291513	0.187	**0.191**	0.194	0.198	0.124	0.049	0.159	0.180	0.085	0.184			
I	FN561562	0.205	**0.212**	0.219	0.219	0.159	0.134	0.177	0.205	0.141	0.201	0.127		
Symbiodinium kawagutii	AF360577	0.449	**0.452**	0.449	0.452	0.484	0.470	0.473	0.466	0.491	0.516	0.459	0.456	

Declarations

Supplementary Material

The supplementary material for this work can be downloaded from: https://cienciasmarinas.com.mx/index.php/cmarinas/article/view/3498/420421145

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Conflict of interest

The authors declare they have no conflict of interest.

Author contributions

Conceptualization: AER-S, IL-V, CJB-S; Data curation: AER-S; Formal analysis: AER-S, IL-V, YBO; Funding acquisition: CJB-S; Investigation: AER-S, IL-V, YBO, CJB-S; Methodology: AER-S, IL-V, YBO; Project administration: CJB-S; Resources: IIL-V, YBO, CJB-S; Software: IL-V, YBO; Supervision: IL-V, CJB-S, YBO; Validation: IL-V, CJB-S, YBO; Visualization: AER-S, IL-V, CJB-S, YBO; Writing–original draft: AER-S; Writing, review, and editing: AER-S, IL-V, YBO, CJB-S.

Data availability

The data for this study are available from the corresponding author by reasonable request.

Use of AI tools

The authors did not employ any AI tools in this work.

References

ABaker AC. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annu Rev Ecol Evol Syst. 34(1):661-689. https://doi.org/10.1146/ANNUREV.ECOLSYS.34.011802.132417

Bigham-Soostani S, Yousefzadi M, Zarei-Darki B, Ranjbar MS. 2021. Evaluation of cytotoxic and antibacterial properties of *Symbiodinium* sp. isolated and purified from *Stichodactyla haddoni* in the Persian Gulf and Gulf of Oman. Aquat Physiol Biotechnol. 9(2):125-144. https://doi.org/10.22124/JAPB.2021.18141.1401

Bustillos-Guzmán J, Band-Schmidt CJ, Durán-Riveroll LM, Hernández-Sandoval FE, López-Cortés DJ, Núñez-Vázquez EJ, Cembella A, Krock B. 2015. Paralytic toxin profile of the marine dinoflagellate *Gymnodinium catenatum* Graham from the Mexican Pacific as revealed by LC-MS/MS. Food Addit Contam. 32A(3):1-14. https://doi.org/10.1080/19440049.2014.1000978

Carlos AA, Baillie BK, Kawachi M, Maruyama T. 1999. Phylogenetic position of *Symbiodinium* (Dinophyceae) isolates from tridacnids (Bivalvia), cardiids (Bivalvia), a sponge (Porifera), a soft coral (Anthozoa), and a free-living strain. J Phycol. 35(5):1054-1062. https://doi.org/10.1046/j.1529-8817.1999.3551054.x

De Palmas S, Denis V, Ribas-Deulofeu L, Loubeyres M, Woo S, Hwang SJ, Song JI, Chen CA. 2015. *Symbiodinium* spp. associated with high-latitude scleractinian corals from Jeju Island, South Korea. Coral Reefs. 34:919-925. https://doi.org/10.1007/s00338-015-1286-y

Figueroa RI, Howe-Kerr LI, Correa AMS. 2021. Direct evidence of sex and a hypothesis about meiosis in Symbiodiniaceae. Sci Rep. 11:18838. https://doi.org/10.1038/s41598-021-98148-9

Graham ER, Parekh A, Devassy RK, Sanders RW. 2015. Carbonic anhydrase activity changes in response to increased temperature and pCO_2 in *Symbiodinium*-zoanthid associations. J Exp Mar Biol Ecol. 473:218-226. https://doi.org/10.1016/j.jembe.2015.08.017

Granados-Cifuentes C, Neigel J, Leberg P, Rodriguez-Lanetty M. 2015. Genetic diversity of free-living *Symbiodinium* in the Caribbean: the importance of habitats and seasons. Coral Reefs. 34(3):927-939. https://doi.org/10.1007/s00338-015-1291-1

Guiry MD, Andersen RA. 2018. Validation of the generic name *Symbiodinium* (Dinophyceae, Suessiaceae) revisited and the reinstatement of *Zooxanthella* K. Brandt. Notulae Algarum. 58:1-5.

Guiry MD, Guiry GM. 2024. AlgaeBase. World-wide electronic publication. Galway (Ireland): National University of Ireland; [accessed Feb 2024]. https://www.algaebase.org/search/genus/detail/?genus_id=44695

Hansen G, Daugbjerg N. 2009. *Symbiodinium natans* sp. nov.: a "free-living" dinoflagellate from Tenerife (Northeast-Atlantic Ocean). J Phycol. 45(1):251-263. https://doi.org/10.1111/j.1529-8817.2008.00621.x

Hirose M, Reimer JD, Hidaka M, Suda S. 2008. Phylogenetic analyses of potentially free-living *Symbiodinium* spp. isolated from coral reef sand in Okinawa, Japan. Mar Biol. 155:105-112. https://doi.org/10.1007/s00227-008-1011-2

Hoppenrath M, Chomerat N, Horiguchi T, Murray SA, Rhodes L. 2023. Marine benthic dinoflagellates - their relevance for science and society. 2nd ed. Stuttgart (Germany): E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller) and Senckenberg Geselschaft für Naturforschung. 376 p.

Hosoi-Tanabe S, Otake I, Sako Y. 2006. Phylogenetic analysis of noxious red tide flagellates *Chattonella antiqua*, *C. marina*, *C. ovata*, and *C. verruculosa* (Raphidophyceae) based on the rRNA gene family. Fish Sci. 72:1200-1208. https://doi.org/10.1111/j.1444-2906.2006.01277.x

Iglesias-Prieto R, Beltrán VH, LaJeunesse TC, Reyes-Bonilla H, Thomé PE. 2004. Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proc R Soc Lond B Biol Sci. 271:1757-1763. https://doi.org/10.1098/rspb.2004.2757

Iglesias-Prieto R, Matta JL, Robins WA, Trench RK. 1992. Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture. Proc Natl Acad Sci. 89(21):10302-10305. https://doi.org/10.1073/pnas.89.21.10302

Iglesias-Prieto R, Trench RK. 1994. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. Mar Ecol Prog Ser. 113:163-175. https://doi.org/10.3354/meps113163

Iglesias-Prieto R, Trench RK. 1997. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. Mar Biol. 130(1):23-33. https://doi.org/10.1007/s002270050221

Jeong HJ, Lee SY, Kang NS, Yoo YD, Lim AS, Lee MJ, Kim HS, Yih W, Yamashita H, LaJeunesse TC. 2014. Genetics and morphology characterize the dinoflagellate *Symbiodinium voratum*, n. sp., (Dinophyceae) as the sole representative of *Symbiodinium* Clade E. J Eukaryot Microbiol. 61(1):75-94. https://doi.org/10.1111/jeu.12088

Keller MD, Selvin RC, Claus W, Guillard RRL. 1987. Media for the culture of oceanic ultraplankton. J Phycol. 23(4):633-638. https://doi.org/10.1111/j.1529-8817.1987.tb04217.x

Kemp DW, Hernandez-Pech X, Iglesias-Prieto R, Fitt WK, Schmidt GW. 2014. Community dynamics and physiology of *Symbiodinium* spp. before, during, and after a coral bleaching event. Limnol Oceanogr. 59(3):788-797. https://doi.org/10.4319/lo.2014.59.3.0788

LaJeunesse TC, Lee SY, Gil-Agudelo DL, Knowlton N, Jeong HJ. 2015. *Symbiodinium necroappetens* sp. nov. (Dinophyceae): an opportunist 'zooxanthella' found in bleached and diseased tissues of Caribbean reef corals. Eur J Phycol. 50(2):223-238. https://doi.org/10.1080/09670262.2015.1025857

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR. 2018. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol. 28(16):2570-2580.e6. https://doi.org/10.1016/j.cub.2018.07.008

Lee SY, Jeong HJ, Kang NS, Jang TY, Jang SH, Lajeunesse TC. 2015. *Symbiodinium tridacnidorum* sp. nov., a dinoflagellate common to Indo-Pacific giant clams, and a revised morphological description of *Symbiodinium microadriaticum* Freudenthal, emended Trench & Blank. Eur J Phycol. 50(2):155-172. https://doi.org/10.1080/09670262.2015.1018336

Méndez-Méndez SM. 2020. Expresión diferencial de genes en *Cladocopium* sp. de dos especies de coral en el Golfo de California [dissertation]. [Mexico]: CIBNOR. 80 p.

Mordret S, Romac S, Henry N, Colin S, Carmichael M, Berney C, Audic S, Richter DJ, Pochon X, de Vargas C, et al. 2016. The symbiotic life of *Symbiodinium* in the open ocean within a new species of calcifying ciliate (*Tiarina* sp.) The ISME J. 10(6):1424-1436. https://doi.org/10.1038/ismej.2015.211

Parkinson JE, Coffroth MA, LaJeunesse TC. 2015. New species of Clade B *Symbiodinium* (Dinophyceae) from the greater Caribbean belong to different functional guilds: *S. aenigmaticum* sp. nov., *S. antillogorgium* sp. nov., *S. endomadracis* sp. nov., and *S. pseudominutum* sp. nov. J Phycol. 51(5):850-858. https://doi.org/10.1111/jpy.12340

Pawlowski J, Holzmann M, Fahrni JF, Pochon X, Lee JJ. 2001. Molecular identification of algal endosymbionts in large miliolid Foraminifera: 2. Dinoflagellates. J Eukaryot Microbiol. 48(3):368-373. https://doi.org/10.1111/j.1550-7408.2001.tb00326.x

Pochon X, Gates RD. 2010. A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. Mol Phylogenet Evol. 56(1):492-497. https://doi.org/10.1016/j.ympev.2010.03.040

Ramos-Santiago AE. 2023. Taxonomía morfológica y molecular de dinoflagelados epibentónicos del Golfo de California [dissertation]. [Mexico]: CICIMAR-IPN. 203 p.

Sampayo EM, Dove S, LaJeunesse TC. 2009. Cohesive molecular genetic data delineate species diversity in the dinoflagellate genus *Symbiodinium*. Mol Ecol. 18(3):500-519. https://doi.org/10.1111/j.1365-294X.2008.04037.x

Santos SR, Taylor DJ, Coffroth MA. 2001. Genetic comparisons of freshly isolated versus cultured symbiotic dinoflagellates: Implications for extrapolating to the intact symbiosis. J Phycol. 37(5):900-912. https://doi.org/10.1046/j.1529-8817.2001.00194.x

Sea temperature. 2024. Water temperature in La Paz (BCS); [accessed Feb 2024]. https://seatemperature.info/august/mexico/la-paz-water-temperature.html

Takabayashi M, Adams LM, Pochon X, Gates RD. 2012. Genetic diversity of free-living *Symbiodinium* in surface water and sediment of Hawaii and Florida. Coral Reefs. 31(1):157-167. http://doi.org/10.1007/s00338-011-0832-5

Takahashi S, Whitney SM, Badger MR. 2009. Different thermal sensitivity of the repair of photodamaged photosynthetic machinery in cultured *Symbiodinium* species. Proc Natl Acad Sci. 106:3237-3242. https://doi.org/10.1073/pnas.0808363106

Taylor FJR, Harrison PJ. 1983. Ecological aspects of intracellular symbiosis. In: Schenk HEA, Schwemmler W, Intracellular Space as Oligogenetic Ecosystem. Berlin (Germany): De Gruyter. p. 828-842. https://doi.org/10.1515/9783110841237-086

Wilcox TP. 1998. Large-subunit ribosomal RNA systematics of symbiotic dinoflagellates: morphology does not recapitulate phylogeny. Mol Phylogenet Evol. 10(3):436-448.

Yamashita H, Koike K. 2013. Genetic identity of free-living *Symbiodinium* obtained over a broad latitudinal range in the Japanese coast. Phycol Res. 61(1):68-80. https://doi.org/10.1111/pre.12004

Yorifuji M, Takeshima H, Mabuchi K, Watanabe T, Nishida M. 2015. Comparison of *Symbiodinium* dinoflagellate flora in sea slug populations of the *Pteraeolidia ianthina* complex. Mar Ecol Prog Ser. 521:91-104. https://doi.org/10.3354/meps11155