International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 14 (8), pp. 001-009, August, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Molecular phylogeny of *Prorocentrum* (Dinoflagellata) from the Pacific Coast of Mexico based on the parsimony analysis of fragments of LSUrDNA and SSUrDNA

Eréndira J. Cohen-Fernández*, Francisco F. Pedroche, Mónica Rodríguez Palacios, Sergio Álvarez Hernández and Esther Meave del Castillo

Departamento de Hidrobiología, Universidad Autónoma Metropolitana - Iztapalapa, Apartado Postal 55-535, México D. F. 09340, México.

Accepted 13 May, 2020

A phylogenetic analysis of the *Prorocentrum* species is presented, that includes the sequences of the large and small ribosomal RNA subunits from 19 cultures from 13 of the 20 species reported in the Pacific coast of Mexico; the results showed that *P. micans, P. gracile* and *P. mexicanum* were the closest of species, that planktonic may be more recent than epibenthonic species and it is suggested that the probable ancestor of the *Prorocentrum* genus could be a round cell without apical spine, toxic and epibenthonic.

Key words: *Prorocentrum*, parsimony analysis, dinoflagellates, LSUrDNA, SSUrDNA.

INTRODUCTION

Phylogenetic relationships between dinoflagellates based on DNA sequences of the small and large subunits of ribosomal RNA (SSUrDNA and LSUrDNA), have showed that this group of organisms consists of several paraphyletic orders, one of which is Prorocentrales. Within this order, species are taxonomically organized in the genus *Prorocentrum, Exuviaella, Mesoporus and Plagiodinium* belonging to Prorocentraceae family. The Prorocentrales are unicellular algae with two apical inserted flagella. The arrangement of the cortical alveoli consists of two dorso-ventrally compressed tecal plates; the rest of the tecal plates are reduced in size and fused together surrounding the apical pore where the flagella emerge. The suture of the main valves may be thick and in some species the right valve may be prolonged in one or two apical spines. Valve surface may be smooth or perforated by pores, poroids or very small spines. Some species develop blooms and produce toxins. With so few characters used to identify and taxonomically classify this group, the number of species changed constantly, with many new descriptions appearing during the last part of the 1800s until the 1970s when many synonyms were established and only one genus *Prorocentrum* was recognized; until now, four genera are accepted (Guiry and Guiry, 2007).

Dodge (1975) was the first to explore the relationship between the *Prorocentrum* species, proposing that the direction of the change in the evolution of the characters, recognized as diagnostic, was from smooth to ornamented valves, from round to elongated cells and towards acquisition of the apical spine that tended to be larger or double. He included 21 species (all the accepted ones) in his analysis. Four species seemed to have an independent origin and do not show a clear relationship with the rest *Prorocentrum minimum* (Pavillard) Schiller, *P. balticum* (Lohmann) Loeblich, *P. maximum* (Gobrret) Schiller, *P. cordatum* (Ostenfeld) Dodge. If Dodge's analysis of morphological characters revealed how difficult it was to unearth ancestor-descendant relationships between species, the analyses of DNA sequences exposed that morphological likeness between the *Prorocentrum* masked deep molecular differences.

Molecular phylogeny of the dinoflagellates shows, from the perspective of SSUrDNA, that *Prorocentrum* species

*Corresponding author. E-mail: erendiracohen@yahoo.com.

Table 1. *Prorocentrum* cultures used in the present study.

Species	Culture	Locality	Coordinates	Mexican Pacific region	Date ddmmyy	Colector	Institution	LSU NCBI accession number	SSU NCBI accession number
P. gracile	51L	L. Cárdenas, Michoacán	17°56'17" N 102°11'6" W	3	081104	MRP/SAH	1	EF517249	EF517263
P. gracile	13A	Acapulco Bay, Guerrero	16°48'58" N 99°53'59" W	3	070604	MRP/CLR	1	EF517251	-
P. gracile	PCGR3	Baja California Sur	-	1 - 2	2004 (yy)	DG	1	EF517248	EF517264
P. gracile	PGCV1	Concepción, Bay BCS	26º40'732" N 111º49'75" O	2	2004 (yy)	LM	2	EF517250	-
P. micans	PMCV1	Concepción, Bay BCS	26º40'732" N 111º49'75" O	2	2004 (yy)	CB	2	EF517254	-
P. micans	23A	Acapulco Bay, Guerrero	16°48'58" N 99°53'59" W	3	070604	MRP/CLR	1		EF517267
P. micans	43A	Acapulco Bay, Guerrero	16°48'58" N 99°53'59" W	3	070604	MRP/CLR	1	-	EF517269
P. micans	33A	Acapulco Bay, Guerrero	16°48'58" N 99°53'59" W	3	070604	MRP/ CLR	1		EF517268
P. micans	12A	Acapulco Bay, Guerrero	16°48'58" N 99°53'59" W	3	040504	MRP/ CLR	1	EF517257	EF517270
P. micans	CCMP 684	La Joya, CA, USA	32º90'00" N 117º25'50" O	4	2004	-	3	EF517255	-
P. mexicanum	24A	Acapulco Bay, Guerrero	16°48'58" N 99°53'59" W	3	241104	MRP/ CLR	1	EF517259	EF517271
P. mexicanum	31L	L. Cárdenas, Michoacán	17°56'17" N 102°11'6" W	3	081104	MRP/ SAH	1	-	EF517272
P. mexicanum	91L	L. Cárdenas, Michoacán	17°56'17" N 102°11'6" W	3	081104	MRP/ SAH	1	EF517258	EF517273
P. mexicanum	PCMX	BCS	-	1,2	2004	DG	1	EF517260	EF517275

Table 1. Contd.

P. mexicanum	VGO680	Ria de Vigo, Spain	-	5	2004	-	4	EF517260	EF517275
P. lima	PRL1	El Pardito, California Gulf, BCS	23º35' N 110º49.6' O	2	2004	-	2	EF517252	EF517266
P. lima	PL7V	Ria de Vigo, Spain	-	5	2004	-	4	EF517253	EF517266
P. compressum	VGO621	Ria de Vigo, Spain	-	5	2004	-	4	EF517256	EF517262
P. minimum	3V	Ria de Vigo, Spain	-	5	2004	-	4	EF517247	-

Mexican Pacific regions: (1) West coast of Baja California, (2) California Gulf, (3) Tropical Pacific, (4) California Current / Subtropical (5) Atlantic; Collectors: MRP-Mónica Rodríguez Palacio, SAH-Sergio Álvarez Hernández, CLR-Cruz Lozano Ramírez, DG-Diana Gongora, LM-Lourdes Morquecho, CB- Lourdes Morquecho; Institutions: (1) Metropolitan Autonomous University-Iztapalapa, Mexico, (2) Northeast Center of Biological Research, Mexico, (3) Provasoli-Guillard Nacional Center for Culture of Marine Phytoplankton, USA, (4) Spanish Instituto of Oceanography- Oceanographycal Center of Vigo, Spain.

are interspersed between the orders Peridiniales, Suessiales, Gymnodiniales and Dinophysiales, while the LSUrDNA analysis are spread between the orders Gymnodiniales, Peridiniales and Dinophysiales (Zardoya et al., 1995; Grzebyk et al., 1998; Daugbjerg et al., 2000; Pearce and Hallegraeff, 2004; Saldarriaga et al., 2001; Saldarriaga et al., 2004; Zhang et al., 2005; Murray et al., 2005). Only *P. micans* Ehrenberg*, P. minimum* (Pavillard) Schiller*, P. mexicanum* Tafall and *P. lima* (Ehrenberg) Dodge, have been included systematically in molecular studies and there are no clear conclusions regarding their phylogenetic relationship because lack of consistency in the results, for example while *P. mexicanum* is the sister species of *P. micans* in most of the ribosomal RNA subunit studies, analyses of cob protein and in one SSUrDNA analysis *P. minimum* appears as the sister taxon of *P. micans*.

In the Pacific coast of Mexico some of the most conspicuous dinoflagellates include 20 species of *Prorocentrum* (Okolodkov and Garate, 2006). By sequencing ribosomal DNA from species collected in this area as well as from cultures, donated and purchased specimens, we try to answer the following questions: Which species of *Prorocentrum* are closely related? Do planktonic species derive from an epibenthonic species? Will phylogeny give us a clue about morphological and ecological characters of a *Prorocentrum* possible ancestor?

MATERIALS AND METHODS

Cultivation

Six species of *Prorocentrum* from the tropical and subtropical pacific collected along the Mexican coast were cultured and sequenced for this study. They were cultured in L2 medium prepared with filtered seawater under a 12:12 h light-dark cycle at 20°C. The same growth conditions were used with cultures donated or purchased, two from Baja California Mexico, one from the coast of California USA, also three cultures from Spain were included: *P. lima, P. compressum* and *P. minimum* with the idea to compare sequences, because the species are also found in Pacific Mexico (Table 1).

DNA extraction, PCR and sequencing

DNA was extracted from 19 *Prorocentrum* strains (Table 2), using 1.5 ml of midlogarithmic phase cultures, also using the DNeasy Plant Minikit from Quiagen and following the manufactures instructions. The only variation to the protocol was the lysis of cells by freezing the harvested cells at -20°C during 10 min and then thawing them at room temperature. The D1-D2 conserved regions and

Table 2. *Prorocentrum* sequences from the GenBank, that were incorporated in this study.

Species	Culture	LSU NCBI accession no.	SSU NCBI code	Location
P. balticum	B	AF042816	-	Massachusetts, USA
P. balticum	D-71	-	DQ887511	South Korea
P. belizeanum	PBMA_01	AJ567460	-	Reunion Island, SW, Indian Ocean
P. compressum	PCPA_01	AY259169	-	Port Arthur, Tasmania
P. concavum	PCRN_01	AJ567464	-	Reunion Island, SW, Indian Ocean
P. dentatum	-	AY833515	-	China
P. dentatum	CCMP1517	-	DQ336057	South Pacific
P. emarginatum	PERN_05	AJ567465	-	Reunion Island, SW, Indian Ocean
P. emarginatum	PREU-2	-	Y16239	Reunion Island, SW, Indian Ocean
P. gracile	PGDW01	AY259165	-	Derwent River, Tasmania
P. gracile	CCCM765		AY443019	Canada
P. lima	PL7V	L38634	-	IEO, Vigo, Spain
P. lima	CRLMN-6	-	AB189778	Limón, Costa Rica
P. mexicanum	-	AF260378	-	Denmark
P. mexicanum	SP3	-	DQ174089	Cat Ba, Hai Phong, Viet Nam
P. micans	EMBL04062	DQ485144	-	China
P. micans	B	AF042814	-	South Korea
P. micans	-	-	AJ415519	Norway
P. minimum	B	DQ054539	-	East Sea China, Fijian Province
P. minimum	PMIN1	L38636	-	IEO, Lisbon
P. minimum	JAOO01		DQ336066	Connecticut, USA
P. rhathymum	PRLS02	AY259167	-	Little Swan port, Tasmania
P. triestinum	MBIC11147	-	AB183673	Japan
P. triestinum	PT5V	L38638	-	IEO, Vigo, Spain
P. triestinum	B	AF042815	-	South Korea
T. gondii	-	X75429	X75429	New York, USA

B = Blooms

intervening variable domains of the (LSU) ribosomal gene were amplified using polymerase chain reaction (PCR) with D1R forward and D2C reverse primers (Bolch, 2001) and the SSU ribosomal gene with 16S1N forward and 16S2N reverse primers (Grzebyk et al., 1998); 20 µL PCR products were amplified in a Touchgene gradient (Techne). LSUrDNA protocol: Initial denaturalization 94°C × 2 m and 30 cycles of denaturalization 94°C x 1 m, annealing 58°C × 1.5 m, extension 72°C × 3 m and final extension 72°C × 6 m. SSUrDNA protocol: Initial denaturalization 94°C × 2 m and 30 cycles of denaturalization 94°C × 1 m, annealing 58.6°C × 2 m, extension 72°C × 3 m and final extension 72°C × 7 m. PCR reactions were checked for successful amplification by electrophoresis of products through 1% agarosa gels. PCR product was cleaned using a QIAquick PCR purification kit (Quiagen, Hilden, Germany) following the manufacturers protocol. PCR product was sequenced using a Big Dye Terminator Cycle Sequencing Kit from Applied Biosystems following the manufacturer's protocol. The product was purified in CentriSep columns (52 mg sephadex G-50 suspended in 800 ml distillated water) and finally sequenced at the Institute of Biology at the National Autonomous University of Mexico and at the Molecular Biology Laboratory at the Metropolitan Autonomous University.

The consensus sequences were obtained by pair wise alignment (optimal GLOBAL alignment, BioEdit 7.0.5.2 [Hall, 1999]) and then aligned with sequences obtained from GenBank (National Center for Biotechnology Information) databases, using Clustal W (full multiple alignment with 1,000 bootstrap) (Thompson et al., 1994). Alignment included both variable and conserved regions.

Phylogenetic analyses

Stimation of phylogeny was carried out using Paup 3.1 (Swofford, 1993). The analyses included the original sequences as well as sequences available in the GenBank database, the ciliate *Toxoplasma gondii* was selected as the out group. This species is used in most phylogenetic analysis of dinoflagellates and its sequence included both SSU and LSU fragments (Table 2).

RESULTS

The molecular diversity of the SSU and LSU calculated by "p" uncorrected distances shows that SSU varies more within the *Prorocentrum* genus (up to 0.71%) than between *Prorocentrum* and *Toxoplasma.* The opposite is true for the LSU region where the interspecific differences were less than the intergeneric ones (up to 0.661%) (Table 3).

The *Prorocentrum* species in this study includes 13 of

Table 3. Optimization model and objective functions used in the parsimony analysis.

Ribosomal subunit	Taxa	Nucleotides	Optimization model	Optimization strategies
LSU	32	639	Full heuristic TBR 100,000 replicas three repetitions w/same result	DELTRAN Gap as 5th base
SSU	23	639	Branch & Bound 100 replicas three repetitions w/ same result	DELTRAN Gap as 5th base

the 20 species reported for the Pacific coast of Mexico. A summary of the main morphological and ecological characteristics of these twelve species is presented in Table 4. The results from the parsimony analysis appear in Table 5 and in Figure 1. LSUrDNA was the fragment with the higher number of parsimony informative characters.

LSUrDNA

The most parsimonious tree proposes that the species are related with each other in sets of trichotomies, the first one formed by *P. gracile* Schütt 1896, *P. micans* and *P. mexicanum*, and this group is part of the second trichotomy that includes *P. triestinum,* and a cluster formed by *P. minimum, P. balticum* and *P. dentatum* Stein. The third trichotomy includes along with the last group *P. emarginatum* Fukuyo and a branching dichotomy of *P. lima* and *P. belizeanum.*

SSUrDNA

Even though the species included in the analysis are a slightly different group than those included in the LSUrDNA analysis, it also includes *P. compressum* in a politomy with *P. gracile, P. micans,* and *P. mexicanum*.

DISCUSSION

P. gracile and P. micans, have been considered as part of a species complex because of its likeness. Its status as a species based on the analysis of their morphometry has been discussed in an earlier paper (Cohen-Fernández et al., 2006).

P. balticum, P. dentatum and *P. minimum* had almost identical sequences, and appeared together consistently. *P. minimum* and *P. balticum* look pretty much alike (Faust and Gulledge, 2002). The closeness of *P. minimum* and *P. dentatum* had been already reported elsewhere (Lin et al., 2006) and it is confirmed here.

P. concavum was closer to the ancestral position, either as the oldest or associated with the cluster that included *P. lima* (Pearse and Hallegareff, 2004); it seems to share molecular characters that place it between the harmless planktonic species and the toxic epibenthonic ones.

Phylogeny and biogeography

The Pacific coast of Mexico has been divided in five (5) regions (Meave del Castillo et al., 2003) (Table 1). The populations of *P. gracile, P. micans* and *P. mexicanum* belong to the west coast of Baja California, the California Gulf and the Tropical Pacific regions. All sequences of *P. micans* from Mexico grouped and were closest to those from the U. S. California coast. The SSUrDNA sequences of *P. micans* from the Acapulco Bay formed a monophyletic clade. *P. mexicanum* from the Mexican tropical Pacific formed a trichotomy with the populations from the subtropical Pacific and the Atlantic (Spain). The sequence of *P. minimum* from Lisbon was closest to the sequence from China than to that from Vigo, Spain.

Phylogeny and ecology

Toxic species were interspersed among non toxic species. *P. lima, P. belizeanum* and *P. concavum* formed one cluster, they are epibenthonic and toxic species that were formerly considered *Exuviaella*. The second cluster was formed by *P. minimum* and *P. dentatum*, they are planktonic as well as toxic species.

The LSUrDNA sequence of *P. rhathymum* Loeblich III, Sherley and Schmidt from Tasmania was clustered along with the *P. mexicanum*, while the SSUrDNA sequence of *P. mexicanum* from Vietnam went to the *P. lima* branch. Probably these sequences may have been misidentified and their names should be switched, Cortés-Altamirano and Sierra-Beltran (2003) suggested *P. mexicanum* to be planktonic and *P. rhatymum* to be toxic and epibentonic.

Table 4. Characteristics of the *Prorocentrum* species[§].

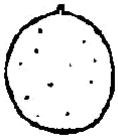
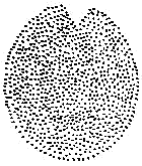
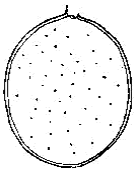
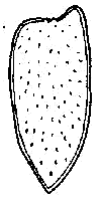
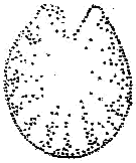
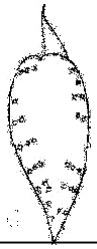
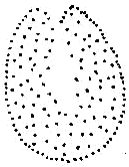
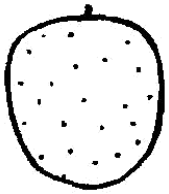
Species	Habit	Shape	Looks like	Ex *Exuviaella*	Toxicity
P. balticum	Planktonic		*P. minimum*	No	None
P. belizeanum	Epibenthonic		*P. compressum*	Yes	Okadaic Acid, DSP, DTX-1
P. compressum	Planktonic		*P. belizeanum*	No	None
P. concavum	Epibenthonic		*P. lima*	Yes	FAT, DSP Okadaic acid
P. dentatum	Planktonic		-	No	None
P. emarginatum	Epibenthonic		*P. rhathymum* *P. mexicanum*	No	None
P. gracile	Planktonic		*P. sigmoides*	No	None

Table 4. Contd.

P. lima	Epibenthonic		*P. concavum*	Yes	Prorocentrolid acid, Okadaic acid, FAT DSP, DTX-1, 2 and 4
P. mexicanum	Planktonic		*P. emarginatum* *P. mexicanum*	No	None
P. micans	Planktonic		*P. gracile*	No	None
P. minimum	Planktonic		*P. balticum*	Yes	Venerupin (hepathotoxina)
P. rhathymum	Epibenthonic		*P. emarginatum* *P. mexicanum*	-	FAT[§§]
P. triestinum	Planktonic			No	None

[§] References: Faust et al. (1999), Hernández-Becerril et al. (2000) and Faust and Gulledge (2002).
[§§] IOC states that all toxicity cases are caused by *P. rhathymum* and not to *P. mexicanum*.

Table 5. Results of the Phylogenetic analysis: Parsimony.

Parsimony analysis	Number of informative characters	Best tree score	Consistency index
LSU	300	1158	0.737
SSU	147	1128	0.884

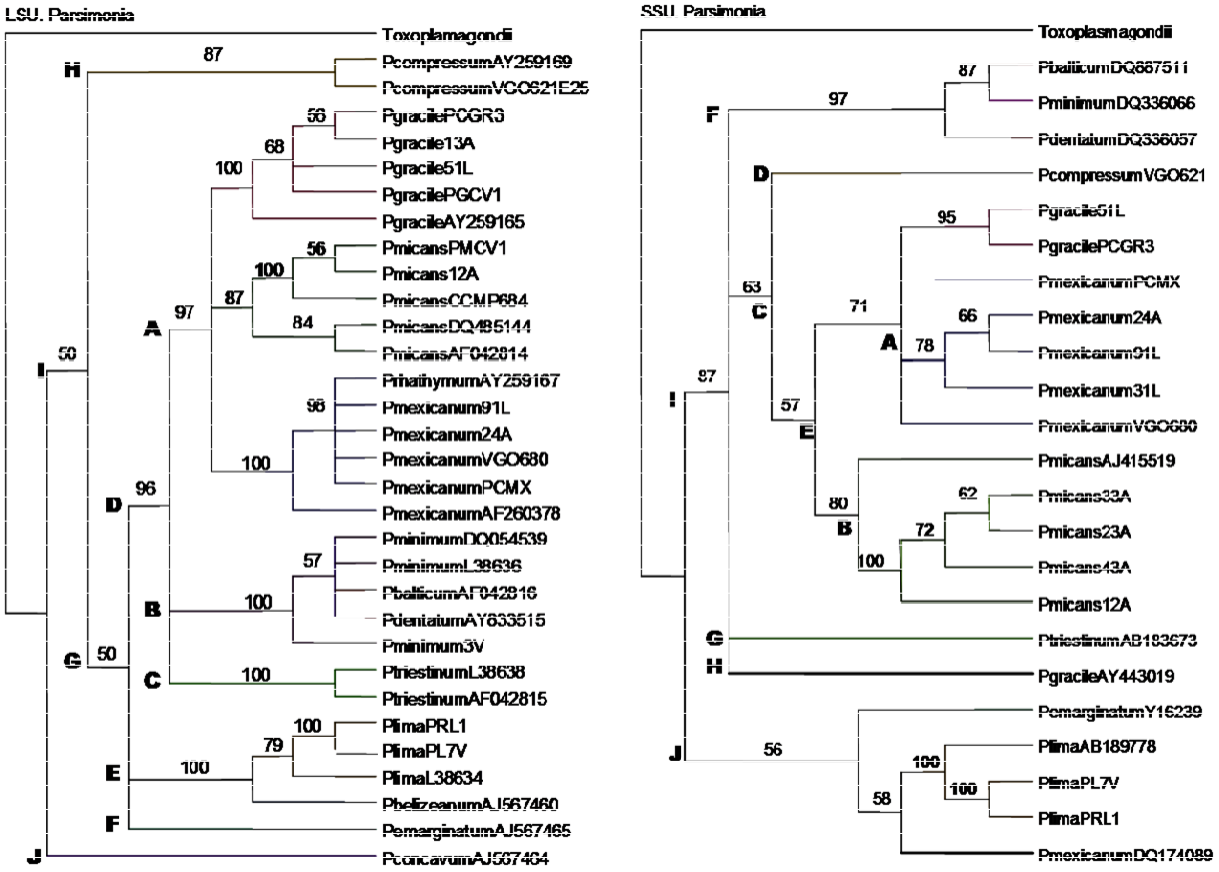


Figure 1. Phylogenetic tree analysis for separate analyses of LSUrDNA and SSUrDNA.

Phylogeny and classical taxonomy

For *P. minimum, P. balticum* and *P. dentatum,* a set of species who's relationship (Dodge 1975) could not clarify; our study showed that they are related to each other.

AKNOWLEGMENTS

Dra. Irene de los Ángeles Barriga-Sosa, Claudia Garnica-Rivera and Abel Chihuahua Laboratorio de Genética y Biología Molecular UAM-I, Johana Díaz-Larrea, Abel Sentíes Granados and Kurt Dreckman Laboratorio de Macroalgas UAM-I, Cruz Lozano Laboratorio de Ficología Aplicada UAM-I, Dra. Alejandra Serrato Díaz Laboratorio Divisional de Biología Molecular UAM-I to Sofía Solórzano Lujano, Laura Márquez, Christine Band Schmidt, Dr. José Bustillos, David Galvez and Anayansi Cohen. Funding was provided by CONACyT proyect G37560-V.

REFERENCES

Bolch CJS (2001). PCR protocols for genetic identification of dinoflagellate cells and cysts. Phycol. 40: 162–167.

Cohen-Fernández EJ, Meave Del Castillo E, Salgado-Ugarte IH, Pedroche FF (2006). Contribution of external morphology in solving a species complex: The case of Prorocentrum micans, Prorocentrum gracile and Prorocentrum *sigmoides* (Dinoflagellata) from the Mexican Pacific Coast. Phycol. Res, 54: 330-340.

Cortés-Altamirano R, Sierra-Beltran AP (2003). Morphology and taxonomy of Prorocentrum mexicanum and reinstatement of Prorocentrum rhathymum (Dinophyceae). J. Phycol., 39: 221-225.

Daugbjerg N, Hansen G, Larsen J, Moestrup Ø (2000). Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSUrDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycol, 39: 302-317.

Dodge JD (1975). The Prorocentrales (Dinophyceae). II. Revision of the taxonomy within the genus *Prorocentrum*. Bot. J. Linn. Soc. 71: 103-125

Faust MA, Gulledge RA (2002). Identifying harmful marine dinoflagellates. Contributions from the United States National Herbarium, Department of Systematic Biology – Botany, National Museum of Natural History, Smithsonian. Instit. 42: 1-141.

Grzebyk D, Sako Y, Berland B (1998). Phylogenetic Analysis of nine species Prorocentrum (Dinophyceae) Inferred from 18S Ribosomal DNA sequences, morphological comparisons and descriptions of Prorocentrum panamensis, sp. nov. J. Phycol. 34: 1055-1068.

Guiry MD, Guiry GM (2007). AlgaeBase version 4.2. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; accessed on 11 May.

Hall TA (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucl. Acids. Symp. Ser. 41: 95-98.

Hernández-Becerril DU, Cortés Altamirano R, Alonso R (2000). The dinoflagellate genus *Prorocentrum* along the coasts of the Mexican

Pacific. Hydrobiol, 418: 111-121
Lin S, Zhang H and Jiao N (2006). Potencial utility of mitochondrial cytochrome b and ITS mRNA editing in resolving closely related dinoflagellates: A case study of Prorocentrum (Dinophyceae). J. Phycol. 42: 646-654.
Meave del Castillo ME, Zamudio-Resendiz ME, Aké-Castillo J, Guerra-Martinez SL and Barbosa-Ledezma IF (2003). Biodiversidad de diatomeas (Bacillariophyta) en la columna de agua del Pacífico Mexicano *in*: Barreiro-Güemes MT, Meave del Castillo ME, Signoret-Poillon M and Figueroa-Torres ME (Eds.) Planctología Mexicana. Sociedad Mexicana de Planctología AC, México.
Murray S, Jorgensen MF, Ho SYW, Patterson DJ, Jermiin LS (2005). Improving the analysis of Dinoflagellate phylogeny based on rDNA. Protist.156: 269-286.
Okolodkov YB, Garate Lizarraga I (2006). An annotated checklist of dinoflagellates (Dinophyceae) from the mexican pacific. Acta Bot Mex. 74: 1-154.
Pearce I, Hallegraeff GM (2004). Genetic affinities, ecophysiology and toxicity of *Prorocentrum playfairii* and *P. foveolata* (Dinophyceae) from Tasmanian freshwaters. Phycol, 43: 271-281.
Saldarriaga JI, Taylor FJR, Keeling PJ, Cavalier-Smith T (2001). Dinoflagellate nuclear SSUrRNA phylogeny suggests multiple plastid losses and replacements. J. Mol. Evol. 53: 204-213.
Saldarriaga JI, Taylor FJR, Cavalier-Smith T, Manden-Deuer S, Keeling PJ (2004). Molecular data and evolutionary history of dinoflagellates. Eur. J. Protistol. 40: 85-111.
Swofford DL (1993). PAUP: Phylogenetic Analysis Using Parsimony, Version 3.1 Computer program distributed by the Illinois Natural Hostory Survey. Champaign. Illinois.pp.1-302
Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic. Acids. Res. 22: 4673-80.
Zardoya R, Costas E, López-Rodas V, Garrido-Pertierra A, Bautista JM (1995). Revised dinoflagellate phylogeny inferred from molecular analysis of long subunit ribosomal RNA gene sequences. J. Mol. Evol. 44: 637-645.
Zhang H, Battacharya D, Lin S (2005). Phylogeny of Dinoflagellates based on mithocondrial cytocrome b and nuclear small subunit rDNA sequence comparisons. J. Phycol. 41: 411-420.