Featured article

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Ostreopsis cf. *ovata* and *Ostreopsis lenticularis* (Dinophyceae: Gonyaulacales) in the Galapagos Marine Reserve

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Summary: The genus of benthic dinoflagellates *Ostreopsis* is of particular interest because some species negatively impact human health and coastal marine ecosystems. *Ostreopsis* populations from a remote area, such as the Galapagos Marine Reserve with its unique biodiversity, can provide significant data. Samples of epibionthic dinoflagellates were collected from two islands (Santa Cruz and Santa Fé) in 2017. Species of the genera *Gambierdiscus, Amphidinium, Coolia* and *Ostreopsis* were found. *Ostreopsis* strains were isolated to characterize their morphology, molecular biology and toxicity. Three different morphotypes of *Ostreopsis* based on dorsoventral and width diameters (n=369) were distinguished. The small cell morphotype was dominant in ten samples, with abundances of up to 33405 cells g^{-1} fresh weight of macroalgae. A total of 16 strains were isolated from field samples with subsequent polymerase chain reaction amplifications of rDNA, 5.8S rDNA and internal transcribed space regions; 13 strains (small cell morphotype) clustered in the *O.* cf. *ovata* Atlantic/Indian/Pacific clade; and 3 strains (large cell morphotype) clustered in the *Ostreopsis lenticularis* genotype from the type locality. The strains proved to be non-toxic. The presence of these genera/species represents a potential threat to marine ecosystems, and it is thus important to consider benthic species in the surveillance of harmful algae blooms in the reserve.

Keywords: dinoflagellates; harmful algal blooms; molecular phylogeny; *Ostreopsis* cf. *ovata*; *Ostreopsis lenticularis*; SEM; taxonomy; toxicity.

***Ostreopsis* cf. *ovata* y *Ostreopsis lenticularis* (Dinophyceae: Gonyaulacales) en la Reserva Marina de Galápagos**

Resumen: El género de los dinoflagelados bentónicos *Ostreopsis* es de particular interés, porque algunas especies afectan negativamente a la salud humana y a los ecosistemas marinos costeros. Las poblaciones de *Ostreopsis* en áreas remotas, como la Reserva Marina de Galápagos con su biodiversidad única, pueden proporcionar datos significativos a su estudio. Se recolectaron muestras de dinoflagelados epibentónicos de dos islas (Santa Cruz y Santa Fé) en 2017. Se encontraron especies de los géneros *Gambierdiscus, Amphidinium*, *Coolia* y *Ostreopsis*. Las cepas de *Ostreopsis* se aislaron para caracterizar su morfología, biología molecular y toxicidad. Se distinguieron tres morfotipos diferentes de *Ostreopsis* basados en tamaño (n=369). El morfotipo de células pequeñas fue dominante en diez muestras, con abundancias de hasta 33405 células g^{-1} de peso fresco de macroalgas. Se aisló un total de 16 cepas y se secuenciaron las regiones de rDNA, 5.8S y ITS para el estudio filogenético. Trece cepas pertenecieron al morfotipo de células pequeñas agrupadas en el clado *O.* cf. *ovata* Atlántico/Índio/Pacífico y tres cepas al morfotipo de células grandes agrupadas en el clado *Ostreopsis lenticularis*. Ninguna de las cepas aisladas resultó ser tóxica. La presencia de estos géneros/especies representa una amenaza potencial para los ecosistemas marinos, por lo que es importante tener en cuenta las especies bentónicas en la vigilancia de la proliferación de algas nocivas en la reserva.

Palabras clave: dinoflagelados; proliferación de algas nocivas; filogenia; *Ostreopsis* cf. *ovata*; *Ostreopsis lenticularis*; MEB; taxonomía; toxicidad.

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INTRODUCTION

Toxic benthic dinoflagellates have been related to seafood poisoning in humans and negative impacts on some marine organisms (Berdalet et al. 2017). Several toxic genera frequently co-exist in epiphytic microalgal assemblages: *Gambierdiscus* Adachi and Fukuyo, 1979, which produce the toxins responsible for ciguatera fish poisoning (Litaker et al. 2017, Munday et al. 2017, Larsson et al. 2018); *Fukuyoa* F. Gómez, D.X. Qiu, R.M. Lopes et Senjie Lin, 2015, which produce haemolytic substances and a maitotoxin-like compound (Holmes 1998, Holland et al. 2013, Laza-Martinez et al. 2016); *Ostreopsis* Schmidt, 1901, associated with clupeotoxicity (Randall 2005), skin irritations and respiratory disorders (Tichadou et al. 2010, Del Favero et al. 2012, Vila et al. 2016); and some toxic species of *Amphidinium* Claparède et Lachmann, 1859, *Coolia* Meunier, 1919, and *Prorocentrum* Ehrenberg, 1834, which may cause human health issues (Laza-Martinez et al. 2011).

In the last two decades, the geographical area of the study of potentially toxic benthic dinoflagellates has increased considerably (Hachani et al. 2018, Irola-Sansores et al. 2018, Durán-Riveroll et al. 2019 and references therein). However, observations on marine diversity are still lacking from low latitudes, which have hitherto been overlooked by the scientific community (Menegotto and Rangel 2018). Sampling efforts should thus be intensified in tropical areas, such as the Galapagos Marine Reserve (GMR), where epiphytic dinoflagellate occurrence has only been reported as preliminary results of the present study (Yépez Rendón et al. 2018). Furthermore, the GMR is known worldwide for its unique biodiversity, influenced by currents, local upwellings and other oceanographic features, representing biodiversity hotspots (Liu et al. 2014). There is little information about microalgae diversity in the Archipelago, and the risk of harmful algal blooms (HAB) in the area has not been assessed. A recent study on the southern islands of the GMR reported 18 harmful taxa (Carnicer et al. 2019), representing an ecological threat for coastal marine ecosystems and for human health that may result in negative economic and social impacts in the GMR (Kislik et al. 2017).

Ostreopsis is of particular interest because some species of this genus are known to negatively impact human health (causing fever, dyspnoea, bronchoconstriction, conjunctivitis and skin irritations) and to cause mortality in marine benthic organisms in temperate regions (reviewed in Accoroni and Totti 2016). *Ostreopsis* cf. *ovata* is the most widely distributed species of the genus; it has been studied in detail, mostly because of its recurrent blooms in the Mediterranean Sea, which pose a health risk to bathers (Vila et al. 2016).

It has been demonstrated, in some cases by bioassay and in others by analytical techniques, that several species/genetic clades of the genus *Ostreopsis* produce palytoxin (PLTX)-like compounds: *O* cf. *ovata* (García-Altares et al. 2014, Tartaglione et al. 2016), *O. siamensis* Schmidt, 1901 (Terajima et al. 2018), *O. mascarenensis* Quod, 1994 (Lenoir et al. 2004), *Ostreopsis* sp. 1 and *Ostreopsis* sp. 6 (Sato et al. 2011, Suzuki et al. 2012), and *O. fattorussoi* Accoroni, Romagnoli et Totti, 2016. *Ostreopsis lenticularis* Fukuyo, 1981 (Ashton et al. 2003), *O. heptagona* Norris, Bomber et Balech, 1985 and *Ostreopsis* sp. 7 (Tawong et al. 2014) have been reported as toxic by mouse bioassay. However, within the *O*. cf. *ovata* strains there is a high infraspecific variability concerning toxin production (Carnicer et al. 2016a), as has been reported in other dinoflagellates such as the *Alexandrium tamarense* species complex (John et al. 2014).

The taxonomic status of the genus *Ostreopsis* is presently in flux and requires extensive revision (Berdalet et al. 2017). Eleven *Ostreopsis* species have been identified on the basis of morphological features, but the characteristics used to delineate those species have proven that unambiguous species identification based on morphology is difficult or even impossible. Instead, molecular characters, particularly the internal transcribed spacer (ITS) region and the D1-D3 large subunit (LSU) ribosomal genes, have proven to be more efficient and consistent for discriminating between dinoflagellate species (Litaker et al. 2007, Penna et al. 2014). For this reason, the two recently described species *O. fattorussoi* (Accoroni et al. 2016) and *O. rhodesiae* Verma, Hoppenrath et Murray, 2016 were defined on the basis of both molecular and morphological criteria.

Morphologically, six species have a tear-drop cell shape: *O.* cf. *siamensis*, *O.* cf. *ovata*, *O. heptagona*, *O. belizeana* Faust, 1999, *O. caribbeana* Faust, 1999, *O. fattorussoi* and *O. rhodesiae*. The other four species of the genus are characterized by a broadly oval, lenticular-shaped cell: *O. lenticularis*, *O. mascarenensis*, *O. labens* Faust et Morton, 1995 and *O. marina* Faust, 1999. All the species share a similar plate pattern, which complicates their identification based on morphology (Penna et al. 2005). Only *O. heptagona* is easily distinguishable under a light microscope because the 2′′′′ plate narrows toward the centre of the hypotheca. Moreover, cell sizes overlap among spe-

cies, and considerable infraspecific variability in cell diameter has been observed both in field samples and in cultures (Aligizaki and Nikolaidis 2006, David et al. 2013, Carnicer et al. 2016b).

In addition, ITS phylogenies based on sequencing the ITS region from numerous *Ostreopsis* isolates indicate the existence of an additional seven genetic clades (*Ostreopsis* spp. 1-7), designated numerically, pending formal taxonomic assignation (Sato et al. 2011, Tawong et al. 2014), apart from an unidentified phylotype (proposed as *Ostreopsis* sp. 8 in Tibiriçá et al. 2019) reported from Reunion Island in the Indian Ocean (Carnicer et al. 2015). Without genetic material from the originally described species location, it is impossible to determine whether the newly sequenced isolates belong to a previously described species. Fortunately, a recent study performed in French Polynesia has associated *Ostreopsis* sp. 5 with *O. lenticularis* (Chomérat et al. 2019) on the basis of the morphological features of the original description of the cells from the same location (Fukuyo 1981). Most recently, *Ostreopsis mascarenensis* has been reinvestigated by morphological and molecular phylogenetic methods using specimens collected from the type locality of the species by Chomérat et al. (2020).

New characterizations of *Ostreopsis* species from unexplored areas, including the study of morphology, phylogeny and toxin profiles, may be helpful in consolidating the original species described in the last century solely by morphology. In addition, reporting existing species will provide valuable data on their geographic distribution and support for current molecularly defined species. The present study aimed to identify the associated epibionthic dinoflagellate assemblage in the GMR and describe the morphology, molecular biology and toxicity of *Ostreopsis* strains found in the area.

MATERIALS AND METHODS

Sampling

Sampling occurred at two southern islands in the GMR. One site was sampled on Santa Fé Island (0°48′16.36″S; 90°5′7.522″W) on 29 March 2017, and two sites were sampled on Santa Cruz Island: Tortuga Bay (0°45′58.43″S; 90°20′42.373″W) on 30 March 2017 and Venecia Bay (0°302′5.755″S; 90°30′56.646″W) on 6 April 2017 (Fig. 1). The surface water temperature was 28.25°C to 28.80°C, salinity was 34.24-34.68, pH was 7.78-7.84 and dissolved oxygen was 5.11-6.34 mL L^{-1} (94.3%-100.6%). Macroalgae and scrapings on the surface of sessile benthic invertebrates, *Tetraclita* sp. (Crustacea: Cirripedia), were collected for analysis of the epibenthic dinoflagellates growing on them. Samples were taken by hand at 1 to 2 m depth and placed in a plastic bag immediately; the volume of the surrounding water was subsequently measured with a plastic graduated cylinder.

Macroalgae and the surrounding water were transferred to a 500 mL plastic bottle, vigorously shaken for one minute and then filtered through a 300 µm mesh. For invertebrates, the surface was scraped off using a

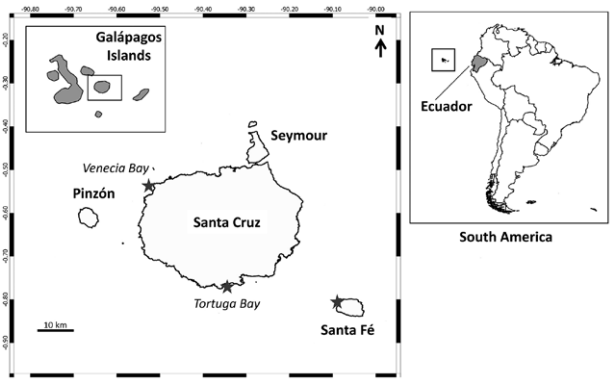

Fig. 1. – Sampling sites: Santa Fé Island (0°48′16.36″S; 90°5′7.522″W), Tortuga Bay (0°45′58.43″S; 90°20′42.373″W) and Venecia Bay (0°302′5.755″S; 90°30′56.646″W) in the Galapagos Marine Reserve.

razor blade and resuspended in the surrounding water sample for filtration through a 300 µm mesh to collect the epibionthic microalgal community. The resulting water with suspended microalgae was fixed in 3% acid Lugol's solution for cell counting. Aliquots of the water samples from Tortuga Bay were kept unfixed for cell isolation. Macroalgae were placed in plastic bags and transported in coolers to the laboratory for weighing (Mettler Toledo SB32001 DeltaRange).

Cell isolation and culture conditions

Cells were isolated by the capillary method (Hoshaw and Rosowski 1973), grown in a 24-well microplate containing f/10 medium (Guillard 1975) for a week and then inoculated in 50 mL flat plastic flasks containing 30 mL f/10 medium. Cultures were transferred during the exponential phase to 500 mL non-treated, sterile polystyrene flat flasks (Thermo Scientific™ Nunc™) and grown at a constant temperature of 24°C. Salinity was adjusted to 36 by adding autoclaved Milli-Q water, and illumination was provided by fluorescent tubes with a photon irradiance of 100 mmol photons m^{-2} s^{-1} under a 12:12-h light:dark photoperiod. Cultures were acclimated to laboratory conditions for at least ten generations (three weeks). The exponential phase lasted for five days, and at the stationary phase (three weeks, density > 10^4 cells L^{-1}) cells were collected on a 0.45 µm nylon filter (Whatman®, GE). Filters were stored at –20ºC until toxin extraction.

Cell counting and measurements

For cell counting, fixed field water samples were settled in 3 mL Utermöhl chambers for three hours before observation with an inverted Nikon Eclipse TE2000-S microscope. The entire bottom of the chamber was examined at 200x magnification to enumerate the larger organisms, and one/two transects at 200x or five/ten fields at 400x magnification were examined to count the small and more abundant organisms. Dinoflagellates were identified to genus except for *Prorocentrum lima*, *O.* cf. *ovata* and *O. lenticularis*. Epiphytic samples were expressed as cells per gram of fresh weight of macroalgae (cells g^{-1} fw) and as cells

per cone surface area (cells cm^{-2}) for conical shaped invertebrates, using the following equation:

$$surface = \neq r\sqrt{h^2 + r^2}$$

where r is base radius, and h is height.

Ostreopsis cells were measured from fixed water samples obtained from macroalgae in Tortuga Bay. In addition, *Ostreopsis* cells from laboratory cultures were measured during the exponential phase (5 days); dorsoventral (DV) and width (W) diameters were recorded using an image capture system (MCDI™ Analysis) with an Olympus DP70 camera connected to an inverted microscope (Nikon Eclipse 80i) at 400× magnification.

Morphological identification

Cultured cells were fixed at the exponential phase with a stock formaldehyde solution (37%) to a final concentration of 4%, examined and photographed in a Hitachi S-3500N scanning electron microscope (SEM) at a working distance of 5 to 6 mm and a voltage of 5.0 kV after a preliminary wash in distilled water followed by dehydration in a series of ethanol solutions of increasing concentration (30, 50, 70, 90 and 100%), critical point drying with pin-type stubs and sputter coating with gold-palladium using a Quarum Q150RS (Quorum Technologies, Newhaven, East Sussex, U.K.). Some strains were analysed under the inverted microscope (Nikon Eclipse 80i) after staining with fluorescent Calcofluor White M2R, based on the Fritz and Triemer (1985) technique.

Molecular identification

For DNA analysis, 15 mL of culture were transferred to plastic Eppendorf vials and centrifuged for 10 min at 2500 rpm. Resulting pellets were stored at –20°C until DNA extraction, following Andree et al. (2011). Primers used for the polymerase chain reaction (PCR) were ITSA (5′ - GTA ACA AGG THT CCG TAG GT - 3′) and ITSB (5′ - AKA TGC TTA ART TCA GCR GG - 3′), previously described by Sato et al. (2011), and the Taq DNA polymerase was from Invitrogen. ITS and 5.8S ribosomal DNA (rDNA) regions were amplified in an Applied Biosytems 2720 Thermal cycler (initial 5 min heating step at 94°C, 30 cycles at 94°C for 1 min, at 55°C for 2 min, and at 72°C for 3 min, and a final extension at 72°C for 10 min. Resulting fragments of approximately 400-base pair (bp) rRNA were evaluated by electrophoresis in agarose gel (1.5% wt/vol) stained with GelRed™ (Biotium Inc., Hayward, CA, USA) and were sent to be sequenced (GENOSCREEN, Paris, France). Amplicons were read by direct sequencing using the same primers as those applied for the initial amplification. Each amplicon was sequenced bi-directionally to resolve any ambiguities in the electropherograms that might have been attributed to polymorphisms.

Sequences were aligned using the CLUSTAL W utility built into MEGA X, and small adjustments were subsequently made to correct the alignment where needed, using the more conserved 5.8S rDNA sequence as an anchor guide to align sequences from all taxa.

The evolutionary history was inferred using the maximum likelihood method and Tamura 3-parameter model+G (Tamura 1992), conducted in MEGA X (Kumar et al. 2018). The least complex phylogenetic model was chosen as that with the lowest BIC score as indicated in the model test utility built into MEGA X. Initial tree(s) for the heuristic search were obtained automatically by applying neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach. The final dataset analysed included 73 nucleotide sequences, each containing 291 positions.

Toxin extraction

Nylon filters from the 16 clonal *Ostreopsis* cultures described above were added to methanol:water (80:20) and sonicated (Vibra-Cell™ Ultrasonic Liquid Processor VCX 750) in pulse mode for 10 min while being cooled in an ice bath before centrifugation (600 × g for 10 min). The supernatant was filtered through 0.22 μm polytetrafluorothylene membrane syringe filters (Kinesis Ltd.). This procedure was repeated twice, and the final volume was adjusted to 10 mL.

Hemolytic assay

The hemolytic assay protocol given in Riobó et al. (2008) was followed. A calibration curve was made using PLTX standard (extracted from *Palythoa tuberculosa*) from Wako Chemicals GmbH, (Neuss, Germany) dissolved in methanol:water (1:1) to a concentration of 25 ng PLTX mL^{-1}. Toxin extracts and PLTX standard were evaporated and refilled with phosphate buffered saline solution (PBS) to eliminate methanol and water from the extraction. A calibration curve was performed with 12 concentrations of standard from 12.5 to 1250 pg PLTX mL^{-1} adjusted to an exponential regression. The working solution was prepared with washed sheep blood (OXOID), centrifuged (4000 × g, 10°C, 10 min) twice and diluted with PBS 0.01 M, pH 7.4 (Sigma), 0.1% bovine serum albumin (BSA), 1 mM calcium chloride ($CaCl_2.2H_2O$) and 1 mM boric acid (H_3BO_3) to a final concentration of 1.5 10^6 cells mL^{-1}. The assay for PLTX specificity was verified by a blank assay with ouabain (1 mM final concentration). The assay was performed in two non-treated 96 well microplates, and samples were analysed in triplicate. After 22 h of incubation at 24°C, microplates were centrifuged (416 × g, 10 min), and 200 μL of the supernatant was transferred to another microplate for absorbance reading by a KC4 microplate reader from BioTec Instruments, Inc. (Winooski, VT, USA) at 405 nm absorbance.

LC-HRMS toxin analysis

The liquid chromatography–high-resolution mass spectrometry (LC-HRMS) conditions were those of Ciminiello et al. (2015). The analyses were performed using a Q-Exactive Orbitrap mass spectrometer coupled to

an Accela AS LC system (Thermo Fisher, San José, CA, USA). The organic solvents (LC-MS grade) and reagents used for LC-MS analysis were purchased from Sigma Aldrich. An Accucore C18 column (2.6 μm, 100×2.1 mm; Thermo Fisher) was eluted at 0.2 mL/min with water (eluent A) and acetonitrile (eluent B), both containing 0.1% formic acid. The gradient elution used was 26% to 29% B over 15 min, 29% to 99% B in 1 min, hold 3 min, 99% to 26% B in 0.5 min, and hold 10.5 min. The injection volume was 10 μl, and the oven temperature was 30°C. HR full MS experiments (positive ionization) were acquired in the range of m/z 700-2000. The following source settings were used: spray voltage = 3200 V; capillary temperature = 250ºC; sheath gas flow = 49; and auxiliary gas flow = 10 arbitrary units. Resolving power was set at 70000 (FWHM at m/z 400).

Palytoxin standard (from *Palythoa tuberculosa*) and strain IRTASMM-11-10 of *Ostreopsis* cf. *ovata* from the northwestern Mediterranean Sea (García-Altares et al. 2014), extracted as described above, were used as references to check for retention times and ionization behaviour of PLTX, isobaric PLTX and ovatoxin (OVTX)-a to -g. The instrumental limit of quantification was estimated to be comparable to that of the method reported in García-Altares et al. (2014), which was 6 ng mL^{-1}. The presence of both known and unknown PLTX-like compounds was investigated using their characteristic ionization profile of PLTXs, typically containing triply charged ions in the region m/z 830-950 and doubly charged ions in the region m/z 1250-1400 (Ciminiello et al. 2011).

RESULTS

Epiphytic dinoflagellate assemblage

Benthic dinoflagellate abundances were estimated in 18 samples: two samples of sessile benthic invertebrates and 16 of macroalgae. Invertebrate samples of *Tetraclita* sp. were collected at Santa Fé Island, and macroalgae were taken from the two other sampling sites at Santa Cruz Island; 11 samples were from Tortuga Bay and five were from Venecia Bay (Table 1).

The "small cell morphotype" of *Ostreopsis* was the dominant species among the benthic dinoflagellate assemblage in 10 out of 18 samples, representing more than 90% of the dinoflagellate assemblage in five samples (Table 1). The maximum abundance of the "small cell morphotype" of *Ostreopsis* (33405 cells g^{-1} fw) was found on *Dictyopteris* sp. (Phaeophyceae: Dictyotales), where a brownish mucilage was easily observed. The "large cell morphotype" of *Ostreopsis* was only found on this macroalgal species (maximum abundance of 4995 cells g^{-1} fw). *Prorocentrum* spp. dominated in three samples, with a maximum abundance of 5300 cells g^{-1} fw on *Pterocladia* sp. (Florideophyceae: Gelidiales). *Amphidinium* spp. showed the highest abundance in three samples, with a maximum of 4344 cells g^{-1} fw on *Gracilaria* sp. (Florideophyceae: Gracilariales). *Gambierdiscus* spp. was present in four samples, two from each site, in low abundances (maximum of 588 cells g^{-1} fw) (Table 1).

Table 1. – Cell abundance estimation in cells g^{-1} fw of macroalgae and in cells cm^2 for invertebrates (*Tetraclita* sp.); percentage of dominance is indicating in bold.

Sampling Site	Date	Substrate	*Ostreopsis* spp. Small-cell morphotype		*Ostreopsis* spp. Intermediate-cell morphotype		Large-cell morphotype		*Coolia* spp.		*Amphidinium* spp.		*Prorocentrum* spp.		*P. lima*		*Gambierdiscus* spp.	
Santa Fé	29/03/2017	*Tetraclita* sp.	2924	**97**	0		0		0		15	**1**	5	**<1**	55	**2**	0	
			11590	**98**	0		0		101	**1**	55	**<1**	0		25	**<1**	0	
Tortuga Bay	30/03/2017	*Gracilaria* sp.	5233	**49**	0		0		341	**3**	1809	**17**	1923	**18**	1339	**13**	0	
			1601	**58**	0		0		45	**2**	19	**1**	370	**13**	708	**26**	0	
			3857	**35**	0		0		136	**1**	4344	**39**	1656	**15**	1071	**10**	0	
		Caulerpa sp.	1950	**12**	150	**1**	0		1500	**9**	2100	**13**	4650	**29**	5700	**36**	0	
			72	**5**	0		0		0	**0**	0	**0**	648	**46**	612	**44**	72	**5**
			126	**31**	0		0		86	**21**	75	**18**	80	**20**	37	**9**	0	
		Chlorophyta	46	**6**	0		0		11	**1**	5	**1**	409	**52**	312	**40**	0	
		Dictyopteris sp.	30171	**97**	461	**1**	230	**1**	0		0		0		230	**1**	0	
			9995	**97**	50	**<1**	165	**2**	0		33	**<1**	0		66	**1**	17	**<1**
			33405	**82**	0		4995	**12**	624	**2**	1561	**4**	0		0		0	
		Dictyota sp.	3037	**92**	0		0		0		269	**8**	0		0		0	
Venecia Bay	06/04/2017	Rhodophyta	882	**10**	0		0		2058	**23**	2940	**33**	2058	**23**	294	**3**	588	**7**
		Padina sp.	214	**36**	0		0		91	**15**	118	**20**	85	**14**	91	**15**	0	
			221	**9**	15	**1**	0		0		88	**3**	1253	**48**	1017	**39**	0	
		Caulerpa sp.	298	**19**	0		0		256	**16**	511	**32**	256	**16**	256	**16**	0	
		Pterocladia sp.	3313	**23**	95	**1**	0		3313	**23**	1798	**13**	5300	**37**	189	**1**	284	**2**

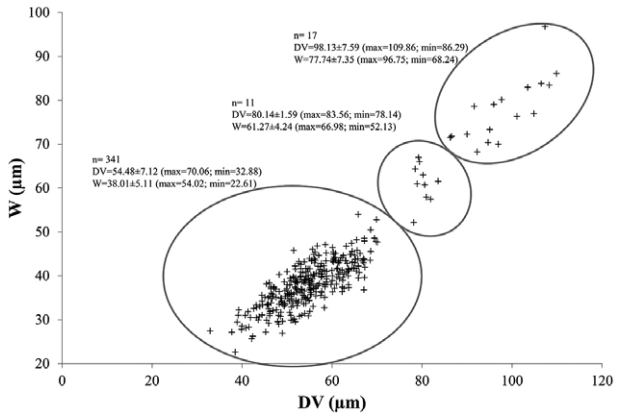

Fig. 2. – Dorsoventral (DV) and width (W) diameters of *Ostreopsis* cells from field samples, n=369.

Field morphology of *Ostreopsis*

A total of 369 *Ostreopsis* cells were measured from the epiphytic dinoflagellate samples obtained from macroalgae in Tortuga Bay, Santa Cruz Island. Three different morphotypes based on DV and W diameters were distinguished (Fig. 2). The small-cell morphotype corresponded to cells with a tear-drop shaped small size (mean±standard deviation; DV=54.48±7.12 µm; W=38.01±5.11 µm; DV/W=1.43). The group with the largest size had a more broadly oval shape, the large-cell morphotype (DV=98.13±7.59 µm; W=77.74±7.35 µm; DV/W=1.26), with a maximum of DV of 109.86 µm and W of 96.75 µm. An intermediate-cell morphotype was observed, having an elongated conical shape compared with the large-cell morphotype, and was larger than the small-cell morphotype (DV=80.14±1.59 µm; W=61.27±4.24 µm; DV/W=1.31) (Fig. 2).

Isolated strains

A total of 16 strains were isolated, 13 from the "small-cell morphotype" and three from the "large-cell morphotype" from field samples in Tortuga Bay. No isolates of the intermediate size were successfully established in culture.

Phylogenetic analysis

The PCR amplifications of 5.8S rDNA and ITS regions obtained from the 16 isolates were aligned to-

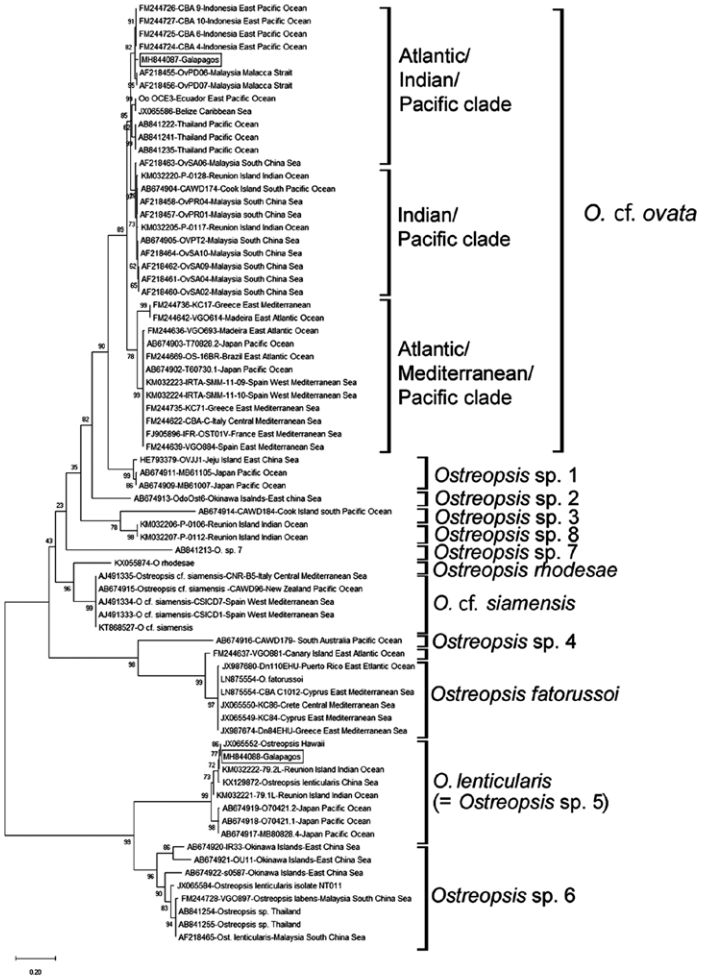

Fig. 3. – Evolutionary relationships of *Ostreopsis* spp. 5.8S rDNA and ITS regions. Bootstrap values (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The tree with the highest log likelihood (-3268.95) is shown and is drawn to scale, with branch lengths measured in the number of substitutions per site. The percentage of trees in which the associated taxa clustered together is shown next to the branches.

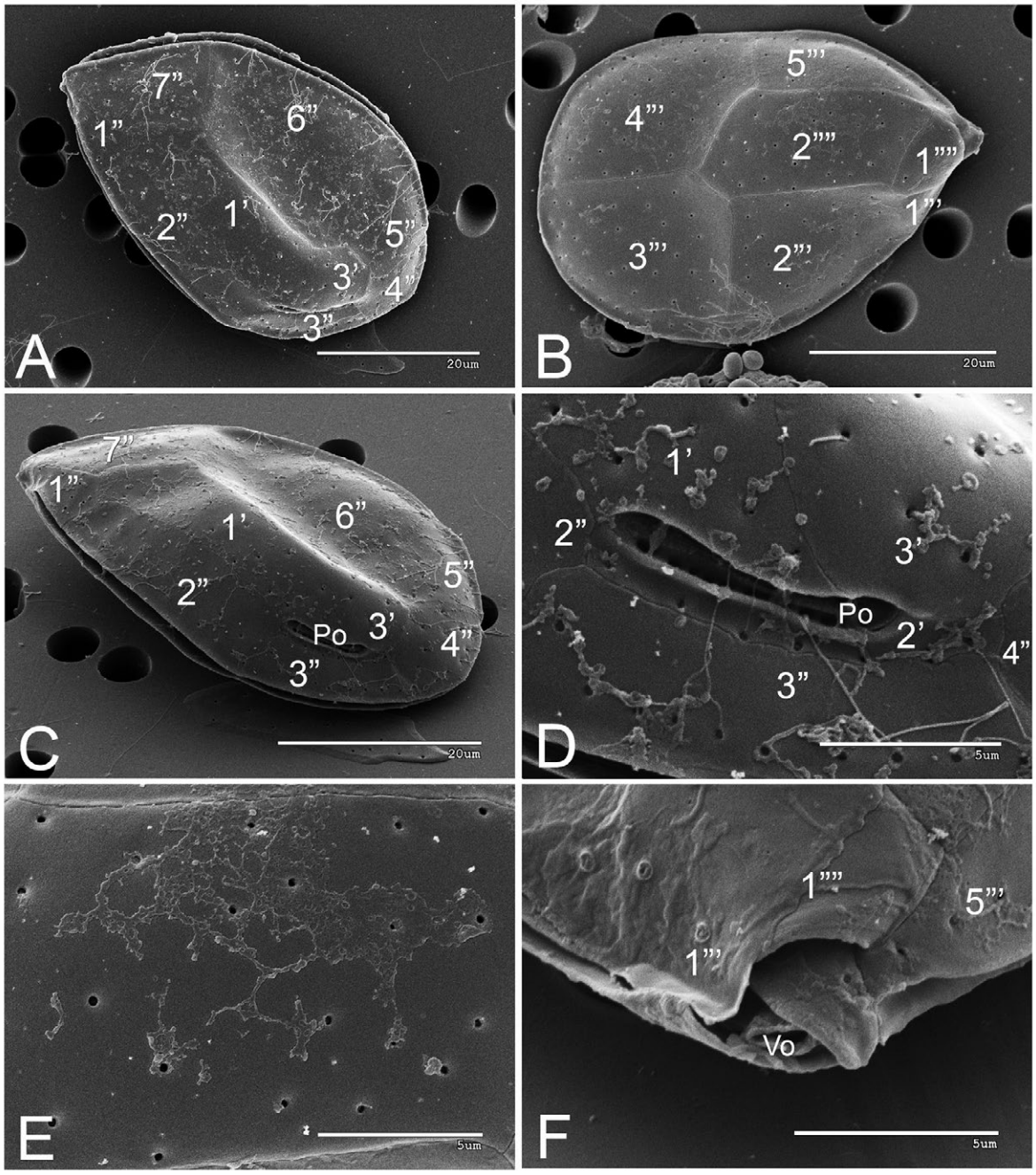

Fig. 4. – Thecal morphology of *Ostreopsis* cf. *ovata* (strain 1G) viewed with scanning electron microscopy. A, apical (epithecal) view; B, antapical (hypothecal) view; C, anterior-dorsal-left-side view; D, the apical pore plate and adjacent epithecal plates in left-side view; E, a fragment of the 1 plate with irregularly scattered trichocyst pores; F, a fragment of the hypotheca and the sulcal area. Plate labels: 1′-3′, the apical plates; 1″-7″ the precingular plates; 1‴-5‴, the postcingular plates; 1⁗ and 2⁗, the antapical plates; Po, the apical pore plate; Vo, the ventral opening. The plates are named according to Hoppenrath et al. (2014). Scale bars: 20 μm in A-C, 5 μm in D-F.

gether with other sequences from GenBank. Thirteen strains that shared identical sequences corresponding to the “small-cell morphotype” clustered in the Atlantic/Indian/Pacific clade of *O.* cf *ovata* (GenBank accession number MH844087 for the strain 1G), and three strains with identical sequences corresponded to the “large-cell morphotype” in *O. lenticularis* (= *Ostreopsis* sp. 5) (GenBank accession number MH844088 for the strain 17G) (Fig. 3). The same tree topology was obtained using Bayesian inference (data not shown), with one caveat being that the Atlantic/Indian/Pacific clade was bifurcated into two clades, one group more distal to the Indian Pacific clade and one group containing the isolates from the Galapagos Islands in the more proximal clade.

Morphological descriptions

Small-cell morphotype – Ostreopsis *cf.* ovata

Cells were oval-pointed and tear-drop shaped, tapering ventrally in apical/antapical view and anter-

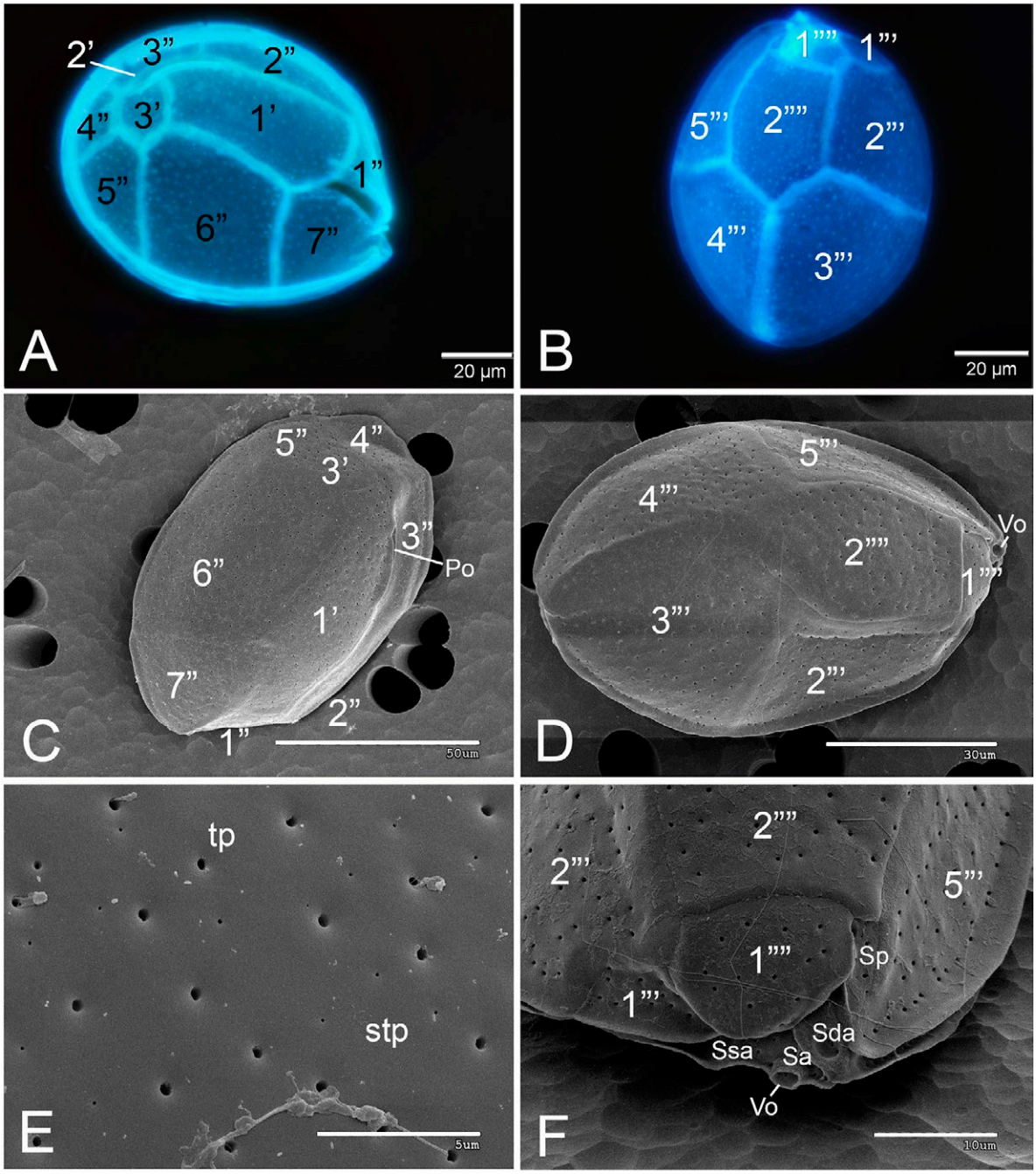

Fig. 5. – Thecal morphology of *Ostreopsis lenticularis* (strain 17G) (A and B, cells stained with Calcofluor White M2R viewed with epifluorescence microscopy; C-F, cells viewed with scanning electron microscopy). A and C, apical (epithecal) view; B and D, antapical (hypothecal) view; E, a fragment of a thecal plate, with the irregularly scattered trichocyst pores and small pores; F, a fragment of the hypotheca and the sulcal area. Plate labels: 1′-3′, the apical plates; 1″–7″, the precingular plates; 1‴-5‴, the postcingular plates; 1"" and 2"", the antapical plates; Po, the apical pore plate; Sa, the anterior sulcal plate; Sda, the right sulcal plate; stp, small thecal pores; Sp, the posterior sulcal plate; Ssa, the left sulcal plate; tp, the trichocyst pores; Vo, the ventral opening (also known as the ventral pore). The plates are named according to Hoppenrath et al. (2014). Scale bars: 20 µm in A and B; 50 µm in C; 30 µm in D; 5 µm in E; 10 µm in F.

oposteriorly compressed. Cells were measured from five different *O.* cf. *ovata* strains (1G, 3G, 5G, 10G and 11G), a total of 477 cells, DV=44.73±5.62 µm (max=57.92 µm; min=28.26 µm); W=32.32±5.35 µm (max=48.71 µm; min=18.57 µm); DV/W=1.39±0.12. Plate 1′ is large, elongated, subhexagonal, slightly shifted to the left side of the cell, about 3.5 to 4 times long as it is wide (Fig. 4A, C). Plate 2′ is as narrow as the latter, contacts plate 4″ (Fig. 4D), and plate 3′ is small and hexagonal. The Po plate is moderately long, slightly shorter than plate 2′ (Fig. 4D). Plate 2"" is pentagonal, relatively short, about half the DV diameter, slightly shifted to the right side of the cell, with almost straight longitudinal sides parallel to each other, of the same width in its anterior and posterior parts, its contact with 4‴ about 1.5-2 times longer than with 3‴ (Fig. 4B). Thecal pores are of one type: 0.19-0.23 µm in diameter (Fig. 4D, E).

Large-cell morphotype – Ostreopsis lenticularis

Cells were broadly oval-pointed and lenticular, tapering ventrally in apical/antapical view, anteroposteriorly compressed. Cells were measured from one *O. lenticularis* (=*Ostreopsis* sp. 5) strain (17G), a total of 61 cells; DV=88.49±7.22 μm (max=105.36 μm; min=70.94 μm); W=67.29±6.11 μm (max=82.69 μm; min=55.5 μm); DV/W=1.32 ±0.06. Plate 1′ large, elongated, subhexagonal, slightly shifted to the left side of the cell, more than twice as long as wide (Fig. 5A, C). Plate 3′ is small, hexagonal. Plate 2′′′′ is somewhat curved longitudinally with its convex side to the right, slightly wider in its posterior part; its contact with 4′′′ is frequently about 1.5 to 2 times longer than with 3′′′ or its contacting sides are about equal (Fig. 5B, D). Thecal pores are of two types, large (the trichocyst pores) and small (Fig. 5E). Large pores (min=0.20 μm; rarely) 0.28 to 0.35 μm, and small thecal pores (min=0.04 μm; rarely) 0.07 to 0.12 μm. The ventral opening (the ventral pore) is 2 μm in diameter (Fig. 5F).

Toxin profile

The 16 toxin extracts analysed (13 from *O.* cf. *ovata* and 3 from *O. lenticularis*) were below the limit of detection of the haemolytic assay (25 pg PLTX mL^{-1}) and proved to be non-toxic. This result was supported by the absence of PLTX-like compounds, both known and unknown, in the analysis by LC-HRMS. Figure 6 shows the total ion chromatograms and full scan MS spectra of reference materials (PLTX standard and *O.* cf. *ovata* IRTASMM-11-10), showing the characteristic clusters of triply charged ions in the region m/z 830–950 and doubly charged ions in the region m/z 1250–1400 (Ciminiello et al. 2011). Mass errors between theoretical and experimental accurate mass of the monoisotopic peak of $[M+3H-H_2O]3+$ions of PLTX and OVTXs (-a to -e and -g) were below 3 ppm.

The LC-HRMS conditions applied in this study were those of Ciminiello et al. (2015), which have been used to report the detection of PLTXs in several studies (García-Altares et al. 2014, Tartaglione et al. 2016, 2017). The instrumental limit of quantitation was estimated to be of the same order of magnitude as in other studies that reported the detection of PLTXs (6 ng PLTX mL^{-1}). Moreover, chromatograms and mass spectra were manually explored to look for the characteristic ionization pattern of palytoxins to find potentially unknown analogues. It is therefore unlikely that the lack of toxicity was due to the insensitivity of the detection methods used.

DISCUSSION

This study is the first accurate report of *O.* cf. *ovata* and *O. lenticularis* in the GMR and confirms the presence of potentially toxic benthic dinoflagellate species. Since the early 20th century, species of the genera *Gambierdiscus*, *Ostreopsis, Prorocentrum, Coolia* and *Amphidinium* have been reported in tropical and subtropical regions such as the eastern (Vargas-Montero et al. 2012, Maciel-Baltazar 2015) and western Pacific Ocean (Rhodes et al. 2017), the Indian Ocean (Car-

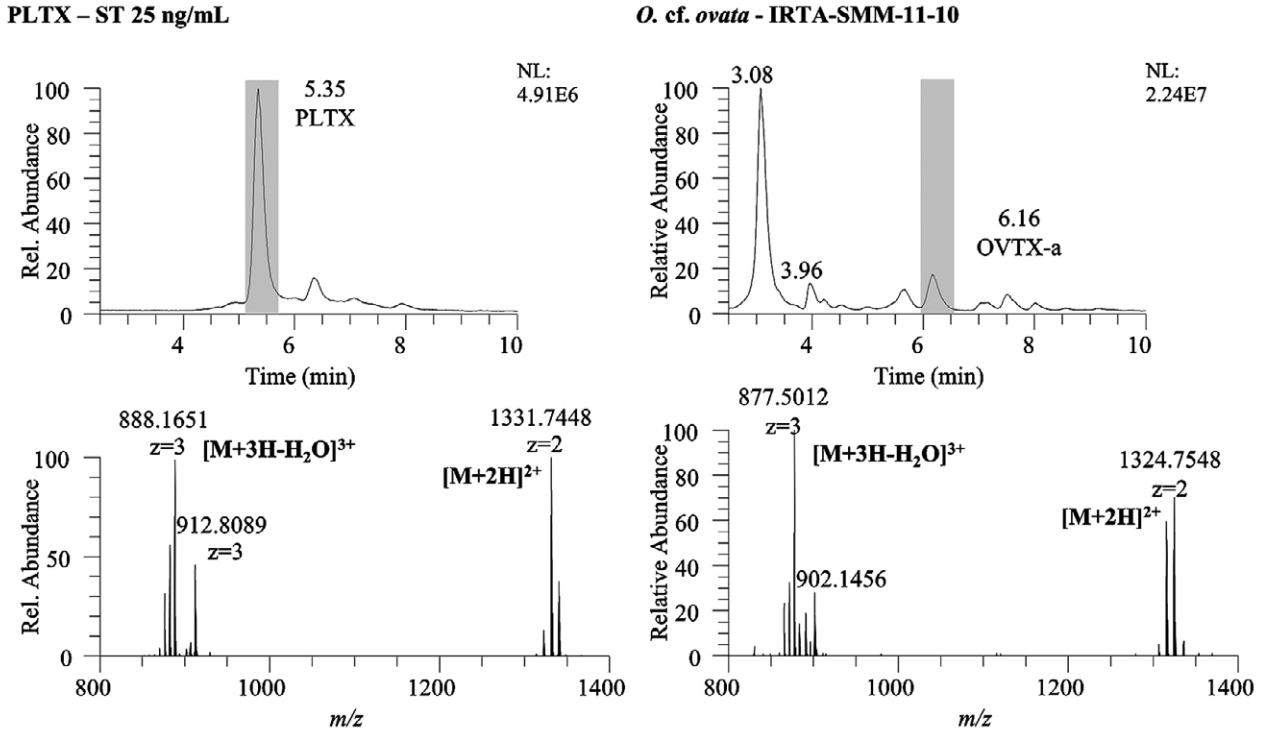

Fig. 6. – LC-HRMS analysis (total ion chromatograms and full scan MS) of PLTX standard (25 ng mL^{-1} from *Palythoa tuberculosa*) and methanolic extracts of *Ostreopsis* cf. *ovata* strain IRTA-SMM-11-10 (toxin profile described in García-Altares et al. 2014), used as a reference sample in the present study for the detection of PLTX-like compounds.

nicer et al. 2015), the west Atlantic Ocean (Mendes et al. 2017) and the Caribbean Sea (Irola-Sansores et al. 2018, Boisnoir et al. 2019). A recent study highlights the potential ecological and sanitary risks to Mexican coasts associated with the presence of *Gambierdiscus*, *Ostreopsis* and *Prorocentrum,* with special attention to the importance of an accurate genetic and toxic identification of these species of these genera (Núñez-Vázquez et al. 2019). The absence of these specific data prevents accurate determination of the potential impacts on marine ecosystems and human health because morphological features are not sufficient to describe a species, and toxin production is unevenly distributed among species and even among strains of the same species (Litaker et al. 2010, Suzuki et al. 2012, Carnicer et al. 2016a).

This is the case for the eastern tropical Pacific (ETP), where there is little information on toxic benthic dinoflagellates (Durán-Riveroll et al. 2019). This area of the globe is of special concern because the marine ecosystem is sensitive to climate change and to El Niño-Southern Oscillation events (Edgar et al. 2010), affecting biodiversity due to changing temperatures and rainfall that, in turn, can influence the distribution of certain species that can adapt to new conditions (Keith et al. 2016). The studies from the ETP are limited to Colombia (Quintana-Manotas and Mercado-Gómez, 2017), where *Coolia* sp., *O. lenticularis*, *O. ovata*, *P. emarginatum* and *P. lima* were recorded, and Costa Rica (Coco Island) (Vargas-Montero et al. 2012), with the presence of *Gambierdiscus* spp., *C. tropicalis*, *C*. cf. *areolota, P. concavum*, *P. compressum, Amphidinium carterae* and *O. siamensis.* Unfortunately, none of these studies included nucleic acid sequencing or toxicity analysis, making the correct identification of species difficult. A third study was performed along the northern and central coasts of Ecuador (Esmeraldas and Manta provinces), where the *Padina* sp. epiphytic community was sampled in 2015 (Carnicer et al. 2016a). *O.* cf. *ovata,* Atlantic/Indian/Pacific clade, non-toxic*, P. lima* and *Coolia* spp were present, but *Gambierdiscus* species were not observed (O. Carnicer, pers. comm.).

In the GMR, investigations have focused on planktonic species during several cruises undertaken by the Naval Oceanographic Institute of Ecuador (INOCAR). The first record of a benthic dinoflagellate was of the genus *Ostreopsis,* reported by the institution's journal (Torres and Andrade 2014). They identified *O. siamensis* on Baltra Island (located north of Santa Cruz Island) from surface seawater samples collected in shallow areas in 2005. However, samples were only observed with a light microscope, so a misidentification may have occurred. For example, small tear-drop shaped cells such as *O.* cf. *ovata*, *O*. cf. *siamensis*, *O. fattorussoi* and *O. rhodesiae* are not distinguishable solely by light microscopy (Accoroni et al. 2016, Verma et al. 2016), and molecular techniques are mandatory for correct identification. In 2017, during the study period in the GMR, *Ostreopsis* cf. *ovata* and *Ostreopsis* cf. *lenticularis* (based on light microscopy observations) were reported from 2 to 10 miles from Santa Cruz Island and islands nearby. Their presence in the water column and the high epibionthic abundances suggest that there may be proliferations in some areas of the GMR that have not been reported. A brownish mucilage has been observed previously in the Archipelago (I. Keith, pers. comm.), but there is no confirmation of the species involved in those events. Further monitoring should be performed covering a larger area of the GMR to evaluate the presence of benthic HAB.

O. cf. *ovata* has been extensively studied, and there are many sequences from different regions around the world, because it is the most widely distributed species of the genus (Accoroni and Totti 2016). According to Hoppenrath et al. (2014), without a genetic characterization of *O. ovata* from the type locality, it is presently not possible to conclude which genotype corresponds to this species; therefore, most authors have reported *O*. cf. *ovata.* Phylogenetically, the *O.* cf. *ovata* species complex has been divided into three clades (Penna et al. 2014). These include the: i) Atlantic/Mediterranean/Pacific, ii) Indian/Pacific, and iii) Atlantic/Indian/Pacific clades. In clade i) all strains produce PLTX-like compounds such as isobaric PLTX and OVTX analogues (e.g., Ciminiello et al. 2013), with the exception of three strains from Japan reported as non-toxic by Suzuki et al. (2012). Clade ii) includes OVTX producing strains (Suzuki et al. 2012, Uchida et al. 2013), ostreol-A producers (a non-PLTX derivative compound) (Hwang et al. 2013) and non-toxic strains (Suzuki et al. 2012, Carnicer et al. 2015). In clade iii) some strains displayed toxicity in mouse bioassays (Tawong et al. 2014) and hemolytic assays (Penna et al. 2010), but the clade also includes non-toxic strains (Carnicer et al. 2016a). The present study contributes additional physiological information on the characterization of the *O*. cf. *ovata* species complex by adding a strain from a geographical area not previously sampled. The *O.* cf. *ovata* strain from the GMR belongs to clade iii), as do the strains sequenced to date from the coasts of Ecuador (Carnicer et al. 2016a), Belize (Penna et al. 2014), Indonesia (Penna et al. 2010), Thailand (Tawong et al. 2014) and Malaysia (Leaw et al. 2001). As for the strains isolated from Ecuador and Belize, *O.* cf. *ovata* strains from the GMR are non-toxic.

The GMR is influenced by the convergence of three major currents that contribute to its unique environmental conditions favouring its high biodiversity (Muromtsev 1963, Banks, 2002, Hickman 2009). The South Equatorial Current flows westward and shows a marked seasonality. More intense cold-salty waters come during the dry season (June-November) with the Humboldt Current influenced by southern winds, while during the wet season (December-May) warmer waters come with the Panama Current. Eastward flowing, the Equatorial Undercurrent upwells in the western islands of the GMR, increasing primary production (Schaeffer et al. 2008). Thus, microalgal colonization from the western Pacific Ocean, as well as from Central America to the Archipelago, may have occurred. It is suspected that *O*. cf. *ovata* populations were separated by the Isthmus of Panama, and subsequent genetic differentiation took place (Penna et al. 2010). This

hypothesis is supported by *O*. cf. *ovata* strains from the western Atlantic (Brazil), which are genetically clustered with the eastern Atlantic and Mediterranean strains (Nascimento et al. 2012) and produce OVTX analogues. However, strains from the Caribbean Sea are genetically clustered with the eastern Pacific and Galapagos strains. Further molecular studies need to be undertaken in the Caribbean Sea and along the eastern Pacific coast to validate the assumption of an introduction of cells through the Panama Chanel with ballast waters (Carnicer et al. 2016a).

At least one other species of *Ostreopsis* has been identified in this study. This species fell in *Ostreopsis* sp. 5 (Sato et al. 2011). Morphologically, it resembles the original description of *O. lenticularis*, but until recently (Chomérat et al. 2019) its known genetic clade assignment could not be used to unambiguously establish these isolates as *O. lenticularis* because it was described by Fukuyo (1981) prior to routine molecular characterization. The absence of the undulation of the cingulum in side view was not verified, although, according to Fukuyo (1981), it is a morphological feature that distinguishes *O. lenticularis*, which possesses additional minute thecal pores, from *O. siamensis*, which does not. This is in agreement with Hoppenrath et al. (2014), who suggested that the species under the name of *O. lenticularis* in Faust et al. (1996) with only one type of pore belongs to another species. In addition, the species illustrated under the name of *O. siamensis* in Faust et al. (1996: Figs 2-8) is described with the two types of pores consistent with the original *O. lenticularis* description. Cortés-Lara et al. (2005) illustrated two pore size classes in *O. siamensis* from the Mexican Pacific and Penna et al. (2005) in *O. ovata* from the western Mediterranean. Aligizaki and Nikolaidis (2006) also reported two types of pores in *O. ovata* and *O*. cf. *siamensis*, which makes delimitation of *O. lenticularis* even more complicated. The terms used in the literature in the description of the cell shape are vague and rather confusing, especially when the dorsoventral diameter/width (DV/W) ratio, which can be a useful feature for separating *Ostreopsis* spp. (Hoppenrath et al. 2014), is not given. The morphology of the sulcal plates in *Ostreopsis* spp. remains poorly examined. Similarly, in our study only close-ups of the sulcal area viewed ventrally-antapically are presented (Figs 4F and 5F), revealing some details that we were unable to compare with the published data on the same plates.

The strains of *O. lenticularis* recently isolated by Chomérat et al. (2019) from the type locality (Tahiti Island) cluster with the sequences previously ascribed to *Ostreopsis* sp. 5. The morphological features of the *O. lenticularis* strains isolated by Chomérat et al. (2019), such as the presence of two types of thecal pores on the theca, are in agreement with the original description, and those authors suggest that this character be used to distinguish *O. lenticularis* from other large species. To confirm the findings obtained by Chomérat et al. (2019), all the known morphological, morphometrical, molecular and toxicity data for *O. lenticularis* and related species are assembled in Table 2 to determine how strongly the preponderance of data supports *Ostreopsis* sp. 5 compared with the closely related *Ostreopsis* sp. 6 being *O. lenticularis.* Comparing cell sizes, strains of *Ostreopsis* sp. 5 (references in Table 2) fit better with the original description of *O. lenticularis* in Fukuyo (1981) (60-100 (DV); 45-85 (W) μm), whereas *Ostreopsis* sp. 6 (references in Table 2) are, in general, smaller cells that do not exceed 85 μm in DV diameter and correspond better to the original description of *O. labens*: 60-86 μm (DV), 70-80 μm (W) (Faust and Morton 1995). The presence of the two types of thecal pores has been considered the main diagnostic feature of *O. lenticularis,* which differentiates it from other *Ostreopsis* spp. From the morphological descriptions available for *Ostreopsis* sp. 5, there are at least two different types of pores, whereas only one type of pore was observed in *Ostreopsis* sp. 6 (Table 2).

The first ribosomal sequences for a strain from Malaysia identified as *O. lenticularis* were presented by Leaw et al. (2001), but the study lacks the necessary morphological description to confirm whether it corresponds to the original description of the species. In subsequent publications, *O. labens* was clustered in the same genetic clade (Penna et al. 2010), and after the addition of three new sequences to the clade it was then called *Ostreopsis* sp. 6 (Sato et al. 2011). However, in a recent study conducted in the China Sea (Zhang et al. 2018), *O. lenticularis* was clustered in another genetic clade, *Ostreopsis* sp. 5, together with the strains from Reunion Island (Carnicer et al. 2015) and three strains isolated from Japan (Sato et al. 2011), posing a new taxonomic question of whether *Ostreopsis* sp. 5 or *Ostreopsis* sp. 6 corresponds to *O. lenticularis*. This was resolved by Chomérat et al. (2019), who found that *Ostreopsis* sp. 5, a non-toxic species, is *O. lenticularis,* and *Ostreopsis* sp. 6 corresponds to a different species.

Another interesting pattern observed is related to toxin content. There is a homogeneity for *Ostreopsis* sp. 5 strains from Japan (Suzuki et al. 2012), Reunion Island (Carnicer et al. 2015) and the Galapagos (this study), which are non-toxic (Table 2). Within *Ostreopsis* sp. 6, there is higher variability in cell toxicity, including observations of toxic strains detected by hemolytic and mouse bioassays (Penna et al. 2010, Tawong et al. 2014), producers of ostreocin-d (Suzuki et al. 2012) and PLTX analogues (Moreira et al. 2012), as well as non-toxic strains (Suzuki et al. 2012) (Table 2).

In summary, the present study provides a description of epibionthic dinoflagellate assemblages from three sites of two southern islands in the GMR (Santa Cruz and Santa Fé) in March and April 2017. The potentially toxic genera of *Amphidinium, Coolia, Gambierdiscus* and *Ostreopsis* were found, the latter with abundances up to 38400 cells g^{-1} fw. The presence of these genera represents a potential threat to humans and to marine ecosystems. Thus, it is important to consider benthic dinoflagellate species in the surveillance of HAB in the GMR. This study also provides the first correct characterization of *Ostreopsis* strains based on molecular, morphological and toxicological data, corresponding to *O.* cf. *ovata* and *O. lenticularis* in the

Table 2. – Description of rounded shaped *Ostreopsis* cells regarding ITS phylogeny, location, toxicity, size, morphology and the number of types of pores; n.d., not determined. [a] First description of the species.

Identification (morphological)	Genetic clade (ITS)	GENBANK	Location	Toxicity	Cell size (µm)	Morphology	Number of pore types	Study
O. lenticularis[a]	n.d.		French Polynesia	n.d.	60-100 (DV); 45-85 (W) field sample	SEM	2	Fukuyo 1981
O. lenticularis	n.d.		Mexican Pacific	n.d.	65-100 (DV); 50-80 (W) field sample	SEM	2	Gárate-Lizarraga et al. 2018
O. lenticularis	n.d.		Colombian Caribbean	n.d.	102.1±7 (DV); 83.8±6.4 (W) field sample	SEM	2	Arbelaez et al. 2017
O. lenticularis	n.d.		New Zealand	n.d.	70-95 (DV); 55-75 (W) field sample	SEM	2	Chang et al. 2000 (doubtful identification; see Chomérat et al. 2019)
O. lenticularis	*O.* sp. 5	AB674917/8/9	Japan	Non-toxic (LC)	n.d.	n.d.		Sato et al. 2011, Suzuki et al. 2012
	O. sp. 5	JX065552	Hawaii (Pacific)	n.d.	n.d.	n.d.		Penna et al. 2014
	O. sp. 5	KX129872	China Sea	n.d.	68-113.5 (DV); 56.5-97.3 (W) culture	SEM, Calcofluor	2-3	Zhang et al. 2018
	O. sp. 5	KM032221/2	Reunion Island (Indian Ocean)	Non-toxic (hemolytic)	103.9±5.1 (DV); 85.3±6.9 (W) field sample	Calcofluor	2	Carnicer et al. 2015
	O. sp. 5	MH844088	Galapagos (Pacific)	Non-toxic (hemolytic; LC)	88.49±7.22 (70.94-105.36) (DV); 67.29±6.11 (W) (55.5-82.69) culture 98.13±7.59 (86.29-109.86) (DV); 77.74±7.35 (68.24-96.75) (W) field sample	SEM, Calcofluor	2	This study
O. lenticularis	*O.* sp. 5	MK227240-48	French Polynesia (South Pacific Ocean)	Non-toxic (CBA-N2a, LC)	81.2±5.7 (DV); 67.5±6.1 (W) culture	SEM, Calcofluor	2	Chomérat et al. 2019
O. marina[a]	n.d.		Caribbean and Indian Ocean	n.d.	83-111 (DV); 73-85 (W) field sample	SEM	1	Faust 1999
O. labens[a]	n.d.		Caribbean and Japan	n.d.	60-86 (DV); 70-80 (W) field sample	SEM	1	Faust and Morton 1995
O. lenticularis	n.d.		Japan, Southwest Indian Ocean, Caribbean	Toxic (mouse bioassay)	65-75 (DV); 57-63 (W) field sample	SEM	1	Faust et al. 1996
O. labens	*O.* sp. 6	FM244728	Malaysia	Toxic (hemolytic)	n.d.	n.d.		Penna et al. 2010
	O. sp. 6 (LSU)	upon request authors	Caribbean	Toxic (hemolytic; LC) ***epiphytic extract***	60-85 (DV); 50-67 (W) culture	SEM	1	Moreira et al. 2012
O. lenticularis	*O.* sp. 6	AF218465	Malaysia	n.d.	64-76 (DV); 52-65 (W) culture	n.d.		Leaw et al. 2001 (doubtful identification; see Chomérat et al. 2019)
	O. sp. 6	AB841255/4	Thailand	Toxic (mouse bioassay)	62.4±8.0 (DV) 48.2±5.5 (W) culture	Calcofluor	1	Tawong et al. 2014
O. lenticularis	*O.* sp. 6	JX065584	South China Sea (Vietnam)	n.d.	n.d.	n.d.		Penna et al. 2014
	O. sp. 6	AB674920/1/2	Japan	Toxic (LC), strain AB674922; non-toxic, strains AB674920/1	n.d.	n.d.		Sato et al. 2011, Suzuki et al. 2012

GMR. The PCR amplifications of rDNA, 5.8S and ITS regions clustered the isolates obtained from 16 strains of the *O.* cf. *ovata* Atlantic/Indian/Pacific clade, and *Ostreopsis* sp. 5 (= *O. lenticularis*). The strains proved to be non-toxic according to the haemolytic assay and LC-HRMS. Morphological characters of *Ostreopsis* sp. 5 are similar to those of *O. lenticularis* according to the original description by Fukuyo (1981) as well as by Chomérat el al. (2019) regarding cell size and type of pores. Furthermore, in our study all the strains of *Ostreopsis* sp. 5 (=*O. lenticularis*) were non-toxic, revealing a possible discriminating character.

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