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Gonyaulax taylorii, a new yessotoxins-producer dinoflagellate species from Chilean waters

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ABSTRACT

In summer 2009, during a survey in Bahía Mejillones, a dense bloom of a dinoflagellate from the genus *Gonyaulax* was detected, as well as the presence of yessotoxin. Phytoplankton samples were analyzed in detail by light and scanning electron microscopy (SEM), revealing the presence of *Gonyaulax taylorii*. Morphological examination showed that the cells in the bloom fit in *Gonyaulax jollifei* Murray et Whitting sensu Dodge, subsequently classified as *Gonyaulax taylorii* by Carbonell-Moore. In this context, some inconsistencies have been found in regard to the holotype; the plate 1''' appears as two plates, 1‴ and 2‴, showing a suture that does not exist in Dodge's figure of *G. jollifei*, from where the holotype was drawn, nor within the samples collected. Therefore, this plate has been originally described erroneously as two plates named 1''' and 2''' instead of only one named 1‴. After this correction, this species has five instead of six postcingular plates. For this reason, the description of this species must be emended. Phytoplankton net samples were found to contain yessotoxin and homoyessotoxin, with concentrations below 1 pg cell^{-1}. The present study identifies, therefore, the dinoflagellate *G. taylorii* as a new source of yessotoxins.

1. Introduction

Yessotoxin (YTX) (Fig. 1) is a marine polyether compound that was first isolated in Japan, 1986 from the scallop *Patinopecten yessoensis* by authors Murata et al. (1987). Since its discovery, numerous analogues and derivatives have been described (reviewed by Domínguez et al., 2010; Paz et al., 2008). These toxins were initially included within the Diarrhetic Shellfish Poisoning (DSP) group, as they yield positive results in the traditional mouse bioassay for DSP toxins (Aune et al., 2002; Terao et al., 1990). Nevertheless, scientific evidence suggests that YTXs should be excluded from the DSP group because these compounds do not produce diarrhea or inhibit protein phosphatase activity (Alfonso et al., 2003; de la Rosa et al., 2001). Yessotoxins have been shown to have cardiotoxic effects in mice (Aune et al., 2002; Terao et al., 1990) and to be potent cytotoxins against human tumor cell lines (Bianchi et al., 2004; Konishi et al., 2004; Pérez-Gómez et al., 2006). These effects prompted European authorities to establish a maximum permitted level of 3.75 mg YTX equivalents kg^{-1} in shellfish meat (European Commission, 2013).

Satake et al. (1997) identified the dinoflagellate *Protoceratium reticulatum* (Claparède & Lachmann) Bütschli, from isolates obtained in New Zealand waters, as the first yessotoxin producer. Approximately 100 YTX analogs have been reported in *P. reticulatum* strains worldwide (reviewed by Paz et al., 2013). Among them, YTX is usually the most prominent toxin (reviewed by Paz et al., 2008), although in some cases the toxic profile is dominated by homoyessotoxin (homo-YTX) (Paz et al., 2007; Suzuki et al., 2007).

Another dinoflagellate, *Lingulodinium polyedrum* (Stein) Dodge has also been reported as a producer of YTXs. In 1996, the presence of YTX and homo-YTX was confirmed by HPLC analysis in net haul samples obtained in the Adriatic Sea during a bloom in which this species represented 99% of the total number of dinoflagellates (Tubaro et al., 1998). Some years later, the presence of YTX was reported in isolates from Spain and the United States (Armstrong and Kudela, 2006; Paz et al., 2004).

The most recent microalgal species to be identified as an YTX producer is the dinoflagellate *Gonyaulax spinifera* (Claparède and Lachmann) Diesing. In 2004, Rhodes et al. (2006) confirmed the presence of YTXs by means of ELISA analysis of single cells of *G. spinifera* from New Zealand. A few years later, Riccardi et al. (2009) reported YTX and homo-YTX in two strains of this species

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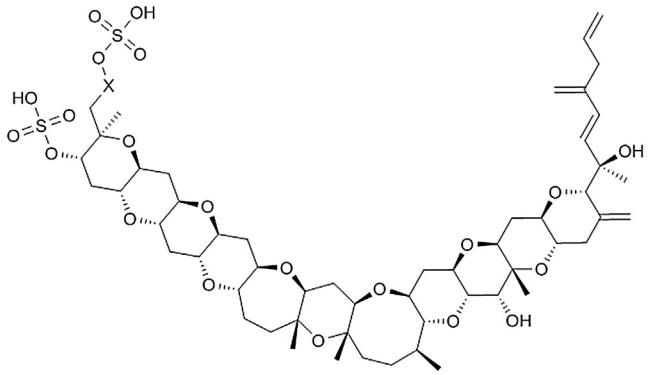

Fig. 1. Chemical structure of YTX and homo-YTX.

isolated from the Adriatic Sea (one containing only YTX, the other containing both YTX and homo-YTX).

In Chile, a country with over 4000 km of coastline, the presence of YTX has been reported in very distant locations. In southern Chile, YTX was detected for the first time in the mussel *Mytilus chilensis* from the Chonos Archipelago (43°S) (Yasumoto and Takizawa, 1997). Later, cysts and motile cells of the dinoflagellate *Protoceratium reticulatum* were also found (Seguel and Sfeir, 2003, 2005), indicating (but not providing unequivocal evidence) to this species as the YTX producer in the area. In northern Chile, YTX was first detected in phytoplankton samples from Bahía Arica (18°S), where it was suggested to be produced by *P. reticulatum* due to the presence of cysts in this species within the water column (Krock et al., 2009). Subsequently, Álvarez et al. (2011) confirmed the presence of YTX in phytoplankton samples obtained from a dense bloom of *P. reticulatum* in Bahía Mejillones (23°S) and unequivocally identified this *P. reticulatum* as the first YTX producer in the northern Chilean coast.

During the period 2007–2009, a study was conducted of toxic phytoplankton in the main scallop aquaculture sites in northern Chile, its main objective being to investigate the occurrence, taxonomy and toxin profile of toxic species. In March 2009, a survey in Bahía Mejillones simultaneously detected a dense bloom of a dinoflagellate from the genus *Gonyaulax* and the presence of YTX. In this work, a new YTX producer *Gonyaulax taylorii* is reported and its taxonomic description is reviewed in detail and emended.

2. Materials and methods

2.1. Biological samples and phytoplankton quantification

Phytoplankton samples were collected from Bahía Mejillones, Chile (23°10′S, 70°45′W) (Fig. 2) on March 29th, 2009 by means of vertical net hauls (20 μm mesh) ($n = 4$) and a 15 m hose ($n = 1$), in order to obtain integrated samples of the entire water column. One aliquot of each sample was preserved – with formaldehyde 4% (net hauls) and with Lugol's iodine (hose) – for taxonomic and quantitative analyses, respectively. Phytoplankton composition and abundance of motile cells of the species reported in this work were quantified by the Utermöhl method (Hasle, 1978), using 10 mL sedimentation chambers on an Olympus IX71 inverted microscope.

In order to obtain an approximation of the cell concentration (cell mL^{-1}) and the proportion of dinoflagellates species present in each of the net haul samples, a 0.2 mL aliquot was diluted in 11 mL of filtered seawater. Cells of *Gonyaulax* cf. *taylorii* and other dinoflagellate species were quantified by the Utermöhl method using 10 mL sedimentation chambers (Hasle, 1978).

2.2. Light microscopy

Light microscopy observations were carried out under a Leica DMLA light microscope (Leica Microsystems, Wetzlar, Germany) with phase contrast, differential interference contrast and epifluorescence. Formalin preserved samples were stained with Fluorescent Brightener 28 (Sigma, St. Louis, MO, USA) following the Fritz and Triemer (1985) technique slightly modified. Cells were dissected by pressing the cover slip over them, sometimes with the aid of sodium hypochlorite. Photomicrographs were taken with an AxioCam HRc (Carl Zeiss, Jena, Germany) digital camera. When the depth of field was insufficient for the entire object, pictures were taken at a series of different foci and automatically merged using Adobe Photoshop (Adobe Systems, San Jose, CA, USA). Any out of focus areas from each layer were erased and the layers flattened, in order to secure a final image with enhanced depth of field.

2.3. Scanning electron microscopy (SEM)

Cells in formalin preserved samples were retained onto a 5.0 μm membrane filter (Isopore, Merck, Darmstadt, Germany), washed with distilled water to remove salts and preservative, and dehydrated in a series of 30, 50, 75, 95 and 100% ethanol, followed by 100% hexamethyldisilazane. After being air-dried overnight, they were coated with gold with a K550 X sputter coater (Emitech, Ashford, Kent, UK) and observed with Phillips XL30 or FEI Quanta 200 scanning electron microscopes (FEI Company, Hillsboro, OR, USA).

2.4. Sample preparation for toxin analyses

Toxin analyses were carried out on concentrated phytoplankton net haul samples preserved with formaldehyde (4%). The cell concentration in these samples was previously quantified by the Utermöhl method. An aliquot of 0.5 mL of concentrated phytoplankton sample was mixed with 1.5 mL of methanol and sonicated with an ultrasonic cell disruptor Branson Sonic Power 450 (Danbury, CT, USA), in order to extract YTXs. The extract obtained was clarified by centrifugation (20,000 × *g*; 20 min) and then filtered through a 0.20 μm Minisart-plus glass fiber syringe filter (25 mm diameter) (Sartorius, Göttingen, Germany). Clean-up was made according to the method described by Paz et al. (2006). A 0.5 mL aliquot of the methanolic extract was mixed with 1 mL of 0.5 M ammonium acetate (pH 5.8) and loaded into a Bond Elut C18 cartridge (Agilent Technologies, Santa Clara, California) previously equilibrated with methanol: 0.5 M ammonium acetate (pH 5.8) (1:1, v/v). The YTXs were eluted using 80% methanol in water. Finally, the resulting extract was concentrated by evaporation under vacuum in a Speed Vac (Thermo Fisher Scientific, San Jose, CA, USA) and redissolved in 0.5 mL of methanol.

2.5. Liquid chromatography mass spectrometry (LC–MS/MS)

Sample analyses were performed by LC–MS/MS using an Accela uHPLC system coupled to a triple quadruple mass spectrometer TSQ Quantum Access Max (Thermo Fisher Scientific) equipped with a heated electrospray ionization (ESI) source. The temperatures in the ion transfer tube and the ESI vaporizer were set at 360 °C and 105 °C, respectively. Nitrogen (>99.98%) was employed as sheath gas and auxiliary gas at pressures of 40 and 10 arbitrary units, respectively. The ESI source was operated in negative mode at a spray voltage of 3.0 kV. The detection was accomplished in multiple reaction monitoring (MRM) mode using Argon as collision

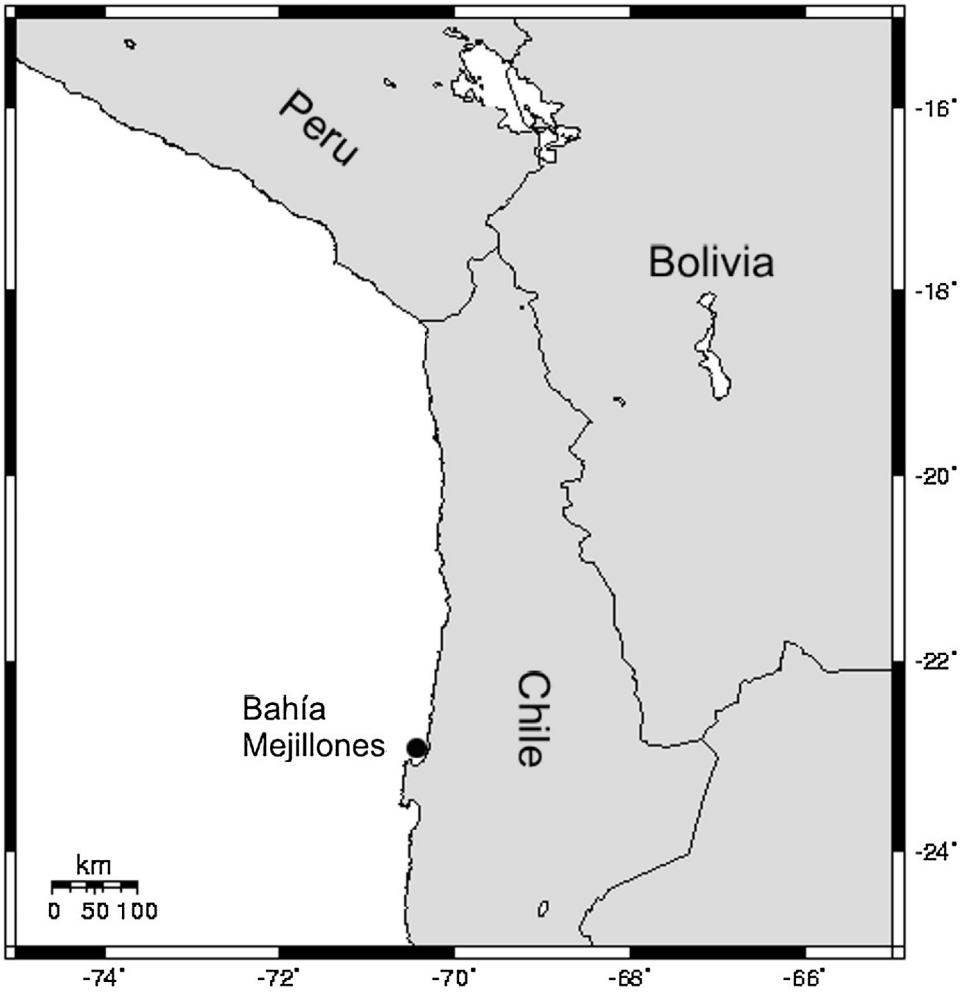

Fig. 2. Location of the sampling station at Bahía Mejillones.

gas at a pressure of 1.5 mTorr. Two MS/MS ion transitions were monitored for each compound; the most intense transition was used for quantification, while the other was employed for confirmation (Table 1).

The concentration of YTXs in the extracts was determined following a method previously validated by Regueiro et al. (2011) with minor modifications. Chromatographic separation was carried out on a reversed-phase column Gemini-NX C18 (50 × 2.0 mm, 3 μm) from Phenomenex (Torrance, California, USA), maintained at 40 °C. Water and acetonitrile/water (9:1, v/v) were used as mobiles phases A and B, respectively, both containing 6.7 mM ammonium hydroxide. The following linear gradient was used: 0 min, 10% B; 1.50 min, 10% B; 3.85 min, 80% B; 4.00 min, 85%B; 4.75 min, 95% B; 6.75 min, 95% B. The flow rate was set to 250 μL min^{-1}, and the injection volume was 10 μL. An on-line solid-phase extraction (SPE) procedure using a security guard

Table 1
Main MS parameters used for the screening and quantification of yessotoxins.

Toxin	Transition	Toxin	Transition
41-keto-YTX	538.4 > 396.4	45-OH-YTX	578.4 > 396.4
41-keto-YTX	538.4 > 467.4	45-OH-YTX	578.4 > 467.4
Trinor-YTX	550.4 > 396.4	45-OH-Homo-YTX	585.4 > 403.4
Trinor-YTX	550.4 > 467.4	45-OH-Homo-YTX	585.4 > 474.4
Trinor-Homo-YTX	557.4 > 403.4	COOH-YTX	586.4 > 396.4
Trinor-Homo-YTX	557.4 > 474.4	COOH-YTX	586.4 > 467.4
44-oxotrinor-YTX	558.4 > 404.4	COOH-Homo-YTX	593.4 > 403.4
44-oxotrinor-YTX	558.4 > 475.4	COOH-Homo-YTX	593.4 > 474.4
44,55-diOH-YTX	565.4 > 396.4	COOH-45-OH-YTX	594.4 > 396.4
44,55-diOH-YTX	565.4 > 467.4	COOH-45-OH-YTX	594.4 > 467.4
41a-homo-44oxotrinor-YTX	565.4 > 411.4	44,55-diOH-41ahomo-YTX	594.4 > 403.4
41a-homo-44oxotrinor-YTX	565.4 > 482.4	44,55-diOH-41ahomo-YTX	594.4 > 474.4
YTX	570.4 > 396.4	32-*O*-monoglycosyl-YTX	636.4 > 462.4
YTX	570.4 > 467.4	32-*O*-monoglycosyl-YTX	636.4 > 533.4
Homo-YTX	577.4 > 403.4	32-*O*-diglycosyl-YTX	702.4 > 528.4
Homo-YTX	577.4 > 474.4	32-*O*-diglycosyl-YTX	702.4 > 599.4

ESI spray voltage: −3.0 kV, sheath gas: 40 (nominal), Aux gas 10 (nominal), vaporizer temp.: 105 °C, capillary temp: 360 °C, collision cell gas pressure: 1.5 mTorr, collision energy: 30 V.

Table 2
Proportions of dinoflagellate species (%) recorded in net haul samples obtained from Mejillones Bay.

	Sample 1	Sample 2	Sample 3	Sample 4
Ceratium azoricum	–	–	0.43	–
Ceratium fusus	–	–	0.43	–
Ceratium pentagonum	–	–	–	0.27
Dinophysis acumuminata	0.30	0.46	0.43	0.27
Gonyaulax taylorii	99.40	99.08	97.84	98.64
Preperidinium meunieri	–	–	0.43	0.27
Prorocentrum gracile	–	0.46	0.43	–
Prorocentrum micans	–	–	–	0.27
Protoperidinium bispinum	–	–	–	0.27
Scripsiella sp.	0.30	–	–	–
Total	100	100	100	100

column Gemini-NX C18 (4.0 × 2.0 mm) was performed as reported by Regueiro et al. (2011).

3. Results

3.1. Biological samples and phytoplankton quantification

Samples collected on March 29th, 2009 from Bahía Mejillones were characterized by the presence of relatively low abundances of phytoplankton, with a total concentration of 140,000 cells L^{-1}. Phytoplankton was dominated by a dinoflagellate preliminarily identified as *Gonyaulax* cf. *taylorii* with a concentration of 64,200 cells L^{-1}. Other phytoplankton species present during the bloom were the dinoflagellates *Dinophysis tripos*, *Preperidinium meunierii*, species belonging to the genus *Gyrodinium* together with abundances below 3500 cells L^{-1} and diatoms of the genus *Pleurosigma* with abundances of 30,000 cells L^{-1}.

Dinoflagellate assemblages in net samples were dominated by *Gonyaulax* cf. *taylorii* with concentrations between 276,187 and 673,664 cells mL^{-1}. The contribution of this species to the total dinoflagellates community was between 97.8–99.4%. Other dinoflagellates recorded in net samples were *Dinophysis acuminata*, *Preperidinium meunieri* and *Prorocentrum gracile*, with contributions below 0.5% (Table 2).

3.2. Morphological observations

Cells 30–36 μm wide and 45–50 long with conspicuous apical horn of about half the length of the epitheca with pronounced shoulders, and one or two antapical spines of variable shape, able to be subdivided evenly (Figs. 3A–C, E and 4). Cingulum displaced about 1.5× its width with a marked overhang (Figs. 3A–C and 4). The theca is strong and heavily ornamented with many round depressions, most of which have a pore, especially abundant in

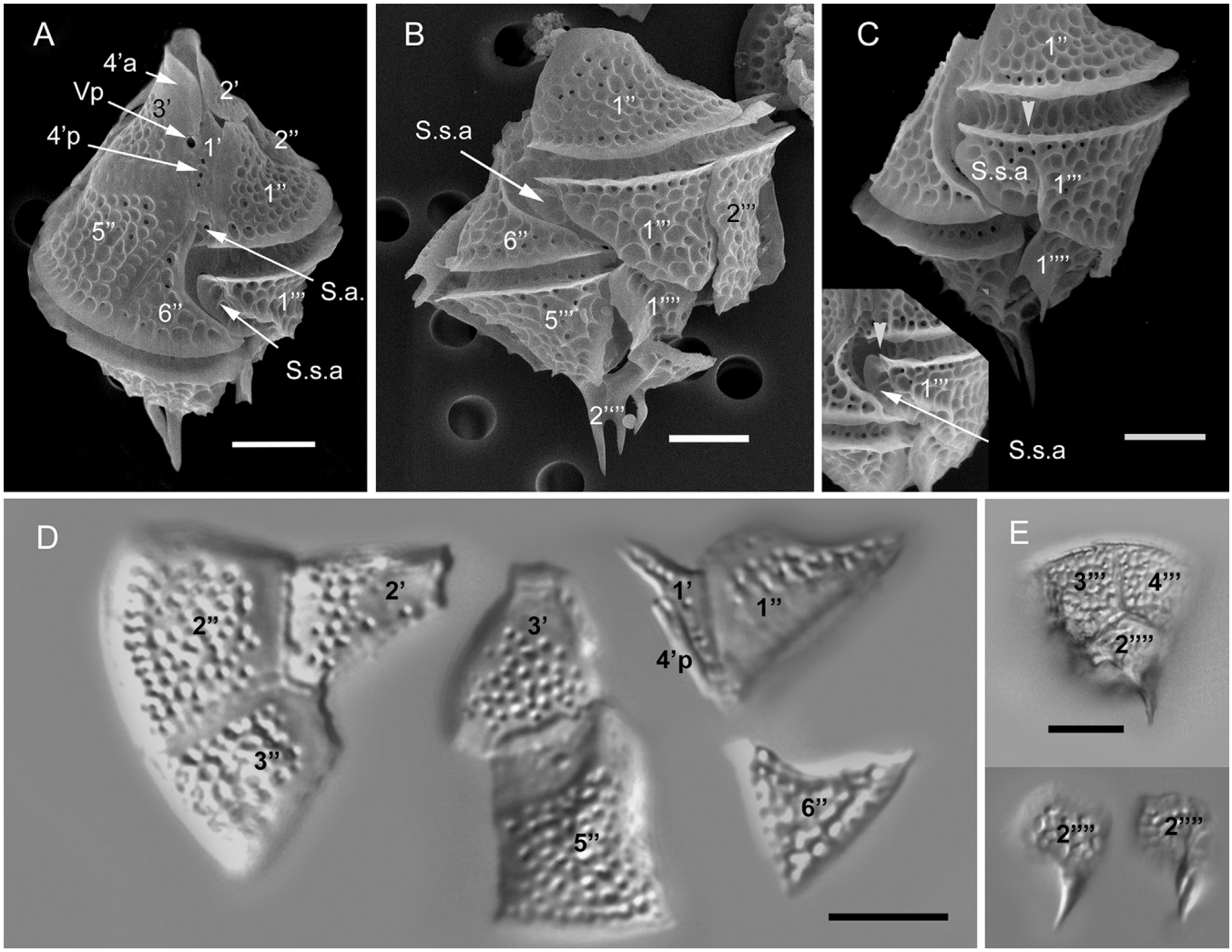

Fig. 3. SEM images of *Gonyaulax taylorii* (a) ventral view of epitheca. (b) Ventral view. (c) Partial ventral view showing a wide S.s.a. (Insert) Detail of a narrow S.s.a. LM images of *G. taylorii* (d) composite of some epithecal plates. (e) Plate 2"" and its relation to plates 3''' and 4'''. All scale bars 10 μm.

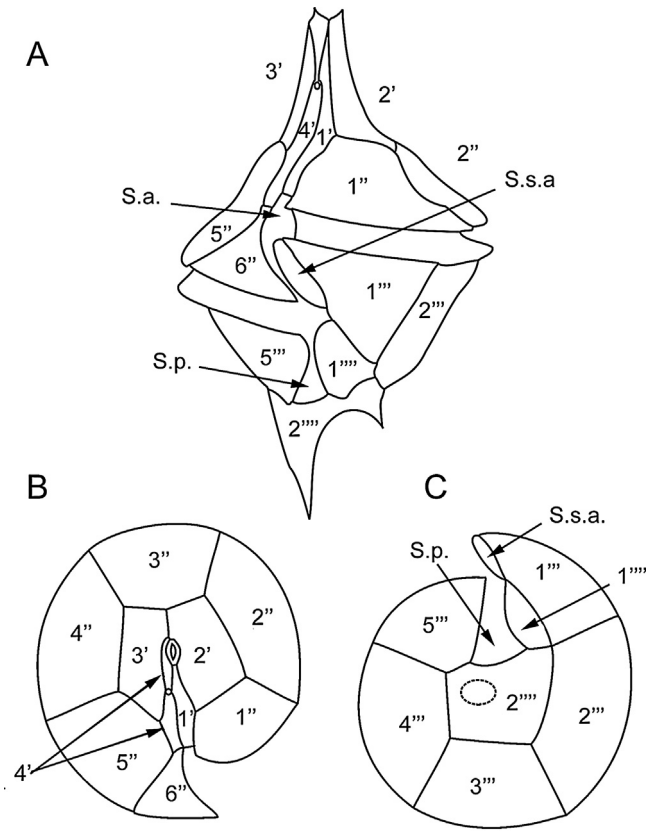

Fig. 4. Ink drawings of *Gonyaulax taylorii*. (a) Ventral view. (b) Apical view. (c) Antapical view.

those near the cingulum. The plate formula is Po, 4', 0a, 6", ?s, 6c, 5"', 0p, 2''''. Po plate is hardly visible, as it is hidden by the apical plates. The ventral side of the apical horn is formed by two long and very narrow plates: 1' which has a single line of round depressions, and 4' with a very conspicuous ventral pore that divides this plate in two parts (named here 4'a and 4'p) that hence look like two plates, as after dissection they normally appear separated (Fig. 3A and D). Plates 2' and 3' are wider than 1' and 4' and form most of the apical horn of the epitheca (Fig. 3A and D). Precingular plates are much bigger than those of the apical series. Plate 6" is triangular (Fig. 3A and D) and the pointed anterior end contacts S.a., 1' and 4' by very short sutures and almost coincident with the level of the anterior side of the left side of the cingulum (Fig. 3A). It was not possible to dissect all the sulcal plates. Sulcal anterior plate has an anterior part that penetrates the epitheca contacting 1", 1', and 6" and, sometimes 4' when 6" is not in contact to 1' as the posterior end of 4' is between them (Fig. 3A). The anterior left sulcal (S.s.a.) appears as the first postcingular plate, but following Balech (1971) and Amorim et al. (2013), it should be considered as S.s.a although it appears as a left wing out of the sulcus (Fig. 3A–C) and hence this species has 5 postcingular plates. This plate, S.s.a., shares the right side of the triangular 1"' with plate 1'''' which other authors considered as 1p. Width of the S.s.a. varies among cells (Fig. 3C). The second antapical plate 2'''' has, in its right side, a strong spine of variable length (Fig. 3H) that sometimes may appear longitudinally divided in two (Fig. 3A–C and E). A shorter spine may be present in the left side of 2'''' and the posterior end of 1'''' may form a wing that looks like another spine.

3.3. Toxin analysis

Analysis of LC–MS/MS revealed the presence of two yessotoxins in all analyzed phytoplankton samples. The chromatograms showed a peak at a retention time of 3.34 min with a doubly charged ion $[M-2H]^{2-}$ at *m/z* 570 corresponding to YTX. The MS/MS fragmentation of this ion yielded the characteristic product ions of YTX at *m/z* 467.4 and *m/z* 396.4, confirming the identification of this toxin (Fig. 5, Supplementary material). The estimated amount of YTX per cell was below 1 pg cell^{-1}.

Analyses showed a second chromatographic peak at a retention time of 3.35 min with a $[M-2H]^{2-}$ ion at *m/z* 577.4, which was

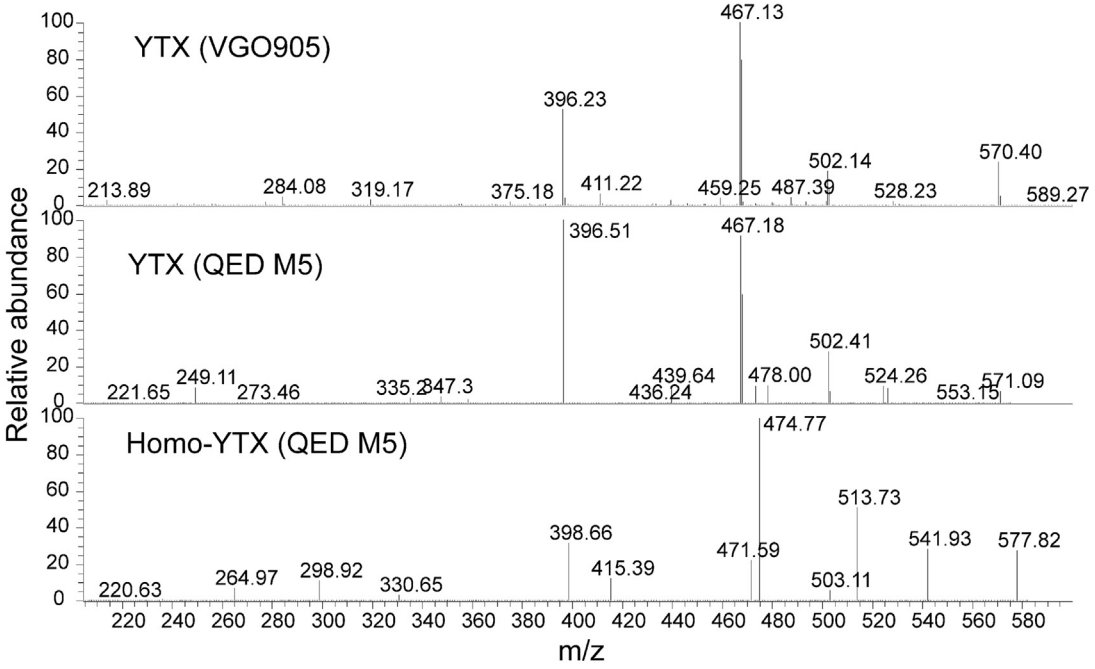

Fig. 5. Fragmentation spectra (QED) corresponding to a reference solution of YTX (extract of *Protoceratiunm reticulatum* VGO905) and to the chromatographic peaks in the samples of this study corresponding to YTX and homo-YTX.

identified as homo-YTX. The presence of this toxin was confirmed by the characteristic MS/MS fragment at *m/z* 474.4 and *m/z* 403.4 (Fig. 5, Supplementary material). The estimated amount of homo-YTX per cell was below 1 pg cell^{-1}.

4. Discussion

4.1. Morphology

The specimens studied in this work are identical to *Gonyaulax jolliffei* Murray et Whitting sensu Dodge (Dodge, 1985, p. 73), and (Dodge, 1989, Fig. 11), but this species differs from the original description of *G. jolliffei* (Murray and Whitting, 1899). Considering that it was originally described as inverted, the plate here interpreted as 6" is wide and pentagonal, instead of triangular. Carbonell-Moore (1996) determined that the species represented by Dodge (1985) in page 73 is her *Gonyaulax taylorii* Carbonell-Moore (1996) and she even used that SEM image to draw her figure 71c, which was considered part of the holotype. Comparing her drawing with the Dodge's SEM image, the suture between the plates labeled 1"' and 2"' in her figure does not exist in the SEM image. These two plates really correspond to only one plate, the first postcingular 1"', in Dodge's image, as it also happens in the Chilean specimens (Fig. 2A–C). Plate 1"' that other authors considered as 2"' is always a big plate in *Gonyaulax* and not as small as in figure 71c of Carbonell-Moore (1996). In that figure, the narrow and small plate to the left of the cingulum has no label. This plate has been interpreted by many authors as 1"', while this study has identified it as S.s.a. Strictly following the Kofoidean notation it should be considered as the first postcingular, however here it is considered as homologous to the left sulcal anterior (S.s.a.) in *Alexandrium* (Balech, 1995) and in *Fragilidium* (Amorim et al., 2013). Hence, *Gonyaulax* has 5 postcingular plates as other Gonyaulacales. In the epitheca, the fourth apical plate 4' is long and very narrow with anterior end in contact to Po and the posterior end to 6". It has a large ventral pore that divides it into two parts able to be separated, or difficult to maintain unbroken after dissection, therefore it is considered by many authors as having two intercalary plates or 4' plus a plate called Cv. The anterior part of 4' (4'a) may encircle Po by its right side, not allowing the contact between 3' and Po; the reason why (Balech, 1977) interpreted the anterior part of the plate considered here to be 4' (4'a) as 3' and the plate 3' in this study as 1a and the posterior part of 4' (4'p) as 2a. Hence considering that 4' is composed of two parts and that it may disconnect 3' from Po.

In summary, the species described has the plate formula Po, 4', 0a, 6", 6c, ?s, 5"', 2"" as other Gonyaulacales such as *Alexandrium* (Balech, 1995), *Ostreopsis* (Besada et al., 1982), *Gambierdiscus* (Besada et al., 1982; Fraga et al., 2011) and *Coolia* (Mohammad-Noor et al., 2013). This article considers this species to be *Gonyaulax taylorii* Carbonell-Moore, and asserts that its original description be emended. In the Dodge's SEM image, the wing shaped S.s.a. is clearly visible as well as in the original description of *G. taylorii*, where it appears without label (Carbonell-Moore, 1996). Nevertheless Dodge (1989) did not draw that plate in his figure 2P.

In the original description of *Gonyaulax taylorii* by Carbonell-Moore (1996), the following descriptions were considered as synonyms of *G. taylorii*, in addition to *Gonyaulax jolliffei* Murray and Whitting sensu Dodge (1985, 1989).

The dinoflagellate *Gonyaulax monacantha* var *minor* sensu Balech (1971) has a shorter 5" as its anterior suture contacts the right side of 6" posteriorly to the posterior edge of 1". Also, *Gonyaulax taylorii* has a much taller 5" (Fig. 2A and D). Plate 4' in Balech's fig. 127 does not have a ventral pore dividing it into two parts. Based on the remainder of the figures, the specimens studied by Balech (1971) were probably *G. taylorii* and the suture between plates 3' and 5" was drawn in error. There is not enough information on the description of this variety by Pavillard (1916) to establish whether or not it is the species assayed here.

The dinoflagellate *Gonyaulax subulata* sensu Taylor 1976 has a 6" quadrangular as *Gonyaulax buxus* Balech 1967. Additionally, *G. taylorii* has a 6" triangular (Fig. 2A–D) with a pointed anterior edge in contact to the posterior end of plate adjacent to the right side of 1'.

The present authors agree that *Gonyaulax jolliffei* Murray and Whitting (1899) is not *Gonyaulax taylorii* as its plate 6" is wide and trapezoidal, has a large 1a, the cingulum has no overhang and the hypotheca is pointed instead of having a large spine.

4.2. Toxin analysis

The present study reveals the dinoflagellate *Gonyaulax taylorii* as a new source of YTXs. This is the first report of these types of toxins being produced by *G. taylorii*, in Chilean waters and possibly worldwide. Yessotoxin and homoyessotoxin were found in phytoplankton samples with concentrations below 1 pg cell^{-1}. The cell quota should be an underestimation of the actual toxin concentration, for several reasons: (a) the determination was made in net haul phytoplankton samples and likely some loss of toxin from the cells took place during the fishing step; (b) the samples were formaldehyde-preserved and, as there exists no study of toxin recovery in preserved samples, the possibility of an incomplete toxin extraction from the preserved cells could not be ruled out; and (c) the recoveries of toxins during the solid phase extraction could have been lower than 100%. Taking into account that (a) *G. taylorii* was the dominant species in the dinoflagellate assemblage in net haul samples (over 97% and over 99% in two out of the four samples) (b) that the other dinoflagellate species found in the samples have never been associated with YTXs production (although most have been frequently studied); and (c) that the presence of feces (of copepods or other grazers) was not observed in significant amounts in the obtained net hauls, it is clear that *G. taylorii* was responsible for the production of YTXs.

The toxin profile of the samples in this study is characterized by the presence of YTX, and one analog corresponding to homo-YTX. Both toxins had been found in other Gonyaulacoid dinoflagellates with differences in their proportions, cell quotas and the presence of other YTX analogues.

In *Protoceratium reticulatum*, YTX is usually the most prominent toxin produced (reviewed by Paz et al., 2008). In some strains, complex toxin profiles have been described; such as that found in a strain collected from Cesenatico, Italy which was dominated by YTX followed by carboxy-YTX, 45-OH-YTX, noroxo-YTX and a lesser amount of homo-YTX (Ciminiello et al., 2003). In Japan, Suzuki et al. (2007) reported that *P. reticulatum* strains isolated from Mutsu and Okirai produced YTX followed by trinor-YTX, trinor-1-homo-YTX, noroxo-YTX-enone and homo-YTX.

In relation to *Gonyaulax spinifera*, homo-YTX seems to be the main toxin produced by this species. In Italy, one strain collected from Cesenatico has a toxic profile dominated by homo-YTX followed by YTX (Riccardi et al., 2009). Chikwililwa (2010) has described a similar profile in strains isolated from Balvis Bay, Namibia in which the profile was dominated by homo-YTX followed by YTX and small amount of 45-OH-YTX.

The YTXs content per cell of *Gonyaulax taylorii* was below 1 pg cell^{-1}. This concentration is lower than those found in the strains of *Protoceratium reticulatum* from Cesenatico, Italy with 12.19 pg cell^{-1} and the strains from Mutsu and Okirai, Japan with 47.2 pg cell^{-1} and 59.8 pg cell^{-1}, respectively. Also, the

concentration is lower than those reported in strains of *Gonyaulax spinifera* from Cesenatico, Italy with 37 pg cell^{-1} and the strains obtained from Walvis Bay, Namibia (14.88–155.88 pg cell^{-1}). Among the gonyaulacoid species, the level of YTXs detected in *G. taylorii* it is only superior to those reported in strains of *Lingulodinium polyedrum* isolated from Spain (0.3 pg cell^{-1}) (Paz et al., 2004), United Kingdom (0.02 pg cell^{-1}) (Stobo et al., 2003) and the United States (0.001–0.2 pg cell^{-1}) (Armstrong and Kudela, 2006; Howard et al., 2008). Notwithstanding, as already commented, the cell quotas of unpreserved cells might be higher.

Unfortunately, the small amount of biological material available in this study made it impossible to obtain the gene sequence of *Gonyaulax taylorii* and consequently to develop phylogenetic analysis or include this species in a gene tree with the other YTXs-producing species. Nevertheless, the results obtained support that YTX production is still confined to the order Gonyaulacales (Howard et al., 2009) and suggest the need of further studies in order to explain which gene or genes are involved in YTX production in species from genera *Gonyaulax*, *Protoceratium* and *Lingulodinium*.

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Appendix A. Taxonomic appendix

Gonyaulax taylorii *Carbonell-Moore (1996) emended S. Fraga*

The holotype of this species was composed of figures 62–66 (SEM images) and 71 (Ink drawing) (Carbonell-Moore, 1996) but according to the figure captions, Figs. 62 and 63 correspond to *Gonyaulax birostris*, and hence only Figs. 64–66 correspond to *Gonyaulax taylorii*. Figure 71c is a drawing based on a SEM image of Dodge (1985) page 73, but it shows a suture between plates 1‴ and 2‴ that does not exist on the original SEM image from it was taken, and hence this plate appears in the drawing as two plates, 1‴ and 2‴, instead as one plate named 1‴, which is the reason why this species description must be emended.

BASIONYM: *Gonyaulax taylorii* Carbonell-Moore (1996) Botanica Marina Vol. 39, pp. 347–370.

LECTOTYPE: Scanning Electron Microscopy image of page 73 (Dodge, 1985) identified there as *Gonyaulax jolliffei* Murray & Whitting. This image was used as a basis for drawing 71c (Carbonell-Moore, 1996) for the holotype (ICN Art. 9.2 and 9.3).

EPITYPE: Fig. 3A based on SEM image of Dodge (1985) page 73 (ICN Art. 9.8).

SYNONYMS: *Gonyaulax jolliffei* sensu Dodge (1985, p. 73, 1989, p. 283, Figs. 2P), 11 non *G. jolliffei* Murray and Whitting 1889, p. 324, pl. 28, Figs. 1a, b.

EMENDED SPECIES DESCRIPTION: Cells 30–36 μm wide and 45–50 μm long, with a conspicuous apical horn of approximately half the length of the epitheca, with shoulders, and one or two antapical spines of variable shape that can be evenly subdivided. Cingulum displaced about 1.5× its width with a marked overhang. The theca is strong and heavily ornamented with many round depressions, many of which have a pore, more abundant in those near the cingulum. The plate formula is Po, 4', 0a, 6", ?s, 6c, 5"', 0p, 2"". The Po plate is hardly visible as it is hidden by the apical plates. The ventral side of the apical horn is formed by two long and very narrow plates: 1' which has a single line or round depressions, and 4' with a very conspicuous ventral pore that divides this plate in two parts that hence look like two plates, one anterior 4'a, and another posterior 4'p, as after dissection they normally appear separated. Plates 2' and 3' are wider than 1' and 4' and form most of the apical horn of the epitheca. Precingular plates are much bigger than those of the apical series. Plate 6" is triangular and the pointed anterior end contacts S.a., 1' and 4' by very short sutures and it is almost coincident with the level of the anterior side of c1. S.a, has an anterior part that penetrates the epitheca contacting 1", 1', and 6" and sometimes 4', when 6" is not in contact to 1' as the posterior end of 4' is between them. Plate S.s.a. is like a wing on the anterior left part of the sulcus and contacts 1"' all along its left side, sharing the right side of the triangular 1"' with plate 1"" which forms a wing on the posterior left side of the sulcus. The second antapical plate 2"" has, in its right side, a strong spine of variable length that sometimes may appear longitudinally divided in two. A shorter spine may be present in the left side of 2"" and the posterior end of 1"" may form a wing that looks like another spine.

LECTOTYPE LOCALITY: East Atlantic Ocean.

DISTRIBUTION: This species is widely distributed, Central Equatorial Pacific, Tropical Atlantic and East Atlantic and Northern Chile.

ETYMOLOGY: This species has been dedicated to Dr. F.J.R. Taylor.

TOXICITY: Yessotoxin (YTX) and homoyessotoxin (homo-YTX) were detected in field net haul samples dominated by this species, appearing as a laboratory culture that was obtained in Mejillones Bay, Chile.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.hal.2016.07.006.

References

Alfonso, A., de la Rosa, L., Vieytes, M.R., Yasumoto, T., Botana, L.M., 2003. Yessotoxins, a novel phycotoxin, activates phosphodiesterase activity. Effect of yessotoxin on cAMP levels in human lymphocytes. Biochem. Pharmacol. 65, 193–208.

Álvarez, G., Uribe, E., Díaz, R., Braun, M., Mariño, C., Blanco, J., 2011. Bloom of the yessotoxin producer dinoflagellate *Protoceratium reticulatum* (Dinophyceae) in Northern Chile. J. Sea Res. 65, 427–434.

Amorim, A., Veloso, V., Rodríguez, F., Fraga, S., 2013. Life-cycle, morphology and phylogeny of species of *Fragilidium* Balech from west Iberia. In: Lewis, J., Marret, F., Bradley, L. (Eds.), Biological and Geological Perspectives of Dinoflagellates. The Micropaleontological Society, London, pp. 215–224.

Armstrong, M., Kudela, R., 2006. Evaluation of California isolates of *Lingulodinium polyedrum* for the production of yessotoxin. Afr. J. Mar. Sci. 28 (2), 399–401.

Aune, T., Sorby, R., Yasumoto, T., Ramstad, H., Landsverk, T., 2002. Comparison of oral and intraperitoneal toxicity of yessotoxins towards mice. Toxicon 40, 77–82.

Balech, E., 1971. Microplancton del Atlántico Ecuatorial Oeste (Equalant I). Serv. Hidrogr. Nav. (Argentina) H 654, 1–103 112 lám.

Balech, E., 1977. Cuatro especies de *Gonyaulax* sensu lato y consideraciones sobre el género (Dinoflagellata). Rev. Mus. Argent. Cienc. Nat. Bernard. Rivadavia V (6), 115–136 113 láms.

Balech, E., 1995. The genus *Alexandrium* Halim (Dinoflagellata). Publ. Sherkin Is. Mar. Station, Sherkin Island, Co., Cork, Ireland p. 151.

Besada, E.G., Loeblich, L.A., Loeblich III, A.R., 1982. Observations on tropical, benthic dinoflagellates form ciguatera-endemic areas: *Coolia*, *Gambierdiscus*, and *Ostreopsis*. Bull. Mar. Sci. 32 (3), 723–735.

Bianchi, C., Fato, R., Angelin, A., Trombetti, F., Ventrella, V., Borgatti, A.R., Fattorusso, E., Ciminiello, P., Bernardi, P., Lenaz, G., Castelli, G.P., 2004. Yessotoxin, a shellfish biotoxin, is a potent inducer of the permeability transition in isolated mitochondria and intact cells. Biochim. Biophys. Acta (BBA): Bioenerg. 1656 (2–3), 139–147.

Carbonell-Moore, M.C., 1996. On *Spiraulax jolliffei* (Murray et Whitting) Kofoid and *Gonyaulax fusiformis* Graham (Dinophyceae). Bot. Mar. 39, 347–370.

Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Magno, S., Guerrini, F., Pistocchi, R., Boni, L., 2003. Complex yessotoxins profile in *Protoceratium reticulatum* from north-western Adriatic Sea revealed by LC–MS analysis. Toxicon 42 (1), 7–14.

Chikwililwa, C., 2010. Marine Biotoxins in the Northern Benguela region—Biological and Chemical Parameters Affecting the Production of Harmful Algal Blooms and Phycotoxin Concentrations. Phd Thesis. University of Rostok, Germany 98 p..

de la Rosa, L.A., Alfonso, A., Vilarino, N., Vieytes, M.R., Botana, L.M., 2001. Modulation of cytosolic calcium levels of human lymphocytes by yessotoxin, a novel marine phycotoxin. Biochem. Pharmacol. 61 (7), 827–833.

Dodge, J.D., 1985. Atlas of Dinoflagellates. Blackwell SCL Publ., Palo Alto p. 119.

Dodge, J.D., 1989. Some revisions of the family Gonyaulacaceae (Dinophyceae) based on a canning electron microscope study. Bot. Mar. 32, 275–298.

Domínguez, H., Paz, B., Daranas, A.H., Norte, M., Franco, J.M., Fernández, J.J., 2010. Dinoflagellate polyether within the yessotoxin, pectenotoxin and okadaic acid toxin groups: characterization, analysis and human health implications. Toxicon 56 (2), 191–217.

European Commission, 2013. Commission regulation No. 786/2013 of 16 August 2013 emending Annex III to Regulation (EC) No. 853/2004 of the European Parliament and of the Council as regards the permitted limits of yessotoxins in live bivalve molluscs. Off. J. Eur. Union L 220/214.

Fraga, S., Rodríguez, F., Caillaud, A., Diogène, J., Raho, N., Zapata, M., 2011. *Gambierdiscus excentricus* sp. nov (Dinophyceae), a benthic toxic dinoflagellate from the Canary Islands (NE Atlantic Ocean). Harmful Algae 11, 10–22.

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing calcofluor white m2r for the visualization of dinoflagellate thecal plates. J. Phycol. 2, 662–664.

Hasle, G.R., 1978. The inverted microscope method. In: Sournia, A. (Ed.), Phytoplankton Manual. UNESCO, Paris, pp. 88–96.

Howard, M., Silver, M., Kudela, R., 2008. Yessotoxin detected in mussel (*Mytilus californicus*) and phytoplankton samples from the U.S. west coast. Harmful Algae 7, 646–652.

Howard, M.D.A., Smith, G.J., Kudela, R.M., 2009. Phylogenetic relationships of yessotoxin-producing Dinoflagellates, based on the large subunit and internal transcribed spacer ribosomal DNA domains. Appl. Environ. Microbiol. 75 (1), 54–63.

Konishi, M., Yang, X.M., Li, B., Fairchild, C.R., Shimizu, Y., 2004. Highly cytotoxic metabolites from the culture supernatant of the temperate dinoflagellate *Protoceratium* cf. *reticulatum*. J. Nat. Prod. 67 (8), 1309–1313.

Krock, B., Seguel, C.G., Valderrama, K., Tillmann, U., 2009. Pectenotoxins and yessotoxin from Arica Bay, north Chile as determined by tandem mass spectrometry. Toxicon 54, 364–367.

Mohammad-Noor, N., Moestrup, Ø., Lundholm, N., Fraga, S., Adam, A., Holmes, M.J., Saleh, E., 2013. Autecology and phylogeny of *Coolia tropicalis* and *Coolia malayensis* (Dinophyceae), with emphasis on taxonomy of *C. tropicalis* based on light microscopy, scanning electron microscopy and LSU rDNA. J. Phycol. 49 (3), 536–545.

Murata, M., Masanori, K., Lee, J.S., Yasumoto, T., 1987. Isolation and structure of yessotoxin, a novel polyether compound implicated in Diarrhetic Shellfish Poisoning. Tetrahedron Lett. 28, 5869–5872.

Murray, G., Whitting, F., 1899. New Peridiniaceae from the Atlantic. Trans. Linn. Soc. Lond. Bot. (Ser. 2, 5) 321–342 pls. 327–333.

Pavillard, J., 1916. Recherches sur les Péridiniens du Golfe du Lion. Traveaux Inst. Bot. Univ. Montpellier 4. Série Mixte Mémoire (4), pp. 9–70 pls. 71–73.

Paz, B., Riobó, P., Fernández, M.L., Fraga, S., Franco, J.M., 2004. Production and release of yessotoxins by the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum* in culture. Toxicon 44, 251–258.

Paz, B., Riobo, P., Souto, M.L., Gil, L.V., Norte, M., Fernández, J.J., Franco, J.M., 2006. Detection and identification of glycoyessotoxin A in a culture of the dinoflagellate *Protoceratium reticulatum*. Toxicon 48 (6), 611–619.

Paz, B., Riobo, P., Ramilo, I., Franco, J.M., 2007. Yessotoxins profile in strains of *Protoceratium reticulatum* from Spain and USA. Toxicon 50 (1), 1–17.

Paz, B., Daranas, A.H., Norte, M., Riobo, P., Franco, J.M., Fernández, J.J., 2008. Yessotoxins, a group of marine polyether toxins: an overview. Mar. Drugs 6 (2), 73–102.

Paz, B., Blanco, J., Franco, J.M., 2013. Yessotoxins production during the culture of *Protoceratium reticulatum* strains isolated from Galician Rias Baixas (NW Spain). Harmful Algae 21–22 (0), 13–19.

Pérez-Gómez, A., Ferrero-Gutiérrez, A., Novelli, A., Franco, J.M., Paz, B., Fernández-Sánchez, M.T., 2006. Potent neurotoxic action of the shellfish biotoxin yessotoxin on cultured cerebellar neurons. Toxicol. Sci. 90 (1), 168–177.

Regueiro, J., Rossignoli, A., Alvarez, G., Blanco, J., 2011. Automated on-line solid-phase extraction coupled to liquid chromatography–tandem mass spectrometry for determination of lipophilic marine toxins in shellfish. Food Chem. 129, 533–540.

Rhodes, L.L., Mcnabb, P., De Salas, M., Briggs, L., Beuzenberg, V., Gladstone, M., 2006. Yessotoxin production by *Gonyaulax Spinifera*. Harmful Algae 5 (2), 148–155.

Riccardi, M., Guerrini, F., Roncarati, F., Milandri, A., Cangini, M., Pigozzi, S., Riccardi, E., Ceredi, A., Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Tartaglione, L., Pistocchi, R., 2009. *Gonyaulax spinifera* from the Adriatic Sea: toxin production and phylogenetic analysis. Harmful Algae 8 (2), 279–290.

Satake, M., MacKenzie, L., Yasumoto, T., 1997. Identification of *Protoceratium reticulatum* as the biogenetic origin of yessotoxin. Nat. Toxins 5, 164–167.

Seguel, M., Sfeir, A., 2003. Floraciones Algales Nocivas en el área Sur de la X Región y los Canales Occidentales de la XI Región. Taller sobre los resultados del Crucero Cimar 8 Fiordos, Valparaíso, pp. 173–180.

Seguel, M., Sfeir, A., 2005. Floraciones algales nocivas en los canales y fiordos del sur de Chile. Cienc. Tecnol. Mar 28 (2), 5–13.

Stobo, L.A., Lewis, J., Quilliam, M.A., Hardstaff, W.R., Gallacher, S., Webster, L., Smith, E., McKenzie, M., 2003. Detection of yessotoxin in UK and Canadian isolates of phytoplankton and optimization and validation of LC–MS methods. In: Bates, S. S. (Ed.), Proceedings of the Eighth Canadian Workshop on Harmful Marine Algae. Gulf Fisheries Centre, Moncton, New Brunswick, Canada, pp. 8–14.

Suzuki, T., Horie, Y., Koike, K., Satake, M., Oshima, Y., Iwataki, M., Yoshimatsu, S., 2007. Yessotoxin analogues in several strains of *Protoceratium reticulatum* in Japan determined by liquid chromatography–hybrid triple quadrupole/linear ion trap mass spectrometry. J. Chromatogr. A 1142 (2), 172–177.

Terao, K., Ito, E., Oarada, M., Murata, M., Yasumoto, T., 1990. Histopathological studies on experimental marine toxin poisoning: the effects on mice of yessotoxin isolated from *Patinopecten yessoensis* an of a desulfated derivative. Toxicon 28, 1095–1104.

Tubaro, A., Sirari, L., Della Loggia, R., Yasumoto, T., 1998. Occurrence of yessotoxin-like toxins in phytoplankton and mussels from the northern Adriatic Sea. In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and IOC of UNESCO, Santiago de Compostela, pp. 470–472.

Yasumoto, T., Takizawa, A., 1997. Fluorometric measurement of yessotoxins in shellfish by high-pressure liquid chromatography. Biosci. Biotechnol. Biochem. 61 (10), 1775–1777.