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First detection of species of the potentially toxic genus *Azadinium* (Amphidomataceae, Dinophyceae) in tropical coastal waters of Brazil

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Abstract

In fall 1995, during a survey in Abrolhos coral reef system (southwestern Atlantic, Brazil), significant densities (143–6174 cells L^{-1}) of small thecate dinoflagellates were detected. Analysis of this material in scanning and transmission electron microscopy confirmed the presence of four taxa assigned to the potentially toxic genus *Azadinium*: *A. dexteroporum* Percopo et Zingone, *A. luciferelloides* Tillmann et Akselman, *A.* cf. *polongum* Tillmann and *Azadinium* sp. The latter taxon showed external morphological features quite distinct from any *Azadinium* taxon yet described, but its formal description as a new species depends on more detailed analysis. Species of *Azadinium* have never been confirmed in Brazilian waters until now, although the toxins produced by these dinoflagellates, the azaspiracids, have been detected in Brazilian southern coast without recognition of their producing organisms. The highest densities of *Azadinium* spp. occurred at stations south of and over the Abrolhos Bank, which receive higher nutrient concentrations due to upwelling of deep and nutrient-rich water masses.

Keywords Dinophyta · Electron microscopy · Harmful algae · Nanoplankton · South America

1 Introduction

Many dinoflagellate species are known as potential toxin producers and may cause serious ecological and economic losses in coastal regions around the world. Among them there are small thecate species of the genera *Azadinium* Elbrächter et Tillmann and *Amphidoma* F. Stein (Family Amphidomataceae), which can produce potent phycotoxins from the azaspiracid group (AZAs, Tillmann et al. 2009, 2014b; Krock et al. 2012). AZAs are lipophilic polyether toxins with more than 30 analogues known (Hess et al. 2014). AZAs accumulate in filter feeders, particularly bivalves' tissues, and may cause azaspiracid shellfish poisoning (AZP), a severe syndrome of gastrointestinal symptoms in humans who consumed contaminated seafood. Similar to Diarrhetic Shellfish Toxins (DSP), human consumption of AZA-contaminated shellfish can result in severe acute symptoms that include nausea, vomiting, diarrhea, and stomach cramps (Twiner et al. 2014). Toxicological studies on animals showed "neuro-toxin-like" symptoms in mice after i.p. injection characterized by sluggishness, respiratory difficulties, spasms, progressive paralysis, and death (Satake et al. 1998). Moreover, AZAs were shown to be teratogenic in fish (Colman et al. 2005), damaging to the gastrointestinal tract in mice (Ito et al. 2002), and to be potential lung tumor promoters (Furey et al. 2010). The first episode of human poisoning by azaspiracid was documented in Ireland in 1995 (McMahon and Silke 1996; Satake et al. 1998). Human poisoning incidents associated with this toxin group have since than been reported in Europe and in the USA, all of which were caused by shellfish harvested in Ireland (Twiner et al. 2014). However, AZA levels above the EU regulatory level have recently been reported from southern Spain as well (Tillmann et al. 2017a), indicating that AZP events may suddenly show up in previously unaffected areas.

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The first toxin-producing organism was only identified in 2009 (Tillmann et al. 2009). Azaspiracids have been recorded in many coastal regions of the world (e.g., James et al. 2002; Braña Magdalena et al. 2003; Taleb et al. 2006; Amzil et al. 2008; Elgarch et al. 2008; Vale et al. 2008; Álvarez et al. 2010; Yao et al. 2010; Trainer et al. 2013; Bacchiocchi et al. 2015; Turner and Goya 2015). This suggests that the dinoflagellates source species are widely distributed throughout the oceans as well, and indeed, records of *Azadinium* spp. have been increasingly reported since studies on the nanoplanktonic communities have advanced (Tillmann and Akselman 2016).

Azadinium spp. are predominantly small (10–15 μm) photosynthetic species with a thin theca, which is difficult to see under light microscopy (LM). Typical Kofoidian tabulation is Po, cp, X, 4′, 3a, 6″, C6, 5S, 6‴, 2⁗, although *A. dalianense* Z. Luo, H. Gu et Tillmann and *A. zhuanum* Z. Luo, Tillmann et H. Gu have reduced numbers of epithecal plates (Luo et al. 2013, 2017). All known species have a distinct ventral pore of variable position among taxa (Tillmann et al. 2014b). Thirteen *Azadinium* taxa were described until now: *A. spinosum* Elbrächter et Tillmann (Tillmann et al. 2009), *A. obesum* Tillmann et Elbrächter (Tillmann et al. 2010), *A. poporum* Tillmann et Elbrächter (Tillmann et al. 2011), *A. polongum* Tillmann (Tillmann et al. 2012), *A. caudatum* (Halldal) Nézan et Chomérat var. *caudatum*, *A. caudatum* var. *margalefii* (Rampi) Nézan et Chomérat (Nézan et al. 2012), *A. dexteroporum* Percopo et Zingone (Percopo et al. 2013), *A. dalianense* (Luo et al. 2013), *A. trinitatum* Tillmann et Nézan, *A. cuneatum* Tillmann et Nézan, *A. concinnum* Tillmann et Nézan (Tillmann et al. 2014a), and the recently proposed *A. luciferelloides* Tillmann et Akselman (Tillmann and Akselman 2016) and *A. zhuanum* (Luo et al. 2017). Infrageneric taxa can be morphologically distinguished by a set of characters, such as presence of an antapical spine, size and shape of epithecal plates, number and arrangement of thecal pores and the position of the ventral pore (Tillmann and Akselman 2016).

Identification and knowledge of the potentially toxigenic species of *Azadinium* present in an area are an important prerequisite to evaluate the risk potential of AZP and to establish early warning monitoring programs in order to protect shellfish consumers. Although AZAs have already been found in the Brazilian coast (Schramm et al. 2010; Massucatto et al. 2014), no record of *Azadinium* spp. was published until now. Proença et al. (2014) preliminarily reported on isolation of an "*Azadinium*-like flagellate" from a shallow mangrove of southern Brazil with the presence of azaspiracid analogues, but this report was not conclusive and still is in need of confirmatory analyses. During observations on samples collected in an oceanographic cruise to the continental shelf of eastern Brazil in fall 1995,

significant densities of small thecate dinoflagellates were detected. Morphological analysis of this material confirmed that those organisms are assigned to the genus *Azadinium*. This work now formally describes the first detection of *Azadinium* spp. along the Brazilian coast (southwestern Atlantic) and shows ecological data related to the occurrence of these species in a Brazilian tropical system.

2 Materials and methods

Samples are from a Brazilian-German Joint Oceanographic Project (JOPS-II-9), collected between April 25 and May 8, 1995, in Abrolhos region (16–21.5°S; 36–40.5°W). The Abrolhos Bank is a large extension of the eastern Brazilian continental shelf, which functions as a topographical barrier for the Brazil Current that flow southward (Ekau and Knoppers 1999). The main water masses in this region are the Brazil Current, a warm, saline oligotrophic tropical water (TW) mass, laying in the upper offshore hundred meters and being the major component of the coastal waters; and the South Atlantic Central Water (SACW), a deep cold and rich water mass flowing northward, that represents the main source of nutrients at the coastal upwelling south of the Abrolhos Bank (Gaeta et al. 1999). Additionally, shallow banks on tidal currents generate internal tides and upwelling over the southern flank of the Abrolhos Bank (Pereira et al. 2005).

Sampling was conducted at 16 stations at two depths: subsurface samples, corresponding to 50–25% of the surface irradiance, and deep euphotic layer samples, with 10–1% of the light intensity (Fig. 1). The hydrographic structure of the area (water temperature and salinity) was obtained by lowering ME-CTD equipment from the surface down to a maximum depth of 450 m. Nitrate, nitrite, phosphate, and silicate were measured by spectrophotometry (Grasshoff 1983), and chlorophyll-a concentration was estimated by fluorometry (Yentsch and Menzel 1963). Plankton was sampled with Niskin bottles. Two to fifteen liters of the plankton samples were immediately concentrated in the dark by gravity using Nuclepore polycarbonate membrane filters with 1 μm pore size to a final volume of 250 mL. Aliquots were preserved with buffered paraformaldehyde (0.3% final concentration) and quantified in settling chambers using a Zeiss Axiovert -35 inverted light microscope (Utermöhl 1958).

For transmission electron microscopy (TEM), 10 mL of these samples were concentrated by centrifugation, prepared on grids covered with Formvar, air-dried at room temperature and observed in a PHILIPS CM-200 microscope. For scanning electron microscopy (SEM), subsamples of the plankton concentrate were fixed with glutaraldehyde (2.5% final concentration) in sodium

Fig. 1 Study area showing numbered sampling stations in Abrolhos Bank, eastern Brazilian continental shelf. Dashed lines correspond to 100 m isobaths
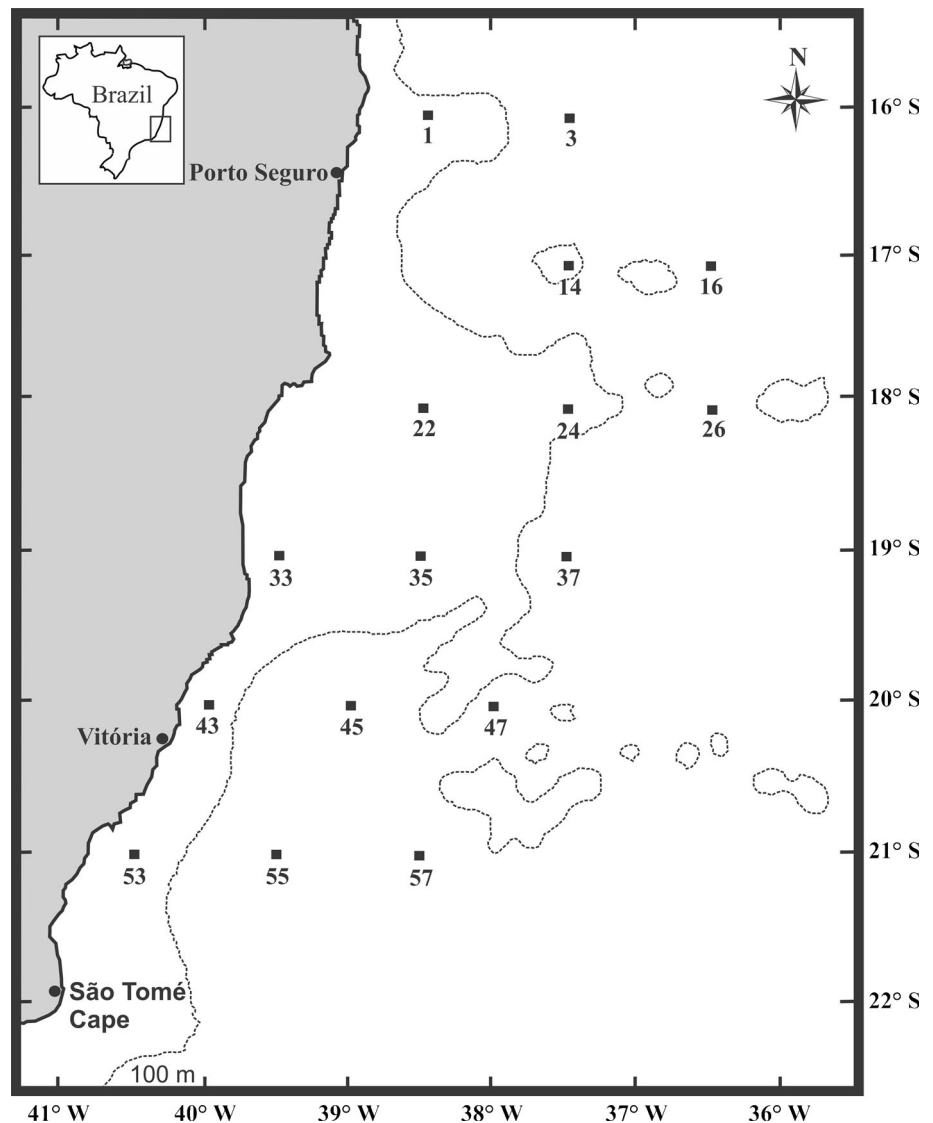
cacodylate buffer 0.1 M and sucrose 0.25 M and stored in a refrigerator (4 °C). In the laboratory, samples were filtered on Nuclepore filters (0.8 μm pore size) coated with poly-L-lysine solution, dehydrated through a graded ethanol series (10, 30, 50, 70, 90 and 100%) and critical point dried. Filters were mounted on stubs, sputter-coated with gold and analyzed with a JEOL JSM-840A microscope. Both electron microscopes are located at the Electron Microscopy Department of Physics Institute—University of São Paulo.

3 Results

Environmental data of the field sampling campaign are summarized in Table 1. High temperature and salinity associated with low nutrient content, especially in surface

layers, evidence the influence of TW from Brazil Current. Nutrients ranged approximately in a tenfold difference from lowest to highest values, except by nitrate, which showed wider variation in different stations. Silicate showed the highest mean level among the nutrient analyzed. Higher chlorophyll-*a* contents (> 0.2 mg m^{-3}) were related to stations south of Abrolhos Bank (especially at the deep euphotic layer) and over the bank.

Because of the small size of the cells and because species-specific features are almost indistinguishable in LM, nanodinoflagellate counts were categorized by shape and size. In these categories, dinoflagellate cells which were *Azadinium*-like in shape and 7–15 μm long ranged from 143 to 6174 cells L^{-1} (Fig. 2). The highest cell densities were observed at subsurface of stations 22 (1 m, 6174 cells L^{-1}), and 24 (10 m, 3812 cells L^{-1}) over the central zone in the bank and at stations 43 (10 m,

Table 1 Range, mean values, and standard deviation (SD) of environmental variables in this study

	Subsurface (n = 16)			Deep euphotic layer (n = 16)		
	Min–Max	Mean	SD	Min–Max	Mean	SD
Temperature (°C)	24.70–28.26	27.22	1.02	21.34–28.21	25.12	2.03
Salinity	36.33–37.40	36.99	0.28	36.36–37.14	36.82	0.24
NO_2 (μM)	< 0.01–0.14	0.04	0.03	< 0.01–0.14	0.06	0.04
NO_3 (μM)	0.04–4.78	0.77	1.24	0.12–2.88	1.08	0.94
PO_4 (μM)	0.05–0.46	0.12	0.10	0.07–0.32	0.15	0.07
$Si(OH)_4$ (μM)	0.63–5.86	2.08	1.55	0.77–7.20	2.79	2.15
Chlorophyll-a (mg m^{-3})	0.06–0.40	0.16	0.09	0.07–0.53	0.25	0.13

Fig. 2 Density (cells L^{-1}) of *Azadinium*-like cells at subsurface and deep euphotic layers by station in Abrolhos Bank
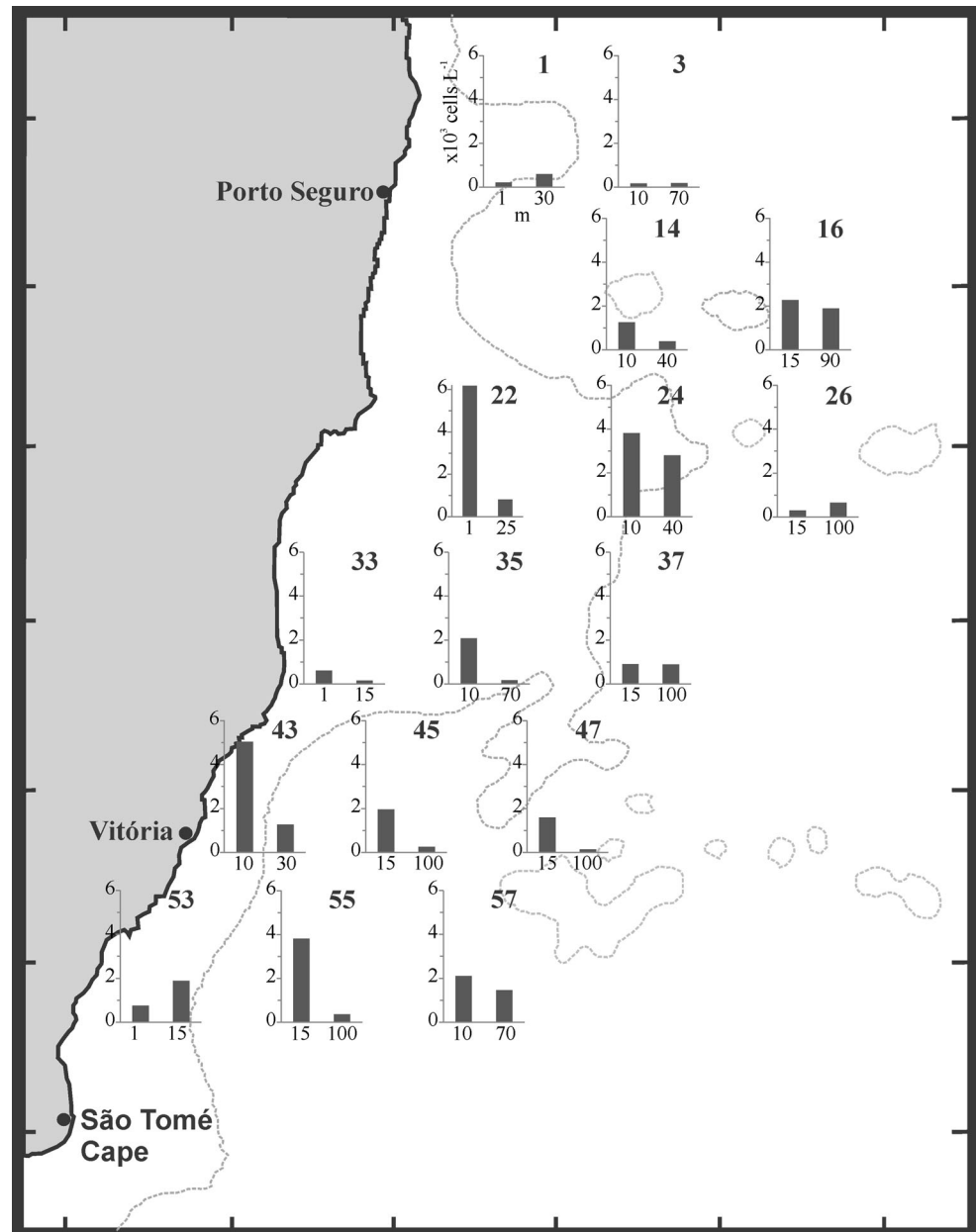
5044 cells L^{-1}) and 55 (15 m, 3812 cells L^{-1}) at south of the bank.

SEM and TEM analysis revealed at least four taxa assigned to the genus *Azadinium* in the samples, which are described below:

Azadinium dexteroporum Percopo et Zingone (in Percopo et al. 2013, J. Phycol. 49, p. 953, Figs. 1–6) (Fig. 3).

Small cell, 7.5 μm long, 5.4 μm wide in SEM image (*n* = 1); epitheca conical, hypotheca hemispherical; cingulum wide and excavated; ventral view not observed; the identification is based on the typical rectangular and concave shape of plate 2a; precingular plates high and antapical spine (0.5 μm long) located at the right side of the cell.

Azadinium luciferelloides Tillmann et Akselman (in Tillmann and Akselman 2016, Phycol. Res. 64, p. 162, Figs. 1–5) (Figs. 4–6).

Small cells, 8–11 μm long, 5.3–8.8 μm wide in SEM images (*n* = 4); epitheca conical, hypotheca hemispherical; cingulum wide and excavated; sulcus not reaching the antapex; plate 1′ narrow; ventral pore located at right ventral edge of the pore plate; precingular plates wide; small antapical spine (ca. 0.5 μm long) at right of the cell, emerging from the plate 2′′′′.

Azadinium cf. *polongum* Tillmann (in Tillmann et al. 2012, Harmful Algae 20, p. 145, Figs. 1–7) (Figs. 7–10).

Small cell, ca. 10 μm long and 7 μm wide in TEM image (*n* = 1); epitheca conical, hypotheca hemispherical;

Figs. 3–6 *Azadinium* spp. in SEM. **3** *A. dexteroporum* in dorsal view. **4–6** *A. luciferelloides* in ventral view (**4**), dorsal-lateral view, showing hypothecal tabulation (**5**), and ventral view of epitheca (**6**). vp = ventral pore. Scale bars = 2 μm
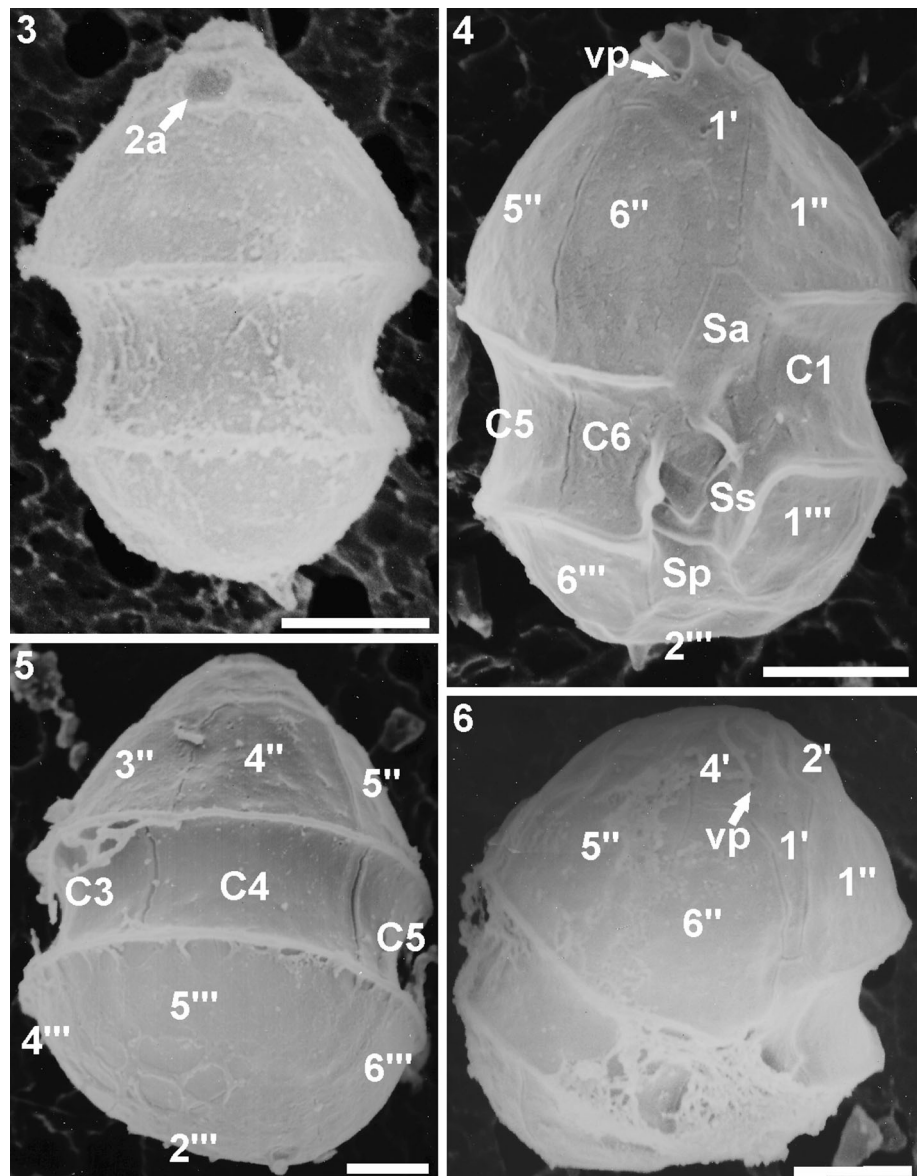
Figs. 7–10 *Azadinium* cf. *polongum*. 7, 8 *A.* cf. *polongum* in ventral view (**7**) and detail of the ventral pore (**8**), TEM. 9, 10. Schematic representation of our interpretation on the tabulation in **7**, ventral view (**9**) and dorsal view (**10**), seen by transparency. Dashed lines indicate sutures assumed but not clearly visible in the image. Scale bars = 2 μm (**7**) and 500 nm (**8**)
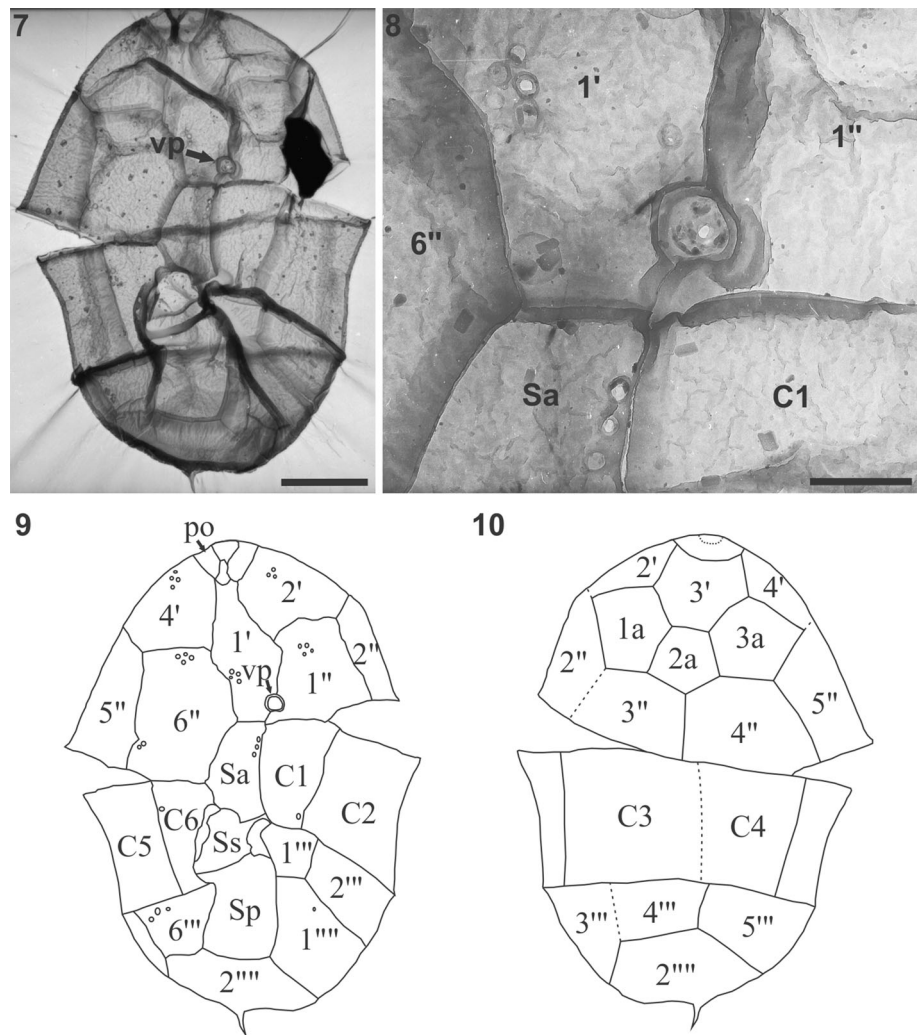
cingulum wide and excavated; sulcus not reaching the antapex; ventral pore located at lower left margin of a wide plate 1′ on the suture with the plate 1″; intercalary plates median in dorsal epitheca (seen by transparency), 2a pentagonal, slightly smaller than 1a and 3a; 1a not in contact to 1″; medium-size antapical spine (ca. 0.6 μm long), located on the right (almost center) of the hypotheca, emerging from plate 2‴′.
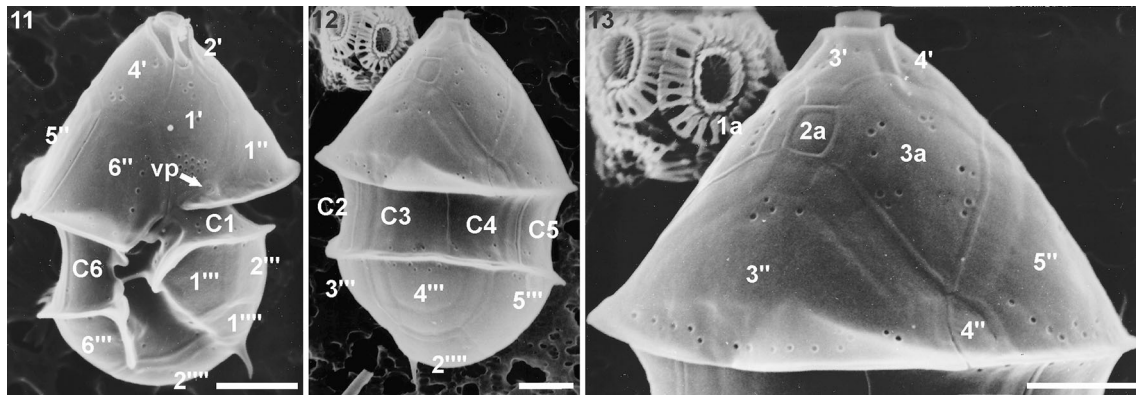
Azadinium sp. (Figs. 11–13).

Small cells, 9.8–11.8 μm long, 7.8–8.7 μm wide in SEM images (*n* = 2); epitheca conical, hypotheca hemispherical; cingulum wide and excavated; sulcus wide, not reaching the antapex; ventral pore located at lower left margin of a wide plate 1′; plate 1′ with a dense group of small pores; plate 2a small and square, median in dorsal epitheca; plate 3a large, with posterior end close to cingulum edge and contacting the tip of a small triangular precingular plate 4″; large antapical spine (0.8–1.0 μm

long) displaced to the left side of the cell emerging from plate 2‴′.

4 Discussion

The identity of the taxa – In fact, the taxa reported here belong to the genus *Azadinium*, based on small size, thecal plate formula, and typical features such as presence of a distinct ventral pore, the apical pore complex structure, with X plate forming a finger-like protrusion in contact to the cover plate, and the sulcal plate Ss running from the cingular plate C1 to C6 (Tillmann et al. 2009; Nézan et al. 2012). Generally, taxa of *Azadinium* at infrageneric level are quite similar, but some key features are proven to be sufficiently conservative among species and are often used in a morphotaxonomic approach (Nézan et al. 2012; Tillmann and Akselman 2016), such as the position of the ventral pore, size, shape and arrangement of intercalary plates, pattern of thecal pores and presence of an antapical

Figs. 11–13 *Azadinium* sp. in ventral (**11**), dorsal (**12**) and detail of dorsal view of epitheca (**13**), SEM. vp = ventral pore. Scale bars = 2 μm

spine (Tillmann et al. 2014b; Tillmann and Akselman 2016).

Azadinium dexteroporum was identified in this study by a unique feature only found in this species so far: the typical concave plate 2a. This character is quite conservative in the type material (Percopo et al. 2013) as well as in specimens recorded from the Argentinean shelf (Tillmann and Akselman 2016), although a subarctic strain identified as *A. dexteroporum* showed a flat plate 2a (Tillmann et al. 2015). Other features, such as high precingular plates, presence of an antapical spine on the right side of the cell and the smaller size (Percopo et al. 2013), as well as the confirmed presence of this taxon in the southwest Atlantic (Tillmann and Akselman 2016) support this identification.

A newly described species forming blooms in Argentinean middle shelf waters, *Azadinium luciferelloides* (Tillmann and Akselman 2016), was also recorded in this study. This species can be distinguished from others by a ventral pore located at right to the pore plate, contacting the suture between the plates 1′ and 4′; plate 1′ is narrow, rhomboid and slightly asymmetrical; and a short spine is present on the antapical plate 2′′′′, at right of the theca (Tillmann and Akselman 2016).

Azadinium spinosum and *A. polongum* are the only taxa so far described as having both a ventral pore located at left margin of the plate 1′ and an antapical spine slightly positioned at the right side of the cell (Tillmann et al. 2009, 2012). However, differences in the shape of the pore plate, in the position of the ventral pore, and in the arrangement of the dorsal epithecal intercalary plates are important in distinction of these species. *A. spinosum* is characterized by a round pore plate, by a ventral pore inserted on the plate 1′ close to (but not on) the suture with the plate 1′′, and by a hexagonal plate 3′ which is almost as high as the plate 2a (Tillmann et al. 2009). In contrast, *A. polongum* has an elongated pore plate, a ventral pore which is more posteriorly positioned in the epitheca and which

insert on the suture between 1′ and 1′′ forming a cavity in plate 1′′, and a plate 3′ with a narrow base which is linked to a more minute plate 2a (Tillmann et al. 2012). The ventral view of the single cell of *A.* cf. *polongum* recorded in this study conforms to the major features of the latter species, except by shape and size of the plates 3′ and 2a. However, these observations should be considered with caution, since the only specimen observed is collapsed and the shape of the plates may be slightly altered. Another interesting feature of the cell of *A.* cf. *polongum* observed here is that 1′′ does not contact 1a. Such a contact was described for the type material of *A. polongum* (Tillmann et al. 2012), but without exception was missing for a Pacific field population of *A. polongum* (Tillmann et al. in revision). In any case, morphological observations of more Brazilian specimens and ultimately molecular data are needed for unequivocal identification of this taxon.

The taxon identified here as *Azadinium* sp. shows external morphological features quite distinct from any *Azadinium* taxon yet described. The position of the antapical spine very distinctly is displaced to the left of the sulcus. Within *Azadinium* an antapical spine position slightly left of the sulcus can be seen for the two varieties of *A. caudatum*, which also have an exceptionally small and three-sided plate 4′ and a very minute (compared to the other epithecal intercalary plates) central epithecal intercalary plate (Nézan et al. 2012). Moreover, for *A. caudatum* the rim running around the pore plate extend on the dorsal side alongside the anterior margins of plate 3′, a feature which is also present in the specimen of *Azadinium* sp. depicted in Figs. 12–13. Based on these similarities a close relation between *Azadinium caudatum* and *Azadinum* sp. might be anticipated. However, the overall shape and size of the cell as well as the arrangement of the thecal plates and pores are evidently different between *A. caudatum* and *Azadinium* sp. The elongated X and pore plates, and the position of ventral pore are very similar to *A. polongum*. However, compared to *A. polongum*, *Azadinium*

sp. showed a more conical epitheca, and the plate Sp is apparently larger, with the margin contacting the plate 2'''' being expressively longer than the suture with the plate 1''''. Moreover, the antapical spine at left of the sulcus and the size and shape of 4'' are important differences between these taxa. A feature of *Azadinium* sp. not described for other species of *Azadinium* is the presence of a group of pores in the posterior part of plate 1'. In conclusion of all morphological details, this taxon seems to be new to science. Previous *Azadinium* species were described based on morphology and molecular data obtained from cultured material. The only exception is *A. luciferelloides*, which was described from a field bloom sample, where the cell number was sufficiently high to evaluate the extent of variation of the observed diacritical features (Tillmann and Akselman 2016). Up to now, our observations about the potentially new *Azadinium* taxon reported here are rather limited and insufficient to evaluate if the morphological differences are conserved in a population. A formal description of this taxon will thus be put on hold until new material for more detailed analysis is available.

Distribution and ecology of *Azadinium* spp. – In the upper mixed layer of Abrolhos Bank region, cell densities of *Azadinium* spp. with maximum of 6×10^3 cells L^{-1} were found at fall, at temperatures between 21 and 28 °C, high salinity and rather low nutrient content (Table 1). In this time, cyanobacteria (64%) and autotrophic nanoplankton (31%) were quite representative of the total phytoplankton in carbon biomass (Susini-Ribeiro 1996, 1999). In addition, phytoplankton carbon biomass was twice as large on the south continental shelf and on the Abrolhos Bank as in the open ocean (Susini-Ribeiro 1999). Similar pattern were observed in the density and distribution of *Azadinium* (Fig. 2) and also of diatoms. The diatoms were more abundant in stations near the coast than offshore. In coastal stations microplanktonic diatoms were most abundant whereas in offshore oceanic stations nanoplanktonic forms predominated (Susini-Ribeiro 1996). A relatively low phytoplankton biomass associated with a high contribution of nanoplanktonic forms in the Abrolhos region is related to the predominance of the oligotrophic, warm and saline tropical water mass from Brazil Current. However, the phototrophic fraction of the micro- and nanoplankton increases in the southern area and over the Abrolhos Bank. In these regions, the input of deep and nutrient-rich water masses originating from SACW justifies an increase in productivity (Susini-Ribeiro 1999; Susini-Ribeiro et al. 2013).

Studies about the spatial and temporal dynamics of *Azadinium* species are scarce, especially due to their small size (about 15 µm length) and the impossibility of an accurate identification during routine quantification at LM

(Tillmann et al. 2014b). Most of the information on the spatial and temporal distribution of *Azadinium* was indirectly inferred by AZA toxins detection (Krock et al. 2013; Tillmann et al. 2014b). In the North Sea, AZA distribution indicated that *Azadinium* populations were more common in the mid to late boreal summer (Tillmann et al. 2014b). Similarly, in northwestern coast of Morocco, maximal concentration of AZAs occurred in July (Taleb et al. 2006). Extensive blooms forming brownish-red patches ca. 60 km long occurred in the northern shelf of Argentina during austral spring at rather low water temperature (8–9 °C) and salinity ($\sim$ 33.7) and high nitrate content (> 3 µM), with maximum *Azadinium* cell density estimated as 9×10^6 cells L^{-1} (Akselman and Negri 2012). According to these authors, *Azadinium* biomass in spring can be greater than typical density of spring-bloom diatoms of the genus *Thalassiosira*, presenting the highest *Azadinium* bloom density known so far.

In many coastal areas of the world, the detection of azaspiracids in shellfish tissues preceded the isolation and identification of the producer organism (Tillmann and Akselman 2016; Tillmann et al. 2017b), and we have the same progression for Brazil. While our samples were taken already in 1995, we only now have accurately determined the first observation of these algae in Brazilian coastal waters. This is partially due to scarcity of researches focusing on the nanoplanktonic size fraction in this region.

Among the four *Azadinium* taxa reported here, AZA production is only confirmed for one of them. The type culture of *A. dexteroporum* from the Mediterranean produces seven (six of which are new to science) different AZAs (Rossi et al. 2017), but a strain from the subarctic Irminger Sea determined as *A. dexteroporum* clearly lack any of these or other known AZA (Tillmann et al. 2015). For *A. polongum*, the type strain from the Northeast Atlantic is negative for AZAs (Tillmann et al. 2012) and a bloom sample with 1×10^6 cells L^{-1} of *A. polongum* from the Pacific coast of Peru was negative for AZAs (Tillmann et al. in revision). For both *A. luciferelloides* and *Azadinium* sp. the AZA production potential is unknown due to the lack of cultured strains.

The knowledge on which AZAs are present in Brazil is rather limited. Azaspiracids have been preliminary detected along the Brazilian southern coast since 2009 in mussels (Schramm et al. 2010), oysters and crabs (Massucatto et al. 2014). The latter authors reported on the presence of six AZAs, with AZA-2 being known as a compound produced by *Azadinium poporum* (Tillmann et al. 2016) and the other AZAs being known as shellfish metabolites. In contrast, Schramm et al. (2010) reported the presence of AZA-1 and -2, and AZA-1 up to now is only known to be produced by *A. spinosum* (Hess et al. 2014). Merging our first data on *Azadinium* diversity with these preliminary

toxin data indicate that both, yet unreported AZAs (as inferred from the presence of *A. dexteroporum*), and yet undetected species (as inferred from the presence of AZA-1 and -2) might be present in Brazil. In any case, in order to evaluate the local AZA production potential, Brazilian strains need to be established and analyzed, as AZA-producing strains and non-toxigenic strains within the same species are known for the genus *Azadinium* (Krock et al. 2014; Tillmann et al. 2015; Rossi et al. 2017).

Our SEM and TEM analysis indicates that in Brazil several species of *Azadinium* co-occur in the environment. A high diversity of species of *Azadinium* in the same area has been emphasized for other areas as well (Tillmann et al. 2010, 2014a; Nézan et al. 2012; Tillmann and Akselman 2016), which makes it generally difficult to identify, quantify, and distinguish between toxic and non-toxic species in light microscopy-based monitoring programs. The *Azadinium* sp. recorded here shows morphological features divergent from others described species, with high potential to be new to science. Altogether, the presented findings corroborate the current notion (Tillmann and Akselman 2016) that the actual distribution and diversity of *Azadinium* species remains underexploited.

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