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A taxonomical study of benthic *Prorocentrum* species (Prorocentrales, Dinophyceae) from Anse Dufour (Martinique Island, eastern Caribbean Sea)

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Abstract :

About 30 benthic *Prorocentrum* species have been described, some of which producing okadaic acid and derivatives involved in diarrhetic shellfish poisoning. The western Caribbean has been extensively studied for benthic dinoflagellates associated with ciguatera, and fifteen *Prorocentrum* species were described from mangroves and coral reefs of Belize. In contrast, no study reported the diversity of this genus in the Eastern Caribbean, especially in the Lesser Antilles. This study adds to the biodiversity knowledge in Martinique Island by investigating one site of the Caribbean coast from 2010 to 2017. Sediment samples were collected each year in March and studied taxonomically. Identification was realized morphologically by scanning electron microscopy, while the partial large subunit (LSU) ribosomal DNA was sequenced for 42 isolated specimens (single-cells) and one strain in culture. A molecular phylogenetic analysis revealed 11 OTUs from Martinique, identified morphologically as *P. concavum*, *P.* cf. *foraminosum*, *P.* cf. *tropicale*, *P. lima*, *P. hoffmannianum*, *P.* cf. *norrisianum*, *P. glenanicum*, *P. panamense*, *P.* cf. *sculptile*, *P.* cf. *fukuyoi*, and *P. rhathymum*. Two morphospecies were also identified (*P.* cf. *maculosum* and *P.* cf. *ruetzlerianum*) but with no sequence obtained. Some species like *P.* cf. *tropicale* and *P.* cf. *norrisinamum* are sequenced for the first time. Our analysis reveals probable former misidentifications of *P.* cf. *foraminosum* and *P.* cf. *sculptile* since the sequences from Martinique form new clades and their geographical origin are closer from the type locality than any other previous studies. Further studies and sequences from the type localities are yet required to assess identifications.

Keywords : Caribbean, Dinoflagellates, LSU rDNA, Phylogeny, *Prorocentrum*, Taxonomy

Introduction

The Caribbean area has been long investigated for potentially toxic microalgae responsible of ciguatera. In the 90s, M.A. Faust published a remarkable series of papers on the biodiversity of benthic dinoflagellates from tropical ecosystems of Belize (Faust 1990a; b, 1991, 1993a; b, 1994, 1997, 2009; Faust et al. 2008). In addition to her investigations of the toxigenic genera *Gambierdiscus* and *Ostreopsis* (Faust 1995, 1999; Faust and Morton 1995), she made a major contribution to the genus *Prorocentrum* for which she described fifteen benthic *Prorocentrum* morphospecies from mangrove habitats of Twin Cays and lagoonal waters at Carrie Bow Bay. To date, some of these species like *P. ruetzlerianum* Faust*, P. sabulosum* Faust or *P. tropicale* Faust are still poorly known, since they have not been reported in any other location than their type locality.

Although the morphology of *Prorocentrum* can be considered as simple owing to the presence of only two main lateral plates and several small platelets in the periflagellar area, the species delimitation in this genus is complex (Hoppenrath et al. 2013). Some morphological characters are now recognized as valuable taxonomic features, such as the number and organization of periflagellar platelets, but they have not been characterized with enough accuracy in several original descriptions (Hoppenrath et al. 2013). In contrast, such features as the size, shape and the ornamentation are variable and not reliable enough for species identification. To date, about 71 species have been described and have been flagged as accepted (Guiry and Guiry 2018), of which half are benthic or epiphytic. More species have been described on a morphological basis, but the use of molecular taxonomy and sequencing of ribosomal DNA genes or internal transcribed spacers ITS1 and ITS2 showed that some closely related morphotypes possessed very similar genotypes and synonymies have been proposed. For instance *P. arabianum* Morton & Faust is now regarded as a junior synonym of *P. concavum* Fukuyo (Mohammad-Noor et al. 2007b). More recently, an extensive study revealed that *P. belizeanum* Faust can be regarded as conspecific with *P. hoffmannianum* Faust (Herrera-Sepúlveda et al. 2015). Nevertheless, some synonymies like *P. arenarium* Faust which has been proposed as a synonym of *P. lima* (Nagahama et al. 2011) are not commonly accepted by all authors. The level of morphological and genetic variability is very high in '*P. lima*' and the species delimitation is not clear, leading to consider this taxon as a species complex (Bouaïcha et al. 2001; Nagahama et al. 2011; Hoppenrath et al. 2013). Some authors have recognized different morphotypes associated with some genetic variability but did not propose them as separate species (Zhang et al. 2015). Only recently, *P.*

caipirignum Fraga, Menezes & Nascimento has been described as a separate species in this complex (Nascimento et al. 2017), on the basis of a molecular analysis of the ITS region since no support was found with LSU rDNA. In this study, the authors suggested that *P. arenarium* could be considered as separate species, while the circumscription of *P. lima* should be reevaluated. Another case of a problematic species complex with a considerable genetic variation among specimens and populations is the group *P. emarginatum*/*P. fukuyoi.* These two species share several morphological characters such as the asymmetry, the thecal pore pattern and a peculiar periflagellar area with a wing on platelet 1. Specimens with this peculiar morphology have been found in various places around the world, but due to their resemblance, their identification was unclear (e.g. Faust 1990a; Mohammad-Noor et al. 2007a; Laza-Martínez et al. 2011), which generated many confusions and misinterpretations (Hoppenrath et al. 2013). Even when molecular data was available, the considerable genetic variability observed in this complex did not allow to conclude on the identity of the specimens (e.g. Laza-Martínez et al. 2011; Hoppenrath et al. 2013). This level of genetic divergence may indicate the existence of cryptic or pseudocryptic species in this complex, but in order to clarify their boundaries, further molecular analyses from various locations in the world are absolutely necessary.

In contrast with the western part of the Caribbean, taxonomic studies focusing on the diversity of benthic dinoflagellates are scarce in French Antilles (also known as French West Indies). In a previous study, Chomérat and Bilien (2014) described *Madanidinium loirii* Chomérat, a new dinoflagellate from Martinique Island. A few other surveys focused on the toxigenic genera *Gambierdiscus* and *Ostreopsis* associated with ciguatera (Besada et al. 1982; Taylor 1985; Litaker et al. 2010; Boisnoir et al. 2018) since intoxications have been recurrently documented in this area (Olsen et al. 1984; Vernoux 1988; Pottier et al. 2001; Rosine et al. 2008; Tester et al. 2010). Although it has been shown that several benthic *Prorocentrum* species produce toxins such as okadaic acid, dinophysistoxins and fast acting toxins (Faust et al. 1999; Faust and Gulledge 2002; Lassus et al. 2016; Luo et al. 2017), no taxonomic study on this genus has been realized in this area. The aim of the present study is to investigate the diversity of benthic *Prorocentrum* species in one site of Martinique Island using a observations of the morphology by microscopy, and sequencing the partial large subunit of ribosomal DNA (LSU rDNA) from single cells. This data will be used in a phylogenetic analysis in order to perform genetic comparisons and better understand the level of genetic variability within species. Furthermore, this analysis will be helpful to better appreciate the biogeography of some

96 species and to bring new insights on the evolution of the Prorocentrales, which is a very distinctive
97 group among dinoflagellates.

98

99 Material and methods

100 Sampling and culturing

101 Surface sediment ("organic dust") samples have been collected recurrently each Spring (in March)
102 from 2010 onwards (2010-2017) at one sampling site located in the Anse Dufour (coordinates
103 14°31.538' N; 61°5.446' W), on the Caribbean coast of the Island (Chomérat and Bilien 2014). Five
104 samples collected the 16 March 2010, 20 and 27 March 2012, 27 March 2015, and 30 March 2017
105 were used in the study. Immediately after being collected in snorkelling, the samples were fixed with
106 acidic Lugol's solution, while one subsample from 2017 was kept fresh and transported to the
107 Concarneau laboratory alive in less than 48h. In the laboratory, Lugol-fixed samples were stored at 4
108 °C in the dark, while the fresh sample was used for isolation of interesting species in order to establish
109 cultures. For cultivation, single cells from the live sediment sample were isolated and processed as
110 described in Chomérat and Bilien (2014). One strain was successfully established (IFR-PTR-01M)
111 and grown in K/5 medium (Keller et al. 1987) in an incubator set up at 22 ± 1.0 °C and a 12:12
112 light:dark illumination cycle with ~ 50 µmol photons m^{-2} s^{-1} provided by white fluorescent tubes.

113

114 Scanning electron microscopy

115 For scanning electron microscopy, cells were first individually isolated and concentrated in 2 ml tubes
116 containing water and a drop of formaldehyde to prevent development of fungi in the water. Then, cells
117 were filtered on polycarbonate membrane filters (Millipore RTTP Isopore, 1.2 µm pore size,
118 Millipore, Billerica, USA), rinsed in deionised water and prepared according to Chomérat and Couté
119 (2008). After gold-coating, SEM examinations were carried out with a Quanta 200 (FEI, Eindhoven,
120 The Netherlands) scanning electron. Cells were measured on SEM digital micrographs using ImageJ
121 software (Rasband 1997). SEM images were presented on a uniform background using GNU Image

Manipulation Program v.2.8.22. The terminology used for thecal plates and periflagellar platelets in this paper follows the system of Hoppenrath et al. (2013).

DNA amplification and molecular analyses

For single-cell isolation, the tube containing Lugol fixed sample was vigorously shaken and a subsample of 30 µl was diluted in 4 ml of filtered seawater in a small Petri dish. A Olympus IX51 (Olympus, Tokyo, Japan) inverted microscope was used to search the cells of interest. They were isolated with a micropipette under the inverted microscope and rinsed in four drops of distilled water before being transferred into a 200 µl PCR tube. Each cell was measured and pictured using a Olympus E–300 digital camera to register the morphological features used for identification. In some cases, when the identification was not possible with LM only, cells were dissected under the inverted microscope using the pipette. Large lateral plates were observed, photographed, and then transferred onto a SEM-stub bearing a poly-L-lysine coverslip with an engraved grid in order to examine them by SEM. The cell protoplast was rinsed and placed in a PCR tube containing 5 µl of ultrapure water. This process allowed us to associate the precise morphology of the theca of a specimen with its sequence. Tubes containing single cells were stored at – 20 °C until further analysis. For PCR, tubes were thawed and processed as described in Chomérat et al. (2012).

All sequences obtained in this study have been deposited in GenBank and accessory numbers are given in Fig. 1.

Phylogenetic analysis

For the phylogenetic analysis, 43 sequences acquired from single cells of *Prorocentrum* were aligned together with 90 LSU sequences of other *Prorocentrum* species and 2 sequences of *Peridiniella* (as outgroup) retrieved from GenBank. The sequences were aligned using MAFFT software version 7 with selection of the Q-INS-i algorithm which considers the secondary structure for the alignment (Katoh and Standley 2013). This step was followed by refinement by eye with MEGA software version 5.2.1 (Tamura et al. 2011). The final data matrix contained 135 LSU rDNA sequences and 643 sites. The best-fitting model of substitution, according to the Akaike Information Criterion (AIC), the Bayesian Information Criterion (BIC) and hierarchical Likelihood-ratio tests (hLRTs), was selected

using ModelGenerator v. 0.82 (Keane et al. 2006). General time reversible (GTR) model with a gamma correction (Γ) for among-site rate variation and invariant sites was chosen from one of the best models for the LSU dataset. The parameters of the model were as follows: Log-likelihood:-8260.79059; Unconstrained likelihood: -3627.54489; Parsimony: 1701; Tree size: 4.35007; Discrete gamma model; Number of categories: 8; Gamma shape parameter: 0.971; Proportion of invariant: 0.086; Nucleotides frequencies: f(A)= 0.22706, f(C)= 0.21750, f(G)= 0.31135, f(T)= 0.24409; GTR relative rate parameters: A↔C: 1.07364, A ↔G: 3.15646, A↔T: 1.02071, C↔G: 0.61559, C↔T: 7.74009 and G↔T: 1.00000. The evolutionary models were examined using maximum likelihood (ML) and Bayesian Inference analysis (BI). Maximum likelihood analyses were performed using PhyML version 3.0 (Guindon et al. 2010), and Bayesian analyses were run using Mr Bayes version 3.1.2 (Ronquist and Huelsenbeck 2003). Bootstrap analysis (1000 pseudoreplicates) was used to assess the relative robustness of branches of the ML tree. Initial Bayesian analyses were run with a GTR model (nst = 6) with rates set to invgamma (gamma for LSU dataset). Each analysis was performed using four Markov chains (MCMC), with two millions cycles for each chain. Trees were saved every 100 cycles and the first 2000 trees were discarded. Therefore, a majority-rule consensus tree was created from the remaining 18,000 trees in order to examine the posterior probabilities of each clade.

Genetic distances were calculated on the same alignment using Kimura 2-parameter (Kimura 1980) model in MEGA 5 software.

Results and discussion

In order to present the taxa in an order reflecting their phylogenetic relationships, this section is presented with the molecular analysis first, allowing to define major clades. In each clade, the morphological features of the species identified in the study are described and discussed.

Phylogenetic analysis

The molecular phylogeny inferred from LSU rDNA revealed that the 43 sequences acquired in this study clustered in six major clades (Fig. 1). The clade A comprises sequences identified as *P. concavum* and related species. It forms a sister clade (with a moderate support) to the clade B which encompasses the species related to *P. lima* forming the *'P. lima* complex'. At the base of these two clades, the clade C groups sequences of *P. borbonicum* and *P. sipadanense* for which no sequence have been obtained in the present study. In contrast, the clade D comprises only new sequences acquired in this study and identified as *P.* cf. *norrisianum*. The clade E encompasses sequences of *P. panamense* and *P. glenanicum*. The clade F groups the sequences attributed to species from the *P. emarginatum*/*fukuyoi* complex and exhibits a high level of genetic variation, with several subclades. The clade G comprises the epibenthic species *P. rhathymum* but also planktonic species of *Prorocentrum.*

Clade A – *Prorocentrum concavum* and related species

Nine sequences from Martinique Island clustered in this clade. Two sequences grouped with other sequences of *P. concavum* retrieved from GenBank (subclade A1), while the other sequences form new branches and did not group with any sequence. The sequence of the strain IFR-PTR-01M (*P.* cf. *tropicale*) branched at the base of the clade A and the remaining 6 almost identical sequences (subclade A3) formed a sister-clade to *P. leve* with a good support, and were ascribed to *P.* cf. *foraminosum* on a morphological basis.

– *Prorocentrum concavum* Fukuyo (subclade A1)

Syn. *P. faustiae* Morton

Cells were broadly oval in shape (Figs 2d-e), 46–48 µm in length (mean 45.8 µm, s.d. 1.8 µm, n = 4) and 38–44 µm in width (mean 40.0 µm, s.d. 3.1 µm, n = 4). The length to width ratio varied from 1.06 to 1.24. The periflagellar area was V-shaped, composed of 9 platelets (1a, 1b, 2, 3, 4, 5, 6, 7 and 8), but platelet '7' was not clearly visible and hidden by platelet 1 (Fig. 2f). The apical part of the left lateral plate was granular (Fig. 2e). The thecal surface was reticulate-foveate (Figs 2d–f) with

scattered thecal pores, except in the centre of lateral plates. Thecal pores were 0.25–0.30 µm in diameter.

The original size range given by Fukuyo (1981) was 44–45 µm in length and 40 µm in width but Faust (1990a) reported larger cells. Morton (1998) gave a size range only slightly wider for *P. faustiae* from reef flats of Heron Island (Great Barrier Reef), 43–49 µm in length and 38–42 in width, while Mohammad Noor (2007a) reported a similar range (43–53 µm in length and 38–48 µm in width) for *P. concavum* but a larger range (45–60 µm in length and 38–53 µm in width) for specimens identified as *P. faustiae*. More recently, a Chinese study reported cells of *P. concavum* being 45.7–50.2 µm long and 37.7–42.4 µm wide (Luo et al. 2017), which is very close to our observations. In the phylogenetic analysis (Fig. 1), all sequences clustered in a well resolved clade with a very low level of genetic variation (mean genetic distance 0.008). Although one of these sequences from Malaysia was ascribed to *P. faustiae* (EF566744), its distance with other sequences in the clade varies from 0.002 to 0.012, which is low. The topology suggests the existence of only one monophyletic species, with slight intraspecific variation as found for specimens from Martinique Island. Therefore, as discussed earlier in Hoppenrath et al. (2013), we consider here that *P. faustiae* and *P. concavum* are conspecific in absence of significant morphological and genetic differences. As *P. concavum* was described earlier (Fukuyo 1981), it has the priority over *P. faustiae* which we consider here as a junior synonym.

– *Prorocentrum* cf. *tropicale* Faust

Cells were broadly oval, with the maximum width behind the middle part. In the culture, they were 35.8–39.8 µm in legnth (mean 37.6 µm, s.d. 1.1 µm, n = 20) and 31.2–35.2 µm in width (mean 33.3 µm, s.d. 1.2 µm, n = 20). The length to width ratio varied from 1.09 to 1.18 (mean 1.13, s.d. 0.02, n = 20). The periflagellar area was in a V-shaped depression of the right lateral plate and was composed of nine platelets (1a, 1b, 2, 3, 4, 5, 6, 7 and 8) (Fig. 2c). The thecal surface was reticulate-foveate (Figs 2a, b) and the intercalary band horizontally striated (Fig. 2c).

Prorocentrum tropicale is a poorly known species described by Faust (1997) from Carrie Bow Cay (Belize). Since its description, this species has never been reported in any another site, making no further comparison possible. In Martinique Island, cells were collected on the sediment while the

specimens studied by Faust (1997) were epiphytic on detritus or coral rubble. In our culture, cells were smaller than the range given in the original description (50–55 µm in length and 40–45 µm in width, Faust 1997), but it might be a bias resulting from culture conditions and a growth rate that is not comparable with field specimens. Except the size, most of the morphological features were in agreement with the description, including the horizontally striated intercalary band. Unfortunately, the pattern of the periflagellar area was not clearly illustrated with enough details by Faust (1997) and it cannot be further compared. Although Faust (1997) mentioned 8 platelets, some may have been overlooked. Because of these discrepancies and in absence of a reinvestigation of the type material, it was not possible to confirm the identity of the specimens from Martinique Island. Provisionally, we considered them as *P.* cf. *tropicale* as they were morphologically closely related to this species but further analyses are necessary.

– *Prorocentrum* cf. *foraminosum* Faust (subclade A3)

Cells were oval oblong in shape (Figs 2g-h), 43–49 µm in length (mean 45.6 µm, s.d. 1.8 µm, n = 12) and 30–36 µm in width (mean 33.2 µm, s.d. 1.6 µm, n = 12). Length to width ratio varied from 1.31 to 1.53 (mean 1.37, s.d. 0.06, n = 12). The periflagellar area was in a small and narrow V-shaped depression of the right lateral plate while the left thecal plate had a nearly flat edge (Figs 2g-i). Nine platelets were identified (1a, 1b, 2, 3, 4, 5, 6, 7 and 8). Platelet 1a was large, almost triangular in shape and beared a large triangular flattened depression . Just below this platelet, a smaller rectangular platelet 1b was present. Both of these platelets were protruding over the accessory pore and platelets located beneath, making this area difficult to study. Platelet 2 was rather small, with a narrow rectangular shape higher than wide. Platelet 3 was distinctive in being narrower towards the sagittal suture and widening on its side in contact with the flagellar pore (Fig. 3j). Platelet 4 was similar in size than platelet 1a, and had roughly the same shape, reversed, but its central depression was smaller. Platelet 5 had a J-shape and is in contact with the flagellar pore. Platelet 6 was small and rectangular, located below platelet 8. Platelet 7 was hardly visible in SEM because of the overlap of platelets 1a and 1b, but its end was seen in some specimens. Platelet 8 was small and separated the flagellar pore (which was partially visible below the platelets 1a and 1b) from the accessory pore. The thecal surface was smooth, and covered with scattered thecal pores, except in the centre. Thecal pores were 0.13–0.15 µm in diameter and were located in shallow depressions (diameter ca. 0.4 µm), which were more

conspicuously visible when cells were in an oblique view. The number of pores on a lateral plate varied from 275 to 320, with an average of 296 (s.d. 16.0, n = 8). The intercalary band was smooth, narrow in young specimens or much wider in older ones (Figs 2i–j).

Morphologically, all the features observed on specimens from Martinique Island were in agreement with the original description of *P. foraminosum* from Hidden Lake, Twin Cays (Belize) (Faust 1993). In addition, we observed the presence of a central pyrenoid and found cells sometimes encased in mucus as mentioned by Faust (1993). Moreover, we found that the organization of the periflagellar area is remarkably similar (cf. Fig. 12 in Faust 1993) and we consider that it can ensure the species identification (Hoppenrath et al. 2013). Apart the original description, it was reported by Mohammad-Noor et al. (2007a) but the morphology was somewhat different from the type material and the identification cannot be ascertained as the ornamentation is a not a reliable feature. Indeed, a species with a typical foveate ornamentation and a similar size has been putatively identified as '*P. foraminosum*' in the northern Atlantic and Sea of Japan (Hoppenrath et al. 2013; Kameneva et al. 2015; Selina 2017) but the sequences acquired for this temperate species are divergent from those obtained in the present study (Fig. 1). In the light of the new molecular data, it appears that the tropical specimens from Martinique Island fit well with *P. foraminosum* while those from the temperate area form a well separate clade (subclade A2) and likely correspond to another, yet undescribed species. Owing to the absence of DNA sequences from the original description and type locality, it is not yet possible to ascertain the identity of specimens from Martinique Island, although the morphology is very similar to *P. foraminosum*. In contrast, the morphology of specimens from the Atlantic and North Sea was not exactly similar and the species was misidentified since it differs more with the original description of *P. foraminosum* than the specimens studied herein. A detailed analysis and a description of this temperate species (subclade A2) is out of focus of the present paper and will be published in a separate study.

Clade B – *Prorocentrum lima* complex and related species

This large clade comprised sequences of *P. lima* and related species such as *P. consutum*, *P. bimaculatum*, *P. lima* (including several morphotypes), *P. caipirignum*, a species previously

292 encompassed within the *P. lima* species complex, and *P. hoffmannianum*. Two sequences acquired in
293 the study were ascribed to *P. lima* and *P. hoffmannianum* on a morphological basis.

294

295 – *Prorocentrum lima (*Ehrenberg) F. Stein

296 Cells with two distinct morphotypes were found in Martinique. Broadly ovate to pyriform specimens
297 (Figs 3a–b) were 31.1–34.6 µm in length (mean 32.6 µm, s.d. 1.5 µm, n = 5) and 28.4–31.3 µm in
298 width (mean 29.6 µm, s.d. 1.3 µm), with a length to width ratio of 1.10–1.11. In contrast, oblong oval
299 cells (Figs 3c–d) were 37.1–38.2 µm in length (mean 37.8 µm, s.d. 0.5 µm, n = 5) and 27.8–32.3 µm
300 in width (mean 30.6 µm, s.d. 1.8 µm, n = 5), with a length to width ratio varying from 1.18 to 1.36.
301 For both morphotypes, the periflagellar area consisted in eight platelets (Fig. 3e). The thecal surface
302 was smooth without any ornamentation (Figs 3a–e). Thecal pores were present on the surface of
303 lateral plates, and in a marginal ring but they were absent in the centre. In the pyriform morphotype,
304 they were round to oval, 0.4–0.5 µm in diameter, but in some specimens, marginal pores were more
305 elongated (Fig. 3b). In the oblong-oval morphotype, the pores were more elongated to kidney-shaped
306 and 0.6–0.8 µm long; the marginal pores were elongated and appeared as short lines (Figs 3c–d).

307

308 From the previous work by Zhang et al. (2015), the broadly-ovate to pyriform morphotype
309 corresponds to 'morphotype 1' and the sequence is closely related to other sequences from various
310 localities including Reunion Island, Malaysia, and tropical Australia. Unfortunately, despite efforts to
311 obtain sequences for both morphotypes, a single sequence corresponding to morphotype 1 has been
312 acquired in the study while the other morphotype can be compared only by its morphology. The
313 broadly oblong specimens with kidney-shaped pores are morphologically very similar to those
314 identified as *P. lima* 'morphotype 4' by Zhang et al. (2015) or more recently as *P.* cf. *maculosum* by
315 Luo et al. (2017). Nevertheless, we consider that this interpretation was mistaken since *P. maculosum*
316 is a typically foveate species (Faust 1993b) and it is very unlikely that all specimens of a foveate
317 species are completely smooth, although ornamentation is variable with cell age (Hoppenrath et al.
318 2013). Interestingly, the sequences associated with the 'morphotype 4' of Zhang et al. (2015) and
319 those of *P.* cf. *maculosum* in Luo et al. (2017) are now genetically related to *P. caipirignum*, a recently
320 described species from Brazil, morphologically very close to *P. lima* (Nascimento et al. 2017). This

species is completely smooth, like *P. lima* and *P.* cf. *maculosum* in Luo et al. (2017). The presence of *P. caipirignum* in Martinique Island cannot be excluded since some specimens had this morphology, but it should be confirmed with a molecular identification since morphology is not sufficient to distinguish it from other morphotypes of *P. lima*. Owing to the great diversity of morphologies shown in the present study, further research in Martinique Island should clarify the genetic diversity in this group.

– *Prorocentrum hoffmannianum* Faust

Cells were broad oval in shape (Figs 3f-g), 46.8–52.5 µm in length (mean 48.8 µm, s.d. 2.6 µm, n = 5) and 39.5–43.1 µm in width (mean 41.9 µm, s.d. 1.5 µm, n = 5). The length to width ratio varied from 1.12 to 1.22 (n = 5). The periflagellar area was V-shaped (Figs 3 h-i), composed of 8 platelets, but platelet '7' was not visible and hidden by platelet 1 (Fig. 3i). The thecal surface was reticulate-foveate with scattered thecal pores, except in the centre of lateral plates. A ring of marginal depressions containing pores was conspicuously visible. The pores were elongated in shape. The intercalary band was smooth, without striation (Fig. 3h).

Although this species is variable in morphology (Hoppenrath et al. 2013; Herrera-Sepúlveda et al. 2015), the specimens from Martinique Island are in agreement with the characteristic features given in the original description (Faust 1990a; Faust and Gulledge 2002). The molecular sequence obtained in this study also confirmed that specimens from Martinique Island were closely related to the strain CCMP683 which has been collected in the Caribbean Sea, off Knight Key (Florida) by J. Bomber and unambiguously ascribed to *P. hoffmannianum*. The level of genetic variability in this clade has been studied in details by Herrera-Sepúlveda et al. (2015) who also proposed that *P. belizeanum* is a junior synonym of *P. hoffmannianum*, as morphological and molecular features to distinguish these species are not strong enough. This species is known for the production of several toxic compounds (Faust and Gulledge 2002; Lassus et al. 2016).

Clade D – *P.* cf. *norrisianum* Faust et Morton

This clade comprised 7 sequences all acquired in the present study.

Cells were oval with more or less straight sides (Figs 4a-d), 31-37 µm in length (mean 33.9 µm, s.d. 2.2 µm, n = 6) and 20-26 µm in width (mean 23.1 µm, s.d. 1.8 µm, n = 6) . The length to width ratio varied from 1.37 to 1.68. The lateral plates were slightly asymmetric with the ventral side more prominent than the dorsal, the anterior end appearing slightly oblique with respect of the longitudinal axis of the cell (Figs 4c–d). The apical area of the left lateral plate was slightly concave (Fig. 4f). The periflagellar area appeared rather flat area and not deeply excavated in the right plate (Figs 4c-d). It was wide (Fig. 4c) and composed of 9 platelets (1a, 1b, 2, 3, 4, 5, 6, 7, 8) (Fig. 4g). The thecal surface was smooth and perforated with pores excempt in the valve center. The intercalary band was striated transversally (Fig. 4e).

Prorocentrum norrisianum has been described from Twin Cays, Belize (Faust 1997). Since its first description, *P. norrisianum* has been only putatively identified by Mohammad-Noor et al. (2007). However, the specimens from Malaysia were much smaller than in the original description, and the number of periflagellar platelets was apparently different. The presence of two sizes of thecal pores, as reported by Faust (1997) is a questionable feature since no illustration show this feature unambiguously. A careful re-examination of the Figs 1 and 2 in Faust (1997) showed that the 'small pores (< 0.05 µm)' correspond better to small pits (depressions) rather than perforations in the theca. Furthermore, the Figs 3-4 revealed that the cells surface was covered by a granular layer that probably resulted from an artefact of preparation (coating) and it does not allow a careful observation of the surface.

The specimens collected in Martinique Island do not fit completely with the features of *P. norrisianum* and all specimens were larger than in the original description. A reinvestigation of this species from Belizean samples is absolutely necessary to confirm its morphological features and provide a reference sequence for subsequent identifications. The sequences clustered in two subclades (Fig. 1) although we were not able to discriminate the specimens morphologically (Figs 4a–b), which either indicate some genetic variations within the species or the existence of two cryptic species. However, specimens were rare and the low number of isolated cells did not allow further interpretations. It cannot be excluded that the species are pseudo-cryptic and that more detailed

observations can reveal discriminating features. This remains to be checked in the future, using clonal cultures.

Clade E – *Prorocentrum glenanicum / P. panamense*

Three sequences from Martinique island clustered in this clade which encompasses species characterized by a 'linear' periflagellar area and an asymmetry (Hoppenrath et al. 2013). One sequence grouped with sequences of *P. glenanicum* and two sequences with *P. panamense*.

– *Prorocentrum glenanicum* Chomérat et Nézan

Cells were almost circular in shape (Figs 5a–b), 23.4–27.1 µm (mean 25.7 µm, s.d. 0.9 µm, n = 15) in length and 22.0–27.0 µm in width (mean 24.4 µm, s.d. 1.1 µm, n = 15). The length to width ratio varied from 0.99 to 1.12. The periflagellar area was almost flat and comprised 9 platelets (1, 2, 3, 4, 5, 6a, 6b, 7, 8) (Fig. 5c). Platelets 1 and 4 possessed several depressions. The thecal surface was smooth with pores in shallow depressions (foveate) distributed in a very distinctive pattern. A ring of marginal pores was present on both lateral plates (Figs 5a–b, d–e), but on the right plate, two areas of densely arranged pores were present in the subcentral part (Figs 5a, f) and in the posterior dorsal part (Figs 5a, d). The intercalary band was smooth and slightly transversally striated in older specimens (Fig. 5d–e).

Compared with the original description from the temperate area, no morphological difference was revealed in the specimens from Martinique Island (Chomérat et al. 2011). In addition, the LSU rDNA sequence acquired in this study is very similar to that obtained from the type locality (Glénan archipelago, South Brittany), indicating a very low genetic variation in this species. Although it was never abundant in the temperate samples, this small species was found in great abundance in some samples from Martinique Island. To date, it has not been reported in any other area than the Atlantic.

– *Prorocentrum panamense* Grzebyk, Sako et Berland

Cells were large, 39.8–46.1 µm in length (mean 42.4 µm, s.d. 2.6 µm, n = 5) and 38.2–42.0 µm in width (mean 40.1 µm, s.d. 1.7 µm, n = 5). They were asymmetrical, with a typical heart-shape. The

405 periflagellar appeared flat and it was not excavated in the right lateral plate. It consisted in 9 platelets
406 (1, 2, 3, 4, 5, 6a, 6b, 7, 8) (Fig. 5i). The ornamentation was reticulate-foveate, with the presence of a
407 large depression with sieve-plate on the posterior dorsal part (Figs 5g, j).

408 From a morphological point of view, this species is very peculiar and morphologically easy to
409 recognize. The type locality of this species was Contadora Island, on the Pacific side of Panama,
410 which is just a little southern to Martinique Island. It has been found also in Arabian Gulf (Saburova,
411 pers. comm.) and more recently in China (Luo et al. 2017). As shown by the sequences, and although
412 specimens were isolated from distant areas (Martinique Island and China), the intraspecific level of
413 genetic variation appears to be low in the LSU (Fig. 1).

414

415 **Clade F – *Prorocentrum emarginatum/fukuyoi* complex**

416 This clade grouped sequences of species characterized by an asymmetry of the anterior dorsal and
417 ventral parts, and a deep, narrow V-shaped periflagellar with a wing. The sequences clustered in two
418 principal subclades: F1, corresponding to *P. emarginatum* complex, and F2 to *P. fukuyoi* complex
419 (Fig. 1).

420

421 *subclade F1* – Prorocentrum emarginatum/sculptile *complex*

422 The subclade F1 was divided into a F1a branch comprising three sequences from GenBank identified
423 as *Prorocentrum emarginatum* and one ascribed to *P. sculptile* (NMN011) but no sequence from
424 Martinique Island while the clade F1b grouped 8 sequences, all from this study, that we identified
425 morphologically as *P.* cf. *sculptile.*

426 – *P.* cf. *sculptile* Faust (Clade F1b)

427 Cells were oval (Figs 6a–f, 7a–e), 28.5–35.8 µm in length (mean 33.4 µm, s.d. 1.7 µm, n = 19) and
428 27.7–32.8 µm in width (mean 30.4 µm, s.d. 1.5 µm, n =19). The length to width ratio varied from
429 1.01 to 1.16 (mean 1.10). The periflagellar area was deep, narrow V-shaped and with a conspicuous
430 wing formed by platelet 1. It was composed of 10 periflagellar platelets (1, 2, 3, 4, 5, 6a, 6b, 7, 8a, 8b)
431 (Figs 7h–k). Some platelets are difficult to observe such as platelet 7 which was located underneath the
432 wing formed by platelet 1 (Figs 7i–k). The division of platelet 8 was seen only when the cell was in a

peculiar position but this feature has been observed in several specimens (Figs 7j–k). The thecal plates were foveate, with depressions more or less visible depending on the thickness of the thecae (Figs 7a–b, d–e). On young specimens, the foveate ornamentation was visible only in the margin of lateral plates, while the centre was smooth and devoid of depressions (Fig. 7c). Sequencing of single cells possessing this peculiar feature (e.g. IFR13-108) revealed that they are genetically identical to completely foveate specimens (e.g. IFR13-107 and IFR13-111, Figs 6a–f) (Fig. 1). The thecal pores were arranged in radiating lines more conspicuously visible in LM or in specimens with a light foveate ornamentation. The intercalary band was smooth and its size increased with cell age (Figs 7f–g). In LM, some cells possessed a central structure resembling a pyrenoid (Fig. 5a) but other specimens genetically identical lacked this feature (Fig. 5d).

The morphological features observed in specimens in the present study are in agreement with the description of *P. emarginatum* but also with that of *P. sculptile* as some cells possessed a pyrenoid-like structure as described by Faust (1994). Many confusions have occurred for species of the '*P. emarginatum* complex'. Back to the protologue in the original description of *P. emarginatum* from the Ryukyu Island (Fukuyo 1981, p. 968), the author clearly stated that 'valve has spinule depressions scattered all over, and in some specimens the depressions are so numerous that the valve seems to [be] densely punctate'. Although he used only the light microscope, these minute depressions (foveate ornamentation) can be clearly seen on the micrographs (Figs 11–12 in Fukuyo 1981) and the careful reading indicated that he observed some variability in the ornamentation. Later, when she putatively identified *P. emarginatum* from Belize, Faust (1990) described specimens with radiating lines of pores and a completely smooth surface, which is clearly in contradiction with Fukuyo's original description and likely correspond to a species in the '*P. fukuyoi* complex'. This interpretation caused many confusions in subsequent works and some authors (e.g. Mohammad-Noor et al. 2007a; Murray et al. 2007; Laza-Martínez et al. 2011) considered that a smooth ornamentation is a typical feature of *P. emarginatum*, which is based on a mistaken interpretation. When Faust (1994) described *P. sculptile* with a foveate thecal ornamentation in contrast with '*P. emarginatum*', she added some confusion since from a morphological point of view, these specimens fit well with the original description of *P. emarginatum* sensu Fukuyo. She did not mention the radiating pore pattern probably because of the strong foveate ornamentation of the theca, but this character is conspicuous in some original pictures by M.A. Faust (e.g. #281001; 281005) available in the Botany collection 2229-2000 from the Smithsonian Institution (https://collections.nmnh.si.edu/search/botany/). Hence, *P. sculptile* possesses

a similar pore pattern than *P. emarginatum*. As pointed out by Hoppenrath et al. (2013), the criteria on which these species can be reliably distinguished are yet unclear. The size range given for *P. sculptile* (32–37 µm long, 30–32 µm wide) is rather similar with the original description of *P. emarginatum* (35–36 µm long, 32 µm wide) and size can not be used to distinguish these two species. A difference in the 'apical collar' has been reported between the two species, but the argument that the wing is curved in *P. scultpile* and straight in *P. emarginatum* is not convincing as no detail was given by Fukuyo (1981) making no comparison possible. Moreover, the variability of this feature in a population has not been checked and the number of specimens shown in Faust (1994) is insufficient. Another feature which may distinguish *P. emarginatum* from *P. sculptile* is the presence of a conspicuous starch-ring pyrenoid in the latter which has not been reported in the former. However, this statement should be considered with care since Mohammad-Noor et al. (2007a) observed a pyrenoid in a Malaysian population of '*P. emarginatum*' and pyrenoids are not always conspicuous in LM. In the present study, this feature was not clear and the fixed nature of the samples might bias our interpretation. Ultrastructural studies using transmission electron microscopy are necessary to check the presence and the type of pyrenoid in the cells (Mohammad-Noor et al. 2007b).

From a molecular point of view, only very few sequences have been ascribed to *P. emarginatum* in GenBank but none are from the type locality, which is a major problem to assess the identity of this species. Since all the sequences in the clade F1a are from Asia/Indian Ocean, we agree with Luo et al. (2017) in identifying them as *P.* cf. *emarginatum* since this species was described from the Japanese Pacific and because the morphology is compatible with the original description. In particular some cells were slightly foveate (cf. Fig. 3H in Luo et al. 2017), as reported by Fukuyo (1981). Furthermore, a single LSU rDNA sequence (NMN011, EF566749) has yet been ascribed to *P. sculptile* but it is very similar to *P. emarginatum,* as emphasized by Luo et al. (2017). In our opinion this sequence corresponds to the same species and specimens with or without a pyrenoid identified by Mohammad-Noor et al. (2007) were likely conspecific, as shown in our study. In contrast, the new sequences provided herein for specimens from the Eastern Caribbean are well divergent from *P.* cf. *emarginatum* and correspond to a genetically separate taxon. The morphology and geographic origin (Caribbean Sea) of the specimens from Martinique Island suggest that they are likely identical to *P. sculptile* but in absence of genetic reference, we can only provide a provisional identification. Owing to the poor morphological differences to distinguish *P. sculptile* from *P. emarginatum* and a description based on a mistaken interpretation of the latter, the existence of two separate taxa was in question

(Hoppenrath et al. 2013). Our molecular data suggest that *P. sculptile* is a distinct, valid species as all our specimens clustered in well supported clade distinct from *P.* cf. *emarginatum*. Nevertheless, further analyses should assess the level of genetic variations within *P. emarginatum* and *P. sculptile* from their type localities and various places in order to clarify the delimitation of these two problematic species. Such molecular data are absolutely necessary to confirm that *P.* cf. *sculptile* from Martinique Island corresponds to the same taxon than described in Belize by Faust (1994) or to another cryptic, and yet undescribed species.

subclade F2 – P. fukuyoi *complex*

In the phylogenetic analysis, the 26 sequences comprised within the clade F2 clustered in five subclades statistically supported: F2a, F2b, F2c, F2d and F2e (Fig. 1). Among the 13 sequences from Martinique Island, 8 clustered in the subclade F2a and 5 grouped in subclade F2b (Fig. 1). Morphologically, some differences were found among the specimens from these two subclades.

– *P.* cf. *fukuyoi* subclade F2a

Cells were oval-oblong in shape, conspicuously asymmetric with the dorsal side more bulging than the ventral side (Figs 6g–i, 8a–b). Cells were 37.0–39.6 µm in length (mean 37.7 µm, s.d. 1.3 µm, n = 6) and 28.6–33.3 µm in width (mean 31.8 µm, s.d. 1.6 µm, n = 6). The length to width ratio varied from 1.15 to 1.26 (mean 1.20). The periflagellar area was deep and narrow, V-shaped and consisted in 10 platelets (1, 2, 3, 4, 5, 6a, 6b, 7, 8a and 8b) (Figs 8c–f). Platelet 1 beared a prominent rectangular wing on almost all its length. The subdivision of platelet 8 was difficult to see but appeared to be present on the margin of the accessory pore (Figs 8e–f). The thecal surface was not completely smooth but some very shallow depressions are present (faint foveate ornamentation). The depressions were 0.6–0.8 µm in diameter. Thecal pores were arranged in radiating rows, with the centre of the lateral plates devoid of pores. Pores of two sizes were observed: large pores were 0.26 µm in diameter, surrounded by a small rim and located in the faint depressions while the small pores were 0.15 µm and not in depressions (Figs 8d–e). A ring of marginal small pores was present.

Morphologically, these specimens differ from the original description of *P. fukuyoi*, which is smooth (Murray et al., 2007). However, they are very similar to the strain Dn33EHU studied by Laza Martínez (2011). Although it was described as smooth, the illustrations of this strain show that the

surface of thecal plates has some shallow depressions (cf. Fig. 41 in Laza Martínez et al. 2011), as observed in this study.

– *P.* cf. *fukuyoi* subclade F2b

Cells were oval oblong in shape (Figs 6j–l, 8g–h), slightly asymmetric, 29.9–37.5 µm in length (mean 34.7 µm, s.d. 3.4 µm, n = 5) and 23.2–31.2 µm in width (mean 27.8 µm, s.d. 3.5 µm, n = 5). The length to width ratio varied from 1.20 to 1.31 (mean 1.25). The periflagellar area was deep and narrow, V-shaped but due to the low number of specimens studied, the number of platelets has not been assessed. Platelet 1 formed a prominent wing (Fig. 8i). Thecal pores were arranged in radiating rows, with the centre of the lateral plates devoid of pores.

The morphology of these specimens is close to the description of *P. fukuyoi*. Moreover, these specimens appear to be morphologically very similar to those observed by Faust (1990) both in shape and ornamentation, but they differ widely from *P. emarginatum* sensu Fukuyo.

As shown by the phylogenetic analysis, the clade F2 contains a considerable level of genetic variability (Fig. 1, Table 1). Although five subclades could be identified, the distinction of different morphoptypes or cryptic species is not yet clear. Murray et al. (2007) described *P. fukuyoi* using the strain SM19 as the type. In the same study, the authors analyzed morphologically the strains SM39 and SM35 and identified them as '*P. fukuyoi*' and '*P. emarginatum*', respectively. Morphologically, the strain SM35 was smooth (Murray et al. 2007) which did not fit with the original description of *P. emarginatum*, and it was probably misidentified. The strain SM39 (putatively *P. fukuyoi*) was not included in the phylogenetic analysis, and except the slight morphological difference in shape with the strain SM19 (type), they were regarded as conspecific. Murray et al. (2009) later showed that the sequence of strain SM39 was more divergent from SM19 than with SM35, which confused the definitions of *P. fukuyoi* and *P. emarginatum.* Laza-Martínez et al. (2011) found a similar topology in their phylogenetic analysis and were unable to identify the strains. They ascribed them to an unidentified complex '*Prorocentrum* sp. *emarginatum/fukuyoi* group'. The sequences from Martinique Island clustered in the subclade F2b which encompasses other sequences from the Atlantic and Mediterranean, including the strains Dn33EHU and Dn34EHU studied by Laza-Martínez et al. (2011), and in the subclade F2a which corresponds to a new ribotype from Martinique not previously

553 sequenced. Interestingly, specimens from the clade F2a were slightly foveate and this morphological
554 feature could appear as a potentially useful character to distinguish among these ribotypes in
555 Martinique samples. Nevertheless, the strains observed by Laza-Martínez et al. (2011) had a smooth
556 (Dn34EHU) and lightly foveate ornamentation (Dn33EHU) and both clustered in the clade F2b,
557 proving that this feature is not reliable. Moreover, the genetic distance between the subclades F2a and
558 F2b is around 0.030, which is lower than between the other subclades (0.041-0.072, Table 1). This
559 value is in the same order of magnitude than the within-group variability for the subclades F2c, F2d
560 and F2e for which the divergence reaches 0.060. (Table 1). Therefore, this variability is yet difficult to
561 interpret and no relationship can be found with the geographical origin of the specimens. The
562 considerable genetic divergence existing among the different strains and field populations may be
563 related to the existence of cryptic species which are not yet understood. With the current knowledge,
564 it is not yet possible to clarify the situation within the clade F2. As emphasized by Hoppenrath et al.
565 (2013), an intensive re-investigation of strains of *P.* cf. *fukuyoi,* from different areas in the world,
566 including the type locality, with morphological and molecular methods is required to redefine the
567 species identity and to understand intra-specific variability of the different features.

568

569 **Clade G – *Prorocentrum rhathymum* and planktonic species**

570 Only one benthic species from Martinique Island was found in this clade comprising mostly
571 planktonic *Prorocentrum* species (Fig. 1).

572 – *Prorocentrum rhathymum* Loeblich, Sherley et Schmidt

573 Cells were oval to oblong, asymmetric, 28.0–35.0 µm in length (mean 31.9 µm, s.d. 2.8 µm, n = 5)
574 and 18.1–24.5 µm in width (mean 21.0 µm, s.d. 2.5 µm, n = 5). The length to width ratio ranged from
575 1.43 to 1.56 (mean 1.52). The periflagellar area was wide V-shaped and comprised 9 platelets (1, 2, 3,
576 4, 5, 6a, 6b,7, 8). Platelet 1 had a conspicuous wing, visible also in the left lateral view and a smaller
577 wing was present on platelet 4 (Fig. 9b). The thecal surface was smooth with a characteristic pore
578 pattern. Large pores were arranged in one apical row of ca. 5–6 large pores and several posterior
579 radial rows in shallow thecal furrows (Figs 9a, b, d). They were located in round depressions. Smaller
580 pores were scattered on the surface of the lateral plates, but some groups of 4–5 small pores were
581 found on the posterior margin of the cell (Fig. 9c). The plate centre was devoid of pores.

582

583 Morphologically, the specimens from Martinique Island were smaller than described by
584 Loeblich et al. (1979) but in agreement with other descriptions of this common species (Hoppenrath
585 et al. 2013). In contrast with the specimens observed by Faust (1990a) in the western Caribbean, the
586 cells found in Martinique Island were completely smooth and without ornamentation. Interestingly, the
587 specimens observed in this study possessed clumps of small pores in the posterior margin of the theca
588 which have not been described by Loeblich et al. (1979) but shown by Laza-Martínez et al. (2011).
589 Such groups of small pores might be related to production of mucous threads for the fixation of cells,
590 as observed in other dinoflagellates (e.g. Hoppenrath et al. 2004; Saburova and Chomérat 2014). The
591 possible synonymy with *P. mexicanum* has been discussed several times (see Hoppenrath et al. 2013)
592 and is out of focus of the present paper. For this reason we follow the taxonomic treatment by
593 Hoppenrath et al. (2013). From a molecular point of view, all sequences ascribed either to *P.*
594 *rhathymum* or *P. mexicanum* are very similar and the partial LSU rDNA cannot help in distinguishing
595 these two species.

596

597 **Unsequenced morphospecies**

598 During the study, we identified a few specimens for which we could not obtain a sequence due to the
599 rarity of cells and/or unsuccessful DNA amplifications. Based on morphological features only, they
600 were ascribed to two different morphospecies: *P.* cf. *maculosum* and *P.* cf. *ruetzlerianum.*

601 **–** *Prorocentrum* cf. *maculosum* Faust

602 Cells were broadly ovate, 50.5–51.5 µm in length and 48.2–48.4 µm in width (n = 2). The length to
603 width ratio was 1.04–1.07. The periflagellar area was located in a wide V-shaped area. All platelets
604 could not be observed and only 7 were seen (1, 2, 3, 4, 5, 6, 7?, 8) (Figs 10c–d). Platelet 1, 3 and 4
605 had depressions while platelet 2 was completely smooth, as for platelet 5, 6 and 8. The platelets 1, 2,
606 3, 5 and 6 developed small lists forming a continuous list around the accessory and flagellar pores
607 (Fig. 10d). The thecal surface was foveate, with small shallow round depressions. Thecal pores were
608 round to kidney-shaped (Figs 10a–b), scattered on the theca, with a conspicuous marginal ring of
609 evenly spaced pores (Fig. 10c) but the centre of lateral plates was devoid of pores.

610

611 The delimitation of this species is problematic as it differs from *P. lima* only by its 'rugose'
612 (i.e. foveate) ornamentation, kidney-shaped pores, and the equal size of the flagellar and accessory
613 pores (Faust 1993b). Morphologically, it appears as intermediate between *P. lima* that is completely
614 smooth and *P. hoffmannianum* that is reticulate-foveate. Specimens from Martinique Island were
615 larger than the original description of *P. maculosum* (Faust 1993b) but closer in size to *P.*
616 *hoffmannianum* (Hoppenrath et al. 2013). The few specimens observed in the study possessed a
617 foveate ornamentation and had kidney-shaped thecal pores, which is in agreement with the original
618 description of *P. maculosum.* During our observations, the accessory pore was not entirely visible
619 because of the overlap by platelet 2 and it could not be measured. Hence this feature could not be
620 evaluated and used as a taxonomic feature. As noticed by Faust (1993b), the periflagellar of *P.* cf.
621 *maculosum* was remarkably similar with that of *P. hoffmannianum*, suggesting that this feature is not
622 taxonomically reliable to distinguish between these taxa. Without rDNA sequence, we cannot
623 ascertain that this morphospecies is genetically distinct from *P. lima* or *P. hoffmannianum* and further
624 studies should resolve this taxonomic issue. In Martinique Island, we cannot conclude whether the
625 specimens studied correspond to immature cells of *P. hoffmannianum* with an incomplete
626 ornamentation, or to a separate species. As discussed above, Luo et al. (2017) identified smooth
627 specimens as *P.* cf. *maculosum* in China but their morphology differs from the description, and there
628 are genetically closely related to *P. caipirignum*.

629

630 – *Prorocentrum* cf. *ruetzlerianum* Faust

631 During the course of our study, only five specimens of this morphospecies were found in the material.
632 For this reason, we observed only two specimens by SEM and 3 were used for unsuccessful DNA
633 amplifications. Cells were broadly oval, 29.0–30.3 µm in length and 24.9–25.8 in width (n = 2). The
634 length to width ratio was ca. 1.17. The periflagellar area was wide V-shaped (Fig. 10e), composed of
635 7 platelets visible (1, 2, 3, 4, 5, 6, 8). The thecal surface was reticulate-foveate (Figs 10e–g), except on
636 the margin of lateral plates and on the intercalary band where elongated depressions formed a
637 conspicuous striated pattern (Figs 10f–g). Thecal pores were scattered on the surface but the centre of
638 lateral plates was devoid of pores (Fig. 10e).

639 Morphologically, *P. ruetzlerianum* is a peculiar species characterized by a strong reticulate-
640 foveate ornamentation formed by deep, pentagonal areolae and a typical striated pattern on the margin

641 of lateral plates and intercalary band, which is unique in the genus (Faust 1990a; Faust et al. 1999).
642 The specimens observed in this study possessed these features, but they were probably younger than
643 those observed by Faust (1990a) because the plates had shallower depressions, and the striated
644 intercalary band was not as wide (cf. Figs 21–24 in Faust 1990a). We did not observe a thecal pore in
645 all the areolae, which contrasts with the description (Faust 1990a), but this fact has already been
646 pointed out by Hoppenrath et al. (2013). Interestingly, the periflagellar area of the specimens studied
647 herein was very similar to that illustrated in the original description of *P. ruetzlerianum* (cf. Fig. 24 in
648 Faust 1990a). In particular, the small and almost square platelet 2, the wide rectangular platelet 3 and
649 the small elongated platelet 6 appear nearly identical. Only the shape of platelet 4 was somewhat
650 different and it appeared to be smaller in the original description (Faust 1990a). The apparent absence
651 of platelet 7 has already been mentioned by Hoppenrath et al. (2013), but unfortunately, the limited
652 number of specimens available did not allow further detailed observations of this area. The rarity of
653 this species has also been mentioned by Faust and Gulledge (2002). The morphological features argue
654 in favour of the identification of *P. ruetzlerianum* in Martinique Island, but further molecular studies
655 are necessary to confirm this in the future. This would be important since Faust and Gulledge (2002)
656 reported that this species produce toxins, but without giving the exact toxin profile. An extended study
657 of this poorly known species is therefore necessary.

658

659 Conclusions

660 The present study revealed a high diversity of benthic species of *Prorocentrum* at Anse Dufour,
661 Martinique Island. Thanks to the sequencing of the LSU rDNA, 11 species were recognized, while 2
662 other morphospecies were identified on a morphological basis only. Hence putatively 13 species are
663 present in the same site, including 5 potentially toxic species: *P. concavum*, *P. lima*, *P.*
664 *hoffmannianum, P.* cf. *maculosum* and *P.* cf. *ruetzlerianum* . Some of the species identified in the
665 study were not previously known from the Caribbean such as *P.* cf. *glenanicum* described from
666 temperate Atlantic. Interestingly, many species were also observed by Faust (e.g. Faust 1996, 2000) in
667 Belizean samples from the western Caribbean, and although the substrates sampled were different,
668 very similar assemblages were found. For instance, among the species found in Martinique Island, we
669 putatively identified the poorly known *P.* cf. *tropicale* and *P.* cf. *ruetzlerianum* which have not been

reported in any study since their discovery. Some morphological features such as cell sizes were not identical with the original descriptions, so further investigations are necessary to confirm the identify of these taxa. The absence of reference sequences from Belizean coral reefs and mangrove habitats that constitute the type locality of several species, prevents genetic comparisons and their unambiguous identification. This issue occurs for several other species described by Faust in Twin Cays, like *P.* cf. *foraminosum*, *P.* cf. *norrisianum* or *P.* cf. *sculptile* for which the identifications only rely on morphological features and hence remain uncertain.

The molecular phylogenetic analysis realized in the study revealed that the topology of major clades is in agreement with previous published phylogenies of the genus (e.g. Hoppenrath et al. 2013). The acquisition of new sequences revealed one new clade, the clade D that encompasses *P.* cf. *norrissianum,* and three subclades that were previously unknown, such as the subclade A3 corresponding to *P.* cf. *foraminosum* and the subclades F1b and F2a corresponding, respectively, to *P.* cf. *sculptile* and a new ribotype of *P.* cf. *fukuyoi*. Interestingly, our phylogentic analysis placed *P.* cf. *tropicale* in the clade A of species related with *P. concavum* although we previously considered it as a putative member of the *P. hoffmannianum* complex (Hoppenrath et al. 2013). The major clades seem to be well supported by morphological differences of the periflagellar area of the taxa. All the species in the clade A appear to possess an excavated periflagellar area comprising 9 platelets with a similar pattern (split 1a/1b), although this is not very clear in *P. leve* (Mertens et al. 2017). This contrasts with the species in clade B related to '*P. lima*' that mostly possess 8 platelets, or 6 as shown in *P. caipirignum* (Nascimento et al. 2017). The molecular phylogeny suggests that the number of periflagellar platelets might reflect a character evolution between the clades A and B. Comparatively, we found also 9 platelets in the clades D (*P.* cf. *norrissianum*) and E (*P. glenanicum*/*P. panamense*) but their periflagellar area is only slightly excavated in the right lateral plate or almost 'flat', respectively. We found 10 platelets arranged in a typical pattern for the specimens from the clade F (*P. emarginatum*/*P. fukuyoi* complex) from Martinique Island. Based on the observations by Luo et al. (2017), 9 platelets have been found in the strain X2P3 of *P.* cf. *emarginatum*, but it cannot be excluded that the subdivision of platelet 8 was obscured by the prominent wings. Finally, *P. rhathymum* (clade G) possesses 9 platelets but with a very conspicuous and typical wing on platelet 1 and a short wing or list on the platelet 4, which is also typical of many planktonic species.

Our study also revealed a higher diversity than previously known in the clade F of *P. emarginatum*/*P. fukuyoi* complex. Thanks to the technique used specifically for taxa in this group, we were able to study the thecal plates of the single cells sequenced using SEM and associate the phenotypes with the genotypes, even in absence of cultures. For instance, the morphological features of specimens from the new clade F1b lead us to identify *P.* cf. *sculptile* since the characters and the geographical origin in the Caribbean were in agreement with the description of this species. As previously shown, the delimitation of *P.* cf. *fukuyoi* is unclear and the existence of two ribotypes in the samples studied confuses even more the taxonomy of this species. As shown by the diverse origins of the sequences ascribed to *P. fukuyoi* complex, this taxon seriously needs a reinvestigation to clarify the species boundaries and eventually identify new, cryptic species.

Although the morphology of the genus *Prorocentrum* appears to be simple because of its peculiar morphology among dinoflagellates, our study confirmed that species identification is complex for some taxa, even with addition of molecular data. As shown by our study, a combination of morphological and genetic features is absolutely necessary to better characterize the taxa. For some species, acquiring sequences from type localities appears as an utmost priority since the lack of molecular data prevents unambiguous identifications. In case of morphologically closely related taxa, genetic information is critical in order to permit descriptions of new species with no risk of redescriptions of already existing taxa. Hence, as recently emphasized by Hoppenrath (2017), there is a serious need of further works on this genus in order to clarify the species delimitations which are still not clear for several taxa.

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872 **Table 1** Mean genetic distances (Kimura 2-parameter) within and net-between subclades of the *P.* cf.
873 *fukuyoi* complex (clade F2), based on the alignment of 26 sequences and 514 characters used
874 in the phylogenetic analysis

Subclades (groups)	F2a (8 sequences)	F2b (9 sequences)	F2c (3 sequences)	F2d (4 sequences)	F2e (2 sequences)
Within groups	0.000	0.007	0.024	0.026	0.060
Net between-groups					
F2a	–	–	–	–	–
F2b	0.030	–	–	–	–
F2c	0.072	0.045	–	–	–
F2d	0.062	0.041	0.051	–	–
F2e	0.070	0.047	0.057	0.044	–

875

Figure legends:

Fig. 1 Phylogenetic tree of *Prorocentrum* sequences rooted on *Peridiniella* spp., inferred from a LSU rDNA matrix (135 sequences, 643 sites). The tree topology (-lnL=8260.79059) was obtained from ML analysis. Symbols indicate branch support (bootstraps in ML/posterior probabilities in BI): thick branch lines indicate a full support (100/1.00), filled triangles a strong support 90–99/0.95–0.99 and filled circles a moderate support 80–89/0.80-0.94). Lower support values are not indicated. Sequences obtained in the present study are highlighted in bold face and with a grey background

Fig. 2 Scanning electron micrographs of species in the clade A. **a–c** *P.* cf. *tropicale*: **a** right lateral view, **b** left lateral view, **c** detail of the periflagellar area; **d–f** *P. concavum*: **d** right lateral view, **e** left lateral view, **f** detail of the periflagellar area; **g–m** *P.* cf. *foraminosum*: **g** right lateral view, **h** left lateral view, **i** apical view, **j** antapical view, **k** oblique view of the dorsal side, **l** detail of the foveate surface and thecal pores, **m** detail of the periflagellar area. Scale bars 10 µm in a–b, d–e, g–h; 5 µm in i–k; 2 µm in c, f, m and 1 µm in l

Fig. 3 Scanning electron micrographs of species in the clade B. **a–e** *P. lima*: **a–b** broadly ovate-piriform morphotype, **b–c** oblong-oval morphotype, **e** detail of the periflagellar area; **f–i** *P. hoffmannianum*: **f** right lateral view, **g** left lateral view, **h** apical view showing intercalary band, **i** detail of the periflagellar area. Scale bars 10 µm in a–d, f–h; 2µm in e, i

Fig. 4 Light and scanning electron micrographs of *P.* cf. *norrisianum* (clade D). **a–b** LM micrographs of two sequenced specimens: **a** IFR15-204, **b** IFR15-205; **c–g** SEM micrographs: **c** right lateral view, **d** left lateral view, **e** apical view showing intercalary band, **f** detail of the apical area of the left lateral plate, **g** detail of the periflagellar area. Scale bars 10 µm in a–e and 2 µm in f–g

Fig. 5 Scanning electron micrographs of species in the clade E. **a–f** *P. glenanicum*: **a** right lateral view, **b** left lateral view, **c** detail of the periflagellar area, **d** dorsal view of a specimen with a thin intercalary band, **e** antapical view of a specimen with a large intercalary band, **f** detail of the central area with pores; **g–j** *P. panamense*: **g** right lateral view, **h** left lateral view, **i** detail of the periflagellar area, **j** detail of the depression with a sieve-like bottom. Scale bars 5 µm in a–b, d–e, g–h; 2 µm in c, f, i and 500 nm in j

Fig. 6 Light (phase contrast) and scanning electron micrographs of single-cells sequenced in the clade F. **a–c** LM micrographs of the specimen IFR13-107, **d–e** LM micrographs of the specimen IFR13-

111, **f** SEM micrograph of the left lateral plate of specimen IFR13-111, **g–h** LM micrographs of the specimen IFR13-122, **i** SEM micrograph of the left lateral plate of specimen IFR13-122, **j–k** LM micrographs of the specimen IFR13-113, **l** SEM micrograph of the left lateral plate of specimen IFR13-113. All scale bars 10 µm

Fig. 7 Scanning electron micrographs of *P.* cf. *sculptile* (clade F1b). **a** right lateral view of cell with strong foveate ornamentation, **b** right lateral view of cell with light foveate ornamentation, **c** right lateral view of cell with a marginal foveate ornamentation and smooth centre, **d** left lateral view of cell with strong foveate ornamentation, **e** left lateral view of cell with light foveate ornamentation, **f** apical view, **g** antapical view, **h–k** different views of the periflagellar area. The asterisk indicates the platelet 8a. Scale bars 10 µm in a–g; 2 µm in h and 1 µm in i–k

Fig. 8 Scanning electron micrographs of *P.* cf. *fukuyoi* (clade F2). **a–f** foveate specimens of the subclade F2a: **a** right lateral view, **b** left lateral view, **c–f** different views of the periflagellar area; **g–i** smooth specimens of the subclade F2b: **g** right lateral view, **h** left lateral view, **i** detail of the periflagellar area. The asterisk indicates the platelet 8a. Scale bars 10 µm in a–b, g–h and 1 µm in c–f, i

Fig. 9 Scanning electron micrographs of *P. rhathymum* (clade G). **a** right lateral view, **b** left lateral view, **c** detail of antapical area showing the groups of small pores and large pores, **d** detail of the periflagellar area. Scale bars 5 µm in a–b and 2 µm in c and d

Fig. 10 Scanning electron micrographs of unsequenced morphospecies. **a–d** *P.* cf. *maculosum*: **a** right lateral view, **b** left lateral view, **c** apical view, **d** detail of the periflagellar area; **e–g** *P.* cf. *ruetzlerianum*: **e** right lateral view, **f** apical view, **g** detail of the periflagellar area. Scale bars 10 µm in a–c, e; 5 µm in f and 2 µm in g