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A brief taxonomy of the genus *Prorocentrum* in the coastal areas along Sanya Bay, Hainan Island

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ABSTRACT: This is the first report of the genus *Prorocentrum* Ehrenberg occurring in the coastal areas along Sanya Bay, Hainan Island. Samples were collected from five coastal stations in 2017, and a brief taxonomy of *Prorocentrum* species was carried out using an inverted microscope. Five species (*P. hoffmannianum*, *P. lima*, *P. micans*, *P. rhathymum*, *P. sigmoides*) were identified, including three toxic species and two red tide-forming species. In particular, *P. hoffmannianum* and *P. lima* were both confirmed as the producers of okadaic acid and responsible for diarrhoetic shellfish poisoning events. This may suggest the potential threats of harmful algal blooms in the coastal areas along Sanya Bay that could cause diarrhoetic shellfish poisoning events.

KEYWORDS: dinoflagellates, planktonic, benthic, toxic, red tide-forming

INTRODUCTION

Dinoflagellates are an important part of the pelagic ecosystem[1], make a large component of primary producers, and are estimated to make up about 40% of the total species of marine phytoplankton[2]. Over 2500 dinoflagellate species have been recognized worldwide[3], and about 76 of these species have been classified as harmful. The harmful species cause water discolorations known as red tides, and sometimes produce toxins that directly kill fish and shellfish or accumulate to lethal levels in higher-trophic level consumers in the food chain, including humans[4]. In any aquatic ecosystem, there hardly exist an environmental condition not exploited by dinoflagellates[5].

High temperature and low salinity are particularly appropriate for the growth of dinoflagellates[6], and the genus *Prorocentrum* Ehrenberg, which includes 56 marine species, belongs to the family Prorocentraceae Stein[7], is one of the most diverse and wide spread genus in the tropical ocean[8,9]. Most of the *Prorocentrum* species are planktonic and benthic[10], at least 9 species have been shown to produce toxins[11], and cause toxic blooms that represent physical danger, such as shellfish poisoning[12,13].

Sanya, the southernmost part of Hainan Island, is located in a tropical climate region, where shellfish industries, fish farming, and tourism have rapidly developed in the coastal areas during the last decade. The development of these activities on land in the coastal areas has resulted in pollutant and nutrient runoff from around those areas and led to serious eutrophication of coastal waters, which is closely related to the frequency of harmful algal blooms (HABs).

In recent years, shellfish poisoning events occurred frequently in Sanya. In this report, we investigated the species of *Prorocentrum* in the coastal areas along Sanya Bay, and a brief taxonomy was carried out.

MATERIALS AND METHODS

Five sampling sites were selected along the coastal areas along Sanya Bay (18°12′39″–18°17’24″N latitude, 109°21’56″–109°28’19″E longitude); all were chosen as shallow, about < 5 m deep areas facing the South China Sea (Fig. 1). Phytoplankton samples were qualitatively collected in January and August 2017, with a 5-μm-mesh plankton net after prescreening with a 200-μm-mesh net. These samples were immediately transferred into 100-ml plastic bottles and preserved with glutaraldehyde at a final

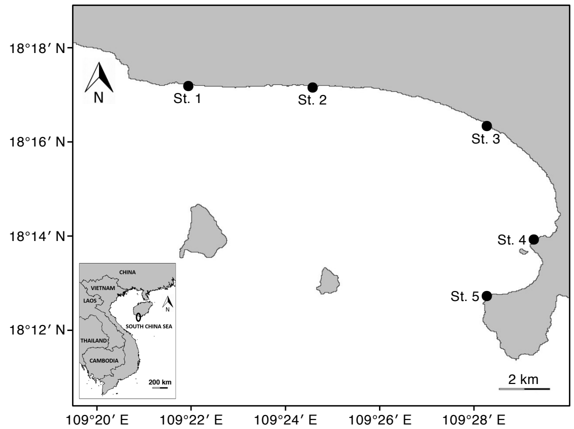

Fig. 1 Map of the Sanya Bay coast showing the sampling locations (•).

concentration of 1%.

Species were observed under an inverted microscope (OLYMPUS, IX71), and if necessary, Calcofluor White M2R at a final concentration of 10 μg/ml was used to stain the outer shells of the dinoflagellates[14], using an inverted differential interference contrast epifluorescence microscope (OLYMPUS, BX60F) equipped with a UV excitation unit. Photographs of these algae were captured with a charge-coupled device camera (OLYMPUS, DP70). *Prorocentrum* spp. were identified according to previous literatures[3,12,15,16].

RESULTS

The genus *Prorocentrum* Ehrenberg, which is a desmokont dinoflagellate, characterized by apical insertion of the flagella, absence of the cingulum and the sulcus[9], and the cell consists of the two lateral thecae, which are joined by the marginal edges[12]. In the present study, five species were from the coastal areas along Sanya Bay as described below.

P. hoffmannianum Faust 1990

Synonym: *Exuviaella hoffmannianum* (Faust) McLachlan et Boalch 1997

Cell is ovoid, broadest in the middle region, tapering slightly apically, 45–55 μm in length and 40–45 μm in width, containing golden-brown chloroplasts, a centrally located pyrenoid, and a posterior nucleus. The valves are apically excavated. The periflagellar area is a wide triangle situated apically on the right valve, and it lacks both valve spines and anterior spines (Fig. 2a).

P. hoffmannianum is a benthic species, can be tychoplanktonic, with warm temperate to tropical coastal waters distribution, reported from Belize[17], and this species was reported to be associated with floating detritus in tropical coastal regions of the Caribbean Sea[17], attached to macroalgae in the Belizean barrier reef ecosystem[18].

P. hoffmannianum is usually considered toxic, producing fast-acting toxin and okadaic acid[19].

P. lima (Ehrenberg) Stein 1878

Basionym: *Cryptomonas lima* Ehrenberg 1860

Synonyms: *E. marina* Cienkowski 1881
Dinopyxis laevis Stein 1883
E. lima (Ehrenberg) Bütschli 1885
E. lima (Ehrenberg) Schütt 1896
E. laevis (Stein) Schroder 1900
E. cincta Schiller 1918
E. marina var. *lima* (Ehrenberg) Schiller 1931
E. ostenfeldi Schiller 1933
E. caspica Kiselev 1940
P. marinum Dodge et Bibby 1973
P. arenarium Faust 1994

Cells are oblong to ovate, broad in the middle region, narrow at the anterior end, 31–47 μm in length and 22–40 μm in width (Fig. 2b, 2c), containing a central pyrenoid and a large posterior nucleus (Fig. 2b). The valve surface is covered with scattered pores, but the centre is devoid of the pores, the right valve having valve pores of two different sizes, and the marginal pores are presented (Fig. 2c). The periflagellar area is a wide triangle containing a curved apical collar, void of valve spines or anterior spines (Fig. 2b).

P. lima is a neritic and estuarine, benthic/epiphytic species (can be tychoplanktonic), worldwide distribution, typically in temperate and tropical waters[8,20], reported from East China Sea and South China Sea[21]; Japan, Indonesia and Philippines[22], and this species was reported to be attached to macroalgae, or was observed swimming close to the bottom substrate, and was associated with coral reefs[17], or can be found attached to floating detritus in mangrove habitats[23].

P. lima is a toxic species known to produce a number of toxic substances, such as fast-acting toxin[24], and diarrhoetic shellfish poisoning (DSP) toxins including okadaic acid[25].

P. micans Ehrenberg 1834

Synonyms: *P. schilleri* Böhrn 1933
P. levantinoides Bursa 1959

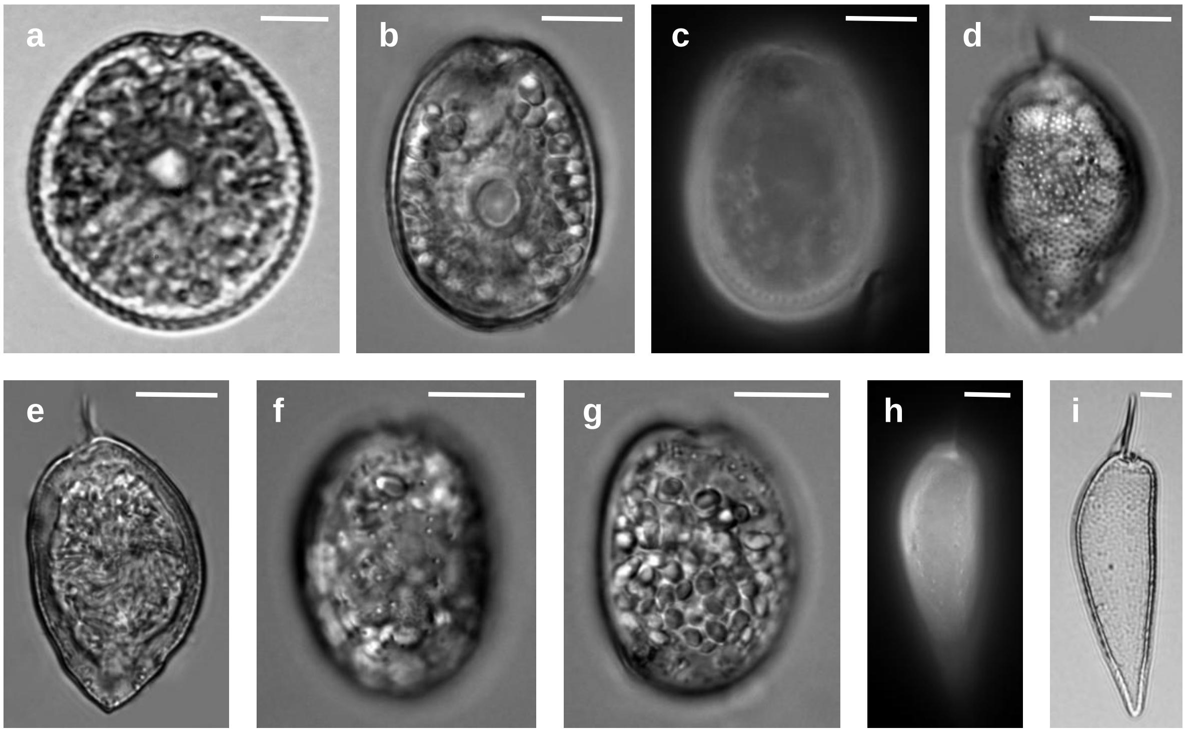

Fig. 2 Light and fluorescence microphotographs of *Prorocentrum* spp.; (a) *P. hoffmannianum*, (b, c) *P. lima*, (d, e) *P. micans*, (f, g) *P. rhathymum*, (h, i) *P. sigmoides*. Scale bars 10 µm.

P. pacificum Wood 1963

Cells are tear-drop to heart shaped, generally rounded anteriorly, tapering posteriorly, broadest around the middle, 35–70 µm in length and 20–50 µm in width (Fig. 2d, 2e). One convex side and one arched side, the convex arch profile is typically in the middle of the cell where is the broadest, and a well-developed winged apical spine is presented (Fig. 2d, 2e). Numerous tubular trichocyst pores are presented in short rows arranged radially (Fig. 2d). The periflagellar area is a relatively small, shallow, broad triangular depression situated apically on the right valve off-centre (Fig. 2e).

P. micans is a neritic and estuarine, planktonic species, but also found in oceanic environments, with cold temperate to tropical waters distribution[20], reported from Japan[16]; Mexican Pacific Coast[13]; East China Sea and South China Sea[21].

Although *P. micans* is capable of forming extensive blooms, it is usually considered harmless[26]. It may excrete substances that inhibit diatom growth, but apparently these substances do not enter the food chain or affect organisms at higher trophic levels[27]. Early reports on *P. micans* being a paralytic shellfish poison producer[28] are unconfirmed, and recent incidents involving shellfish mortality have been attributed to oxygen depletion[29].

P. rhathymum Loeblich III, Sherley et Schmidt 1979

Cells are asymmetric, ovate to oblong with straight sides, 30–38 µm in length and 20–25 µm in width (Fig. 2f, 2g). The valve pores form the line patterns in the posterior part of the valve (Fig. 2f), the marginal pores are presented (Fig. 2g), and the centres of the valves are devoid of pores (Fig. 2f, 2g). The periflagellar area, located apically and off-centre on the right valve, is a relatively small, V-shaped, shallow depression, and the curved periflagellar collar may appear as an apical spine (Fig. 2f, 2g).

P. rhathymum is a neritic and estuarine, benthic species, can be tychoplanktonic, with tropical and subtropical waters distribution[20], reported from New Caledonia and Ryukyu Islands (Japan)[30], and this species was reported to be associated with microalgae, dead coral and seagrasses in Malaysia[31].

P. rhathymum may produce toxins with haemolytic activity[32], and water-soluble fast-acting toxin[24].

***P. sigmoides* Böhm 1933**

Cells are elongated, in 'S' shape, one convex side and one generally straight side, and tapering posteriorly, 60–85 µm in length and 20–30 µm in width (Fig. 2h, 2i). Numerous trichocyst pores are presented (Fig. 2h, 2i). A sharp and tiny apical spine protruded from the anterior side, adjacent to the periflagellar area, sometimes slightly sigmoid, with the presence of an antapical mucron (Fig. 2i).

P. sigmoides is a planktonic species, with temperate to tropical waters distribution[16], reported from South China Sea and Japan[21].

P. sigmoides has never been reported to be a toxin producer, but it is a fish killer[33], capable of forming extensive blooms, which can consume dissolved oxygen and cause biota kills[34].

DISCUSSION

P. hoffmannianum is similar in shape to *P. lima*, but larger and broader with dense areolae, and misidentified as *P. concavum* frequently, but can be distinguished by its ovoid shape and presence of areolae in the centre of the valve[30]. Both *P. hoffmannianum* and *P. belizeanum* have a prominent flared curved apical collar on the left valve bordering the periflagellar area[35], and lack an apical spine of the periflagellar area[17,35], although the latter species has a rounder and more prominent collar than the former one, and *P. hoffmannianum* has a more complex platelet configuration[17,20,35], these two species were still considered as conspecific based on the subtle morphological overlaps found in cell shape, size, and ornamentation[36]. The areolae of *P. hoffmannianum* are distinct from the similar known species *P. ruetzlerianum*, *P. ruetzlerianum* has about 550 pentagonal-shaped areolae per valve while *P. hoffmannianum* has approximately 670 round to oval areolae[17], although electron microscope was not performed in the present study, we still consider the current species (Fig. 2a) as *P. hoffmannianum*, for the cells of *P. ruetzlerianum* are round to ovoid with an average diameter of 28–35 µm[17].

P. lima can be distinguished by its size, shape, narrow periflagellar area and the presence of the valve and the marginal pores. The marginal pores can be used to differentiate *P. lima* at the light microscope level from the other species which are similar in shape, such as *P. concavum* or *P. compressum*[37].

P. micans varies considerably in shape and size, can be confused with closely related species, such as *P. gracile*, *P. scutellum*, and *P. caribbaeum*. *P. gracile* has a strong winged apical spine, and the length-width ratio usually larger than two, while *P. micans* usually smaller than two[8]; *P. scutellum* and *P. caribbaeum* are in the same size range as *P. micans*, but *P. scutellum* bears a shorter and broader apical spine, *P. caribbaeum* is heart-shaped and broadest around the anterior end, and *P. micans* is more tear-drop shaped and broadest around the middle[15,35].

P. rhathymum was always considered as a synonym of *P. mexicanum*[8], but some studies pointed out, the pores are arranged in organized pattern in *P. mexicanum* while disorganized in *P. rhathymum*[38], and the former one has an apical wing-shaped spine terminating with two or three tips whereas the latter one only one simple spine[10,11].

P. sigmoides was proposed as a synonym of *P. gracile*[15], then treated as a distinct species for it has been consistently reported in Mexico[9,13]. *P. sigmoides* and *P. gracile* can be distinguished by the length-width ratio and the apical spine, for the former one, the length-width ratio usually larger than three, and the apical spine is on the right valve, sometimes sigmoid, while the latter one has a winged apical spine, sometimes like an arrow-head[8,9], but it is still difficult to distinguish as either *P. sigmoides* or *P. gracile* for some cells, because they showed a sigmoid valve margin and a straight and long spine, or a slightly sigmoid spine with a straight valve margin, and mainly because there were no morphometric characters exclusive to *P. sigmoides*, it was suggested as a junior synonym of *P. gracile*[13].

P. hoffmannianum, *P. lima*, and *P. rhathymum*, these three species occurred worldwide in the coastal areas, mostly in benthic and epiphytic habitats[8], however, in this study, they were found in the water column, and although quantitative analysis of species populations was not performed, *P. rhathymum* showed a high concentration clearly. Actually, some literature indicate the benthic species can be pelagic, such as *P. lima*, this species has been found in substantial concentrations in the water column in Eastern Canada[39], and high concentrations of this species were also found free-living in the subsurface waters of Northern Tunisia[40], and on the other hand, we should note that, in the present study, all of the sites were chosen as shallow, about < 5 m deep areas, and these areas always suffering strong waves, and this may also explain why the benthic species appeared.

In this short report, five species of *Prorocentrum* were described from Sanya Bay, including three toxic species, *P. hoffmannianum*, *P. lima*, and *P. rhathymum*, and two red tide-forming species, *P. micans* and *P. sigmoides*. In particular, both

P. hoffmannianum and *P. lima* confirmed a s the producers of okadaic acid and responsible for the DSP events[19,25,40], while DSP is the most recurrent threat for the shellfish industries[40], this information may indicate that, the coastal areas along Sanya Bay potentially face the danger of HABs, and close attention must be paid to the DSP events.

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