

## Morphotype diversity of *Prorocentrum lima* in the western part of Indonesian waters

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**Abstract.** Widiarti R, Zamani NP, Bengen DG, Madduppa H. 2021. Morphotype diversity of *Prorocentrum lima* in the western part of Indonesian waters. *Biodiversitas* 22: 607-614. *Prorocentrum lima* is one of the toxic benthic dinoflagellates, known to produce various toxins, including okadaic acid and dinophysins toxins. The species have a wide range of morphological variability, and possess morphotype diversity, which makes it essential to have detailed morphology observation for identification and other purposes. However, such comprehensive observation has never been reported from Indonesian waters. This study aims to determine the morphological characteristics (including pore size and number of pores) of *P. lima* morphotypes, in the western part of Indonesian waters (Bintan Island, Belitung Island, Seribu Islands, and Karimunjawa Islands). The results showed three different and unique morphotypes of *P. lima*, namely morphotype 1, morphotype 2, and morphotype 3. Three clusters were presented by Cluster Analysis, corresponded to the three morphotypes, which were Belitung Island clusters, Seribu Islands and Karimunjawa Islands cluster, and Bintan Island cluster, respectively. These findings support distribution of *P. lima* and its potential risk of toxicity in Indonesian waters, which prompts the necessity of conducting future research, to avoid the negative impact.

**Keywords:** Cluster Analysis, morphological variability, morphology characters, reef islands, toxic dinoflagellates

### INTRODUCTION

Some benthic dinoflagellates produce toxic substances, which causes several poisonous syndromes experienced by humans or other mammals, which commonly occur after the consumption of various seafood products. Approximately nine species of *Prorocentrum* have been known to produce okadaic acid (OA) and dinophysins toxins (DTX's) (Hoppenrath et al. 2013; Hoppenrath et al. 2014), which causes Diarrhetic Shellfish Poisoning (DSP). *Prorocentrum lima* is one of the benthic dinoflagellates, known to produce those various toxins (Nishimura et al. 2019). Furthermore, *P. lima* associated with other toxic benthic dinoflagellates, *Gammaridiscus toxicus*, also causes Ciguatera Fish Poisoning (CFP) in tropical areas (Burkholder 1998; Lehane and Lewis 2000).

*Prorocentrum* cells show similar shape and size, making it necessary to have detailed feature observations of cell surface morphology, for identification and classification purposes (Faust 1990). *Prorocentrum* has been reported in Indonesian waters. For instance, in Seribu Islands (Penjalaran Barat Island, Pramuka Island, Panggang Island, Semak Daun Island, Pari Island, Air Island and Tidung Island) (Widiarti 2002; Widiarti 2011; Widiarti and Pudjiarto 2015), Belitung Island (Buyut Island, Kelayang Cape, and Keran Island) (Widiarti 2010), Bali waters (Kuta, Sanur, and Nusa Dua), Lombok (Gili Trawangan) (Skinner et al. 2011), west coast of South Sumatera and Bintan Island coast-Riau Islands (Thamrin 2014), Padang

city beach waters (Dwivayana 2015; Eboni et al. 2015; Oktavian et al. 2017; Seygita et al. 2015), North Lombok (Gili Meno and Gili Air) (Widiarti et al. 2016a), South Lampung waters (Pahawang Besar Island and Kelagian Kecil Island) (Widiarti and Adi 2016), and Weh Island waters-North Aceh (Rubiah Island) (Widiarti et al. 2016b). However, no detailed observation on cell surface morphology has been recorded.

*Prorocentrum lima* has a wide range of morphological variability, and possesses morphotype diversity (Hoppenrath 2013; Zhang et al. 2015; Chomérat et al. 2018). The morphotype diversity of *P. lima* has been revealed by several studies, such as in South China Sea waters (Zhang et al. 2015) and Caribbean Sea waters (Chomérat et al. 2018) (Table 1). Zhang et al. (2015), showed the cell shape, cell length and width, ratio length and width (ratio L/W), pore shape, and the number of pores. Similarly, Chomérat et al. (2018) showed cell shape, cell length and width, ratio L/W, and pore shape and dimension. However, the studies lacked information on both pore dimension and number of pores, which should have been used to complete the database on morphotype diversity of *P. lima*. The morphotype diversity of *P. lima*, has never been reported in Indonesian waters. Therefore, this research is being conducted to determine the morphological characteristics (including pore size and number of pores) of *P. lima* morphotype, from several sites in the western part of Indonesian waters: Bintan Island, Belitung Island, Seribu Islands, and Karimunjawa Islands.

**Table 1.** *Prorocentrum lima* characters by previous studies

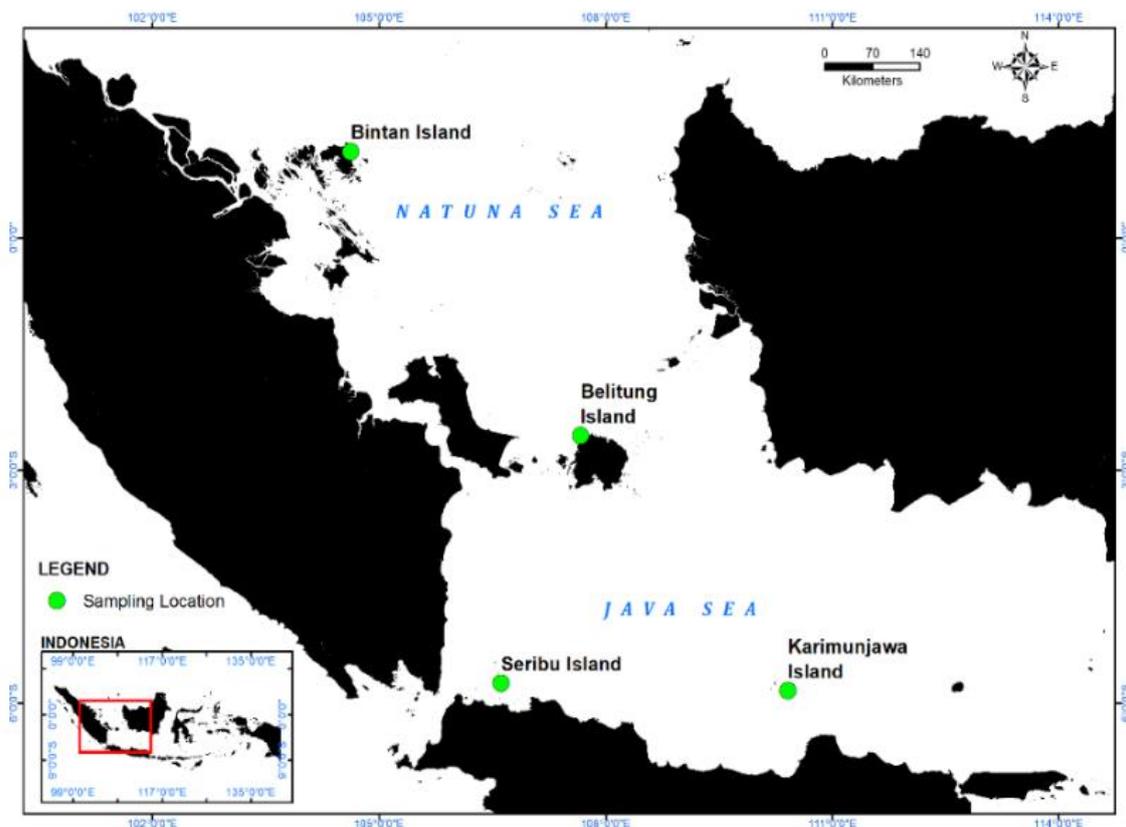
Characters	Results	
	South China Sea Waters (Zhang et al. 2015)	Caribbean Sea Waters (Chomérat et al. 2018)
Cell Shape	Ovate, broadly ovate, oblong oval, broadly oblong	Oblong oval, broadly ovate
Cells Length ( $\mu\text{m}$ )	32-48.1	31.1-38.2
Cells Width ( $\mu\text{m}$ )	25.2-38.2	27.8-32.3
Ratio L/W	1.06-1.61	1.10-1.36
Pore Size ( $\mu\text{m}$ )	-	0.4-0.8
Number of Pores (valve and marginal pores)	28-94	-
Pore Shape	round/ oblong/ kidney-shaped	round/ oval/ kidney-shaped

## MATERIALS AND METHODS

### Study area

Sampling was conducted from April to September 2018 in four islands, located in the western part of Indonesian waters, including Bintan Island, Belitung Island, Seribu Islands, and Karimunjawa Islands (Figure 1). The sampling locations focused on coral reefs with poor conditions, because it potentially provides a new surface for various

macroalgae growth, where the toxic dinoflagellates are more likely to attach (deSylva 1994; Lehane and Lewis 2000). The coral reefs in all four locations have already been disrupted by domestic, tourism, and local fishery activities (Estradivari et al. 2009; Yusuf 2013; Susetiono et al. 2016), so that the macroalgae were also found abundantly in the areas. Sampling locations were situated on the eastern coast of Bintan Island, the northern coast of Belitung Island, Pramuka Island on Seribu Islands, and Karimunjawa Island on Karimunjawa Islands.



**Figure 1.** Sampling locations in Bintan Island (Station II), Belitung Island (Station IV), Seribu Islands (Station I), and Karimunjawa Islands (Station III)

## Procedures

### Data-collection

The macroalgae collected, were only limited to *Sargassum* and *Padina*. As has been observed by Widiarti (2002), benthic dinoflagellates are commonly found attached to both macroalgae. Macroalgae's thallus was randomly harvested on the reef flat areas with 45 cm-100 cm depth, and placed inside wide-mouthed plastic bottles containing ambient seawater (Tester et al. 2014). The whole process was conducted underwater (Tester et al. 2014; Jauzei et al. 2018), to avoid sample disruptions due to air and sunlight exposures.

After collection, plastic bottles containing macroalgae and seawater were shaken using vortex machine (1250 rpm for 1 minute), to detach benthic dinoflagellates from the surface of macroalgae. Furthermore, the samples were filtered through a series of sieves with a mesh size of 125 and 20  $\mu\text{m}$ , to separate samples from sediments and other larger organisms. For morphological observation using LM and SEM, samples were fixed using glutaraldehyde to reach the final concentration of 4% (v/v).

### Morphological-character-observation

Cell shape and size were observed, by using a light microscope (LM) (Leica DM 500). The fixed cells were isolated using modified Pasteur pipette, dripped onto the object-glass, and sealed with a cover glass. The slides were then observed under a microscope with 200 magnification. Furthermore, cell length and width were measured digitally, using LEICA LAS EZ 2.0.

Cell pores (shape, size and the number of pores) and periflagellar area were observed, using Scanning Electron Microscope (SEM) (ZEISS with EVOIMA10 type). The isolated cells were dripped onto 2 x 1  $\text{cm}^2$  filter paper (Whatman 125 mm, pore size 3  $\mu\text{m}$ ), which was already placed on top of the object-glass covered with carbon tape. The samples were air-dried and coated with gold (Au), using Sputter Coater (Quorum type Q150R ES), with 20 (mA) Sputter Current and 60 second Sputter Time. After the coating process, SEM samples were analyzed, and the images were taken using the SE (Secondary Electron) detector, with 8.0 mm Working Distance (WD) and EHT 16.00 kV. Pore length and width measurements were obtained using biometric program tpsUtil32 dan tpsDig232.

### Environmental-factors-measurement

During sample collections within the four locations, measurements of the environmental condition were also conducted. These measurements included, salinity by refractometer (ATAGO), water temperature and dissolved oxygen by DO-meter (HANNA), light intensity by luxmeter (LX-1010B), water acidity by pH indicator (MColorpHast 6.5-10), water current by current meter, with nitrate and phosphate using APHA Analysis Method (2012) in the laboratory.

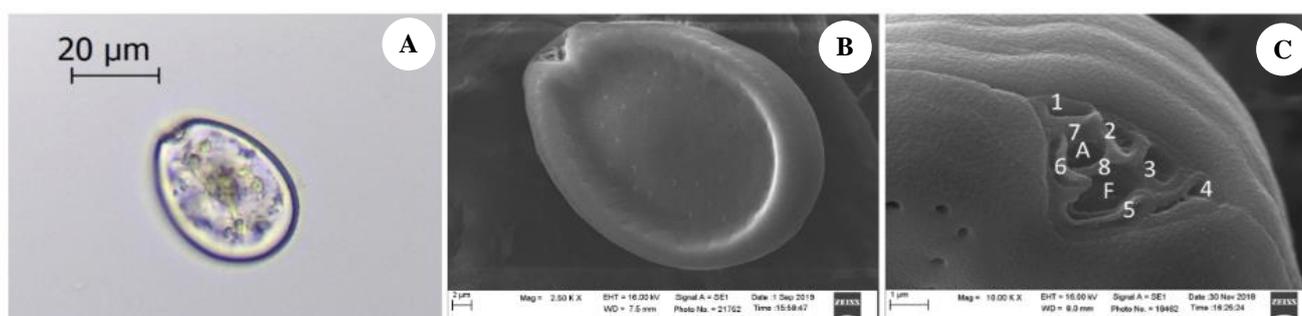
### Data analysis

Morphological character data were tabulated, and compared with morphotypes grouping suggested by Zhang et al. (2015). Character similarity of each Operational Taxonomic Units (OTU) was analyzed by cluster analysis methods, with the use of Hierarchical Cluster Analysis, which was further processed by R version 3.6.2. The cluster analysis results were presented in the form of a dendrogram (treelike diagram) (Sneath 2005).

## RESULTS AND DISCUSSION

All samples collected from the four sampling locations, showed that *Prorocentrum lima* cells possessed ovoid/ovate shape, visible pyrenoid at the center of the cell, scattered pores on both valves (except in the center of the cell), and V-shaped periflagellar areas with 8 platelet plates (Figure 2).

Based on the observation using LM, the length and width of the cells ranged from 35.49-44.94  $\mu\text{m}$  (mean 38.27  $\mu\text{m}$ , s.d 2.77  $\mu\text{m}$ , n = 20), and 25.57-31.56  $\mu\text{m}$  (mean 28.63  $\mu\text{m}$ , s.d. 2.05  $\mu\text{m}$ , n = 20), with the ratio L/W being 1.17-1.44 (mean 1.34, s.d 0.09, n = 20). Furthermore, based on the observation using SEM, the pore length and width ranged from 0.21-0.40  $\mu\text{m}$  (mean 0.30  $\mu\text{m}$ , s.d 0.05  $\mu\text{m}$ , n = 20), and 0.15-0.33  $\mu\text{m}$  (mean 0.21  $\mu\text{m}$ , s.d 0.05  $\mu\text{m}$ , n = 20). The number of pores observed was around 40-68 (mean 50.3, s.d 9.2, n = 20), with the valve shape being round to kidney-shaped (Table 2). Due to the unclear images obtained by SEM, the observation of pores in this study was based only on valve pores.



**Figure 2.** Morphology characters of *Prorocentrum lima* using LM and SEM. A. Cell shape, pyrenoid in the center of the cell; B. Scattered pores except in the center of the cell; C. V-shaped periflagellar area with eight platelets

**Table 2.** Morphology characters of *Prorocentrum lima* from all sampling locations (n = 20)

Characters	Sampling locations			
	Seribu Islands	Bintan Island	Karimunjawa Islands	Belitung Island
Cell Length ( $\mu\text{m}$ )	36.05-41.16	40.26-44.94	35.81-37.30	35.49-37.79
Cell Width ( $\mu\text{m}$ )	26.32-30.05	29.45-31.56	25.57-27.17	28.86-31.51
Ratio L/W	1.33-1.42	1.34-1.42	1.35-1.44	1.17-1.23
Pore Length ( $\mu\text{m}$ )	0.29-0.40	0.28-0.37	0.23-0.31	0.21-0.38
Pore Width ( $\mu\text{m}$ )	0.23-0.33	0.19-0.21	0.15-0.19	0.16-0.32
Number of Pores (Valve Pores)	40-48	51-68	42-53	48-59
Pore Shape	round/kidney-shaped	round/kidney-shaped	round/kidney-shaped	round

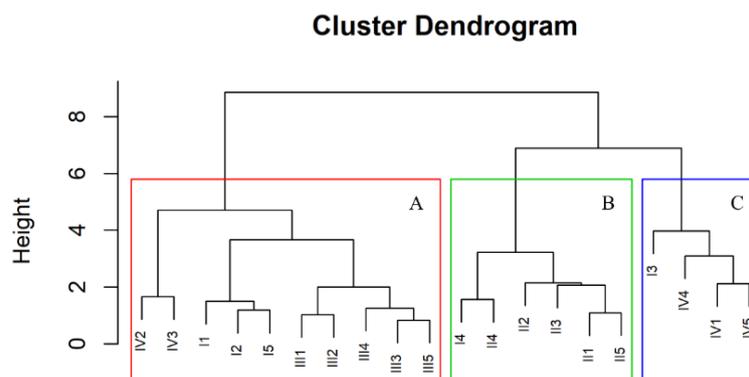
Further examination using Cluster Analysis, showed that the optimal number of the cluster could be obtained through the use of a Scree and Silhouette Plot. There were three optimal numbers of the cluster, showed by the elbow and peak points at the Scree and Silhouette Plots, respectively (both showed cluster  $k = 3$  at axes 1). Cluster Analysis showed three clusters, where cluster 1 consists of four members, cluster 2 consists of ten members, and cluster 3 consists of six members. Cluster 1 represented by I3, IV1, IV4 and IV5, was described as one *P. lima* specimens at station 1 (Seribu Islands), and three specimens at station 4 (Belitung Island). Based on the cluster dendrogram, three specimens from Belitung Island were closely related to each other (Figure 3). Cluster 1 was characterized by a low mean value of ratio L/W. Specimens of *P. lima* obtained from Belitung Island possessed the lowest ratio L/W values compared to other sampling locations (Table 2).

Cluster 2 represented by I1, I2, I5, III1, III2, III3, III4, III5, IV2 and IV3, which were described as three *P. lima* specimens at station 1 (Seribu Islands), all specimens at station 3 (Karimunjawa Islands) and two specimens at station 4 (Belitung Island). Based on the cluster dendrogram (Fig 3), three specimens from the Seribu Islands were closely related to all specimens from Karimunjawa Islands. At cluster 2, variables III1, III2, III3, III4 and III5 possessed closer distance, compared to other members of the cluster, which is showed by the shorter height of Euclidian distance at axes 2. Cluster 2 was characterized by the lowest mean values of cell length (36.4040), cell width (26.9190), pore length (0.2740), and pore width (0.1920). The *P. lima* cells in Seribu Islands

possessed high values of pore dimensions (0.29-0.40  $\mu\text{m}$  in length and 0.23-0.33  $\mu\text{m}$  in width), while showing low mean values of both characters, based on Cluster Analysis. These occurred as a mean result of pore dimensions between values from Seribu Islands and from the Karimunjawa Islands, which possessed the lowest values from all sampling locations (0.23-0.31  $\mu\text{m}$  in length and 0.15-0.19  $\mu\text{m}$  in width) (Table 3).

Cluster 3 represented by I4, I11, I12, I13, I14 and I15, was described as one *P. lima* specimens at station 1 and five *P. lima* specimens at station 2 (Seribu Islands and Bintan Island), respectively. Based on the cluster dendrogram, four specimens from Bintan Island, were closely related to each other (Figure 3). Cluster 3 was characterized by a high mean value of ratio L/W, and the number of pores. In this research, *P. lima* specimens from Bintan Island possessed the highest pore number (51-68), compared to other sampling locations (Table 3). In previous studies, different pore shapes were frequently observed in one cell (Hoppenrath et al. 2013; Zhang et al. 2015), same as this study where both round and kidney-shaped pores, were also found in one cell. Pore shape character was not used as a variable in the Cluster Analysis.

Environmental parameters data in four different sampling locations showed, salinity values ranged from 30-34.7‰, temperature from 27.5-30.8°C, dissolved oxygen (DO) from 7.6-13.5 ppm, light intensity from 1392-46983 lux, acidity from 7.1-7.8, water current from 0.039-0.115  $\text{ms}^{-1}$ , nitrate from 0.087-0.101  $\text{mg.L}^{-1}$  and phosphate from 0.002-0.009  $\text{mg.L}^{-1}$  (Table 4).

**Figure 3.** Closely related groups at each cluster based on Euclidian distance. A. Cluster 2, Seribu Islands and Karimunjawa Islands; B. Cluster 3, Bintan Island; C. Cluster 1, Belitung Island

**Table 3.** Mean values of morphology characteristic from each cluster (n = 20)

Group	Cell Length	Cell Width	Ratio L/W	Pore Length	Pore Width	Pore Number
1 (n = 4)	37.5175	30.66500	1.225000	0.3475	0.2775000	49.25
2 (n = 10)	36.4040	26.91900	1.356000	0.2740	0.1920000	45.20
3 (n = 4)	41.8750	30.12667	1.388333	0.3150	0.2066667	59.50

**Table 4.** The average values of environmental factors measurement at four sampling stations

Location	Salinity	Temperature (°C)	DO (ppm)	Light (lux)	pH	Water current (ms <sup>-1</sup> )	Nitrate (mg.L <sup>-1</sup> )	Phosphate (mg.L <sup>-1</sup> )
Seribu Islands (Station I)	30.0	27.5	13.4	1392	7.8	0.048	0.094	0.002
Bintan Island (Station II)	32.0	30.5	7.6	37270	7.1	0.039	0.087	0.009
Karimunjawa Islands (Station III)	34.7	30.8	11.7	30850	7.2	0.115	0.101	0.003
Belitung Island (Station IV)	34.0	29.6	13.5	46983	7.2	0.055	0.098	0.003

The cells of *P. lima* have specific morphological characters, which are generally used to differentiate this *Prorocentrum* species from others. Those characters were symmetric cells with oblong oval, ovoid (Hoppenrath et al. 2013) or ovate shape (Zhang et al. 2015), visible pyrenoid in the center of the cell, scattered pores on both valves but void in the center of the cell (Fukuyo 1981; Faust 1990), and wide V-shaped periflagellar areas consisting of 8 platelet plates (Hoppenrath et al. 2013; Zhang et al. 2015). In this study, observation using LM or SEM showed that benthic dinoflagellates obtained from Bintan Island, Belitung Island, Seribu Islands, and Karimunjawa Islands, possessed all the mentioned characters, so all the specimens collected could be defined as *P. lima* (Figure 2).

Based on the observation using LM, the cells showed normal and broadly ovate shape, with dimensions ranging from 35.49-44.94  $\mu\text{m}$  (mean 38.27  $\mu\text{m}$ , s.d. 2.77  $\mu\text{m}$ , n = 20) and 25.57-31.56  $\mu\text{m}$  (mean 28.63  $\mu\text{m}$ , s.d. 2.05  $\mu\text{m}$ , n = 20) (Table 2). The cell sizes were within the range of *P. lima* length and width, which were at 31-47  $\mu\text{m}$  and 22-40  $\mu\text{m}$  for Grzebyk et al. (1998), and 30-57  $\mu\text{m}$  and 21-46  $\mu\text{m}$  for Zhang et al. (2015). The ratio L/W from this study was 1.17-1.44 (mean 1.34, s.d. 0.09, n = 20), which was also within the range of *P. lima* by Zhang et al. (2015) at 1.06-1.61, or that of Chomérat et al. (2018) at 1.10-1.36. Based on the observation using SEM, the cells showed pore length and width ranging from 0.21-0.40  $\mu\text{m}$  (mean 0.30  $\mu\text{m}$ , s.d. 0.05  $\mu\text{m}$ , n = 20), and 0.15-0.33  $\mu\text{m}$  (mean 0.21  $\mu\text{m}$ , s.d. 0.05  $\mu\text{m}$ , n = 20), which were still within the range of 0.37  $\mu\text{m}$  by Nagahama and Fukuyo (2005), and 0.31-0.70  $\mu\text{m}$  by Hoppenrath et al. (2013). The number of pores obtained in this study ranged from 40-68 (mean 50.3, s.d. 9.2, n = 20), relatively close to Zhang et al. (2015), which was 42-94.

*P. lima* showed five morphotypes, which differ in cell length, cell width, ratio L/W, and pore number (Zhang et al. 2015). Meanwhile, other characters such as pore shape, pore length, and pore width, were also characters that could help differentiate morphotype (Grzebyk et al. 1998). Observation on *P. lima* cells obtained from four sampling locations also showed different results, and descriptions on each sampling locations are as follows,

### Seribu Islands

Observation on *P. lima* cells obtained in Seribu Islands waters showed ovate shape, cell length and width ranging from 36.05-41.16  $\mu\text{m}$  (mean 38.39  $\mu\text{m}$ , s.d. 2.17  $\mu\text{m}$ , n = 5) and 26.32-30.05  $\mu\text{m}$  (mean 27.90  $\mu\text{m}$ , s.d. 1.58  $\mu\text{m}$ , n = 5), respectively, with the ratio L/W from 1.33-1.42 (mean 1.38, s.d. 0.04, n = 5). The shape and size were within the range of *P. lima* "morphotype 2" by Zhang et al. (2015), with cell length and width ranging from 36.8-40.0  $\mu\text{m}$ , and 25.6-28.9  $\mu\text{m}$ , respectively, and the ratio L/W at 1.33-1.45 (Table 2, Figure 4.1). However, observation on pores showed different results, where the pore number with 40-48 (mean 44, s.d. 5.2, n = 5) is closely within the range of "morphotype 1" by Zhang et al. (2015), which was 42-84. The shape of pores observed in this study were round to kidney-shaped, showing pore shape variation of "morphotype 2" which is round, and "morphotype 3," which is kidney-shaped/oblong.

### Bintan Island

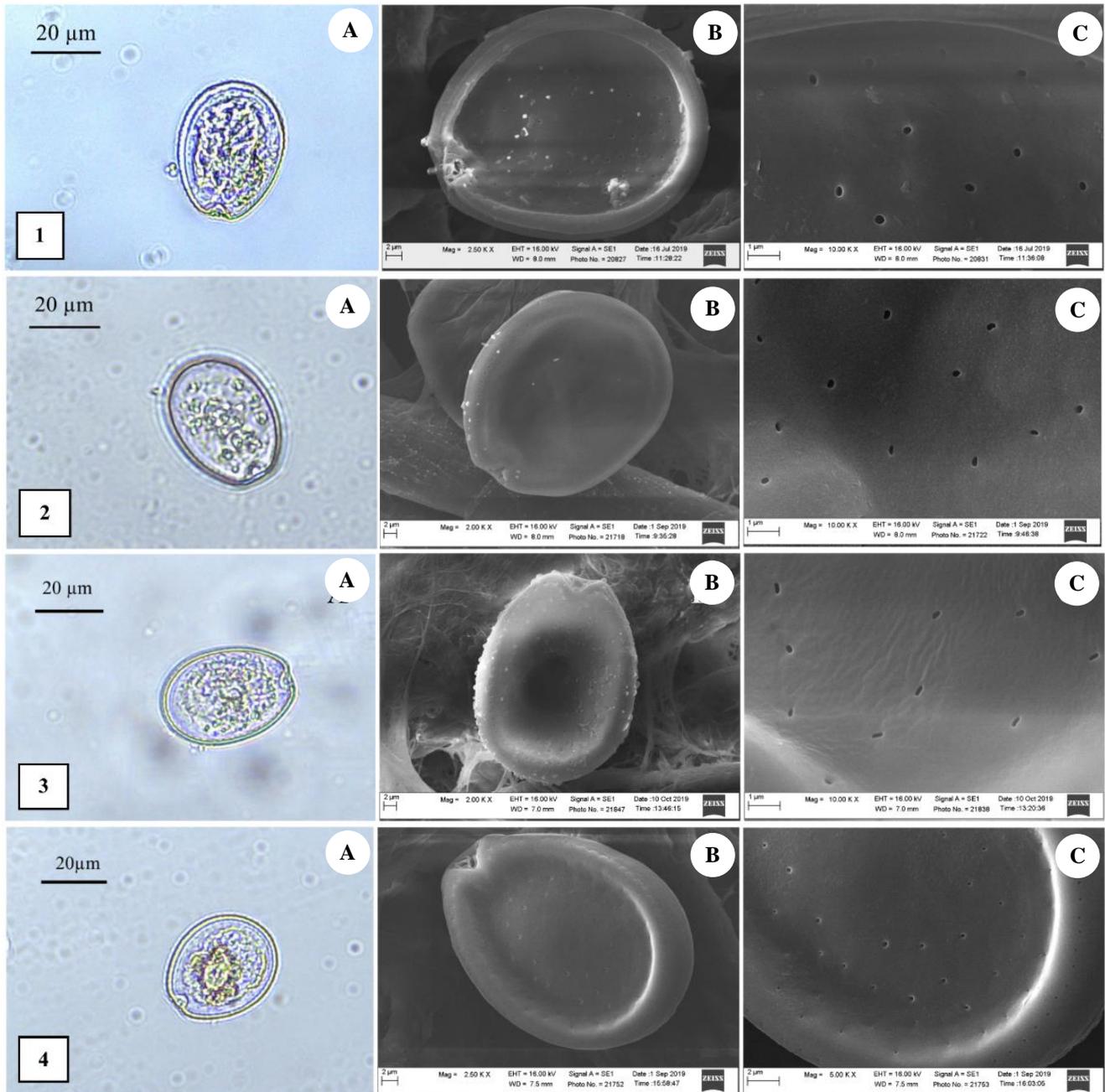
Observation on *P. lima* cells collected in Bintan Island waters showed ovate shape, cell length and width ranging from 40.26-44.94  $\mu\text{m}$  (mean 42.02  $\mu\text{m}$ , s.d. 1.86  $\mu\text{m}$ , n = 5) and 29.45-31.56  $\mu\text{m}$  (mean 30.34  $\mu\text{m}$ , s.d. 0.87  $\mu\text{m}$ , n = 5), respectively, with the ratio L/W from 1.34-1.42 (mean 1.38, s.d. 0.03, n = 5). The shape and size were within the range of *P. lima* "morphotype 3" by Zhang et al. (2015), with cell length and width at 37.5-48.1  $\mu\text{m}$  and 27.3-38.2  $\mu\text{m}$ , respectively, and the ratio L/W at 1.23-1.5 1 (Table 2, Figure 4.2). Furthermore, observation on pores showed number of pores with a count of 51-68 (mean 61.2, s.d. 6.3, n = 5), which corresponds to "morphotype 2" by Zhang et al. (2015). The shape of pores observed in this study were round to kidney-shaped, showing pore shape variation of "morphotype 2" which is round, and "morphotype 3," which is kidney-shaped/oblong.

### Karimunjawa Islands

Observation on *P. lima* cells obtained in Karimunjawa Islands waters showed ovate shape, cell length and width ranging from 35.81-37.30  $\mu\text{m}$  (mean 36.43  $\mu\text{m}$ , s.d. 0.57  $\mu\text{m}$ , n = 5) and 25.57-27.17  $\mu\text{m}$  (mean 26.11  $\mu\text{m}$ , s.d. 0.65

$\mu\text{m}$ ,  $n = 5$ ), respectively, with the ratio L/W from 1.35-1.44 (mean 1.40, s.d. 0.04,  $n = 5$ ). The shape and size were within the range of *P. lima* "morphotype 2" by Zhang et al. (2015), with cell length and width from 36.8-40.0  $\mu\text{m}$  and 25.6-28.9  $\mu\text{m}$ , respectively, and the ratio L/W from 1.33-1.45 (Table 2, Figure 4.3). Furthermore, observation on pores showed number of pores with a count of 42-53 (mean

47.8, s.d. 4.2,  $n = 5$ ), corresponds to "morphotype 1" by Zhang et al. (2015), which was 42-84. The shape of pores observed in this study were round to kidney-shaped, showing pore shape variation of "morphotype 2" which is round, and "morphotype 3," which is kidney-shaped/oblong.



**Figure 4.** Morphology characters of *Prorocentrum lima*. 1. Seribu Islands. 2. Bintan Island. 3. Karimunjawa Islands. 4. Belitung Island. A. Cell shape and size under LM observation; B. Cell shape and size under SEM observation; C. Pore shape and size observation using SEM

### Belitung Island

Observation on *P. lima* cells obtained in Belitung Island waters showed broadly ovate shape, cell length and width ranging from 35.49-37.79  $\mu\text{m}$  (mean 36.24  $\mu\text{m}$ , s.d. 0.94  $\mu\text{m}$ , n = 5) and 28.86-31.51  $\mu\text{m}$  (mean 30.17  $\mu\text{m}$ , s.d. 1.05  $\mu\text{m}$ , n = 5), respectively, with the ratio L/W from 1.17-1.23 (mean 1.20, s.d. 0.02, n = 5). The shape and size were within the range of *P. lima* "morphotype 1" by Zhang et al. (2015), with cell length and width from 32.0-44.4  $\mu\text{m}$ , and 27.3-36.5  $\mu\text{m}$ , respectively, and the ratio L/W from 1.06-1.36 (Table 2, Figure 4.4). Furthermore, observation on pores showed number of pores with a count of 48-59 (mean 48.2, s.d. 10.4, n = 5), corresponds to "morphotype 1" by Zhang et al. (2015), which was 42-84. The shape of pore observed in this study was round, and similar to that of "morphotype 1".

*Prorocentrum lima* is known as a common species, widely distributed in tropical, subtropical and temperate waters (Hoppenrath et al. 2013; Nishimura et al. 2019). In this study, the cells were found in all sampling locations. Previous studies showed that *P. lima* was also found in several locations in Indonesian waters, namely Seribu Islands (Widiarti 2002; Widiarti 2011; Widiarti and Pudjiarto 2015), Belitung Island (Widiarti 2010), North Lombok (Widiarti et al. 2016a), South Lampung waters (Widiarti and Adi 2016) and Weh Island waters-North Aceh (Widiarti et al. 2016b), with environmental condition ranging from 28-33 °C for temperature, 26.0-34.5 ‰ for salinity, 6.5-7.4 for water acidity, 6.17-8.30 ppm for dissolved oxygen, and <0.1-0.2  $\text{ms}^{-1}$  for water current. The environmental data measured in this study, also showed similar range of values, which were temperature from 27.0-31.3°C, salinity from 30-35‰, water acidity from 6.5-7.9, dissolved oxygen (DO) from 6.1-14.0 ppm, and water current from 0.0001-0.196  $\text{ms}^{-1}$  (Table 4). All of the sampling locations showed relatively slow water current and high concentration level of nitrate (0.087-0.101  $\text{mg.L}^{-1}$ ) and phosphate (0.002-0.009  $\text{mg.L}^{-1}$ ). Nitrate concentrations in the four locations, were above the water quality standard from the Ministry of Forestry and Environment for marine biota, which is 0.002  $\text{mg/L}$  (Ministry of Forestry and Environment 2004). The slow water current and high level of nutrients, are likely to become factors that enhance eutrophication in the waters, as the potency of *P. lima* blooms should be at alert. The study on the density of *P. lima* in Tanjung Gelam, Karimunjawa Islands waters, showed that the abundance of cells obtained in the area, correlates to the higher phosphate value in the waters (Widiarti et al. 2018).

Due to the morphological observations and morphotype grouping by Zhang et al. (2015), the *P. lima* in this study could be determined into three morphotypes, namely morphotype 1 for specimens collected from Belitung Islands, morphotype 2 for specimens collected from Seribu Islands and Karimunjawa Islands, and morphotype 3 for specimens collected from Bintan Island (Table 2). All three morphotypes corresponded to those of the clusters based on Cluster Analysis, which were the Belitung Island clusters, the Seribu Islands and Karimunjawa Islands cluster, and the Bintan Island cluster, respectively.

The results of this study prompt the necessity of conducting an abundance survey, establishing strains, toxins and molecular phylogenetic analysis, and growth experiment, to assess the negative impact in the future.

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