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Taxonomic characterization of a brackish water, bloom-forming *Peridiniopsis* species (Peridiniales, Dinophyceae) from Iraq

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Abstract

Background: The occurrence of a small brackish water bloom-forming dinoflagellate belonging to the genus *Peridiniopsis* is reported for the first time from the Shatt Al-Arab River in Iraq. Based on the study of cell morphology, this taxon was attributed to the recently described *Peridiniopsis minima*, recorded so far only from the Jiulongjiang River, China.

Results: The description of *P. minima* was extended to include some previously unreported details of the thecal surface, apical pore complex, and sulcus structure using light and scanning electron microscopy. Cells of this species from Iraq were oval to rhombic in shape and slightly dorso-ventrally compressed, with distinct red eyespot and endosymbiont nucleus. Cells were covered by a thin theca with the Kofoidian plate formula Po, X, 3′, 1a, 6″, 5c, 5 s, 5‴, 2⁗. Complementing the original description of the species, five plates were discerned in the composition of the sulcal area, and the presence of small median sulcal plate was confirmed.

Conclusion: The finding of *P. minima* in the waters of Shatt Al-Arab River represents a new addition to the Iraqi aquatic microflora. Our observations of this dinoflagellate in brackish waters of the Shatt Al-Arab River system extend the known range of salinity tolerance for this species.

Keywords: Dinophyceae, Dinoflagellates, Iraq, Morphology, Peridiniopsis, Shatt Al-Arab River, Taxonomy, SEM

Background

Over last decades, contributions to the knowledge of microalgal flora inhabiting fresh and brackish water ecosystems of Iraq have revealed the remarkable predominance of diatoms in the composition of phytoplankton (e.g., Huq et al. 1978; Al-Handal 1989; Maulood et al. 1993, 2013; Al-Zubaidi et al. 2006), whereas a little is known in respect of occurrence and diversity of non-diatom algae including dinoflagellates so far. However, some dinoflagellates, particularly species belonging to the genera *Ceratium* Schrank, *Peridinium* Ehrenberg, and *Peridiniopsis* Lemmermann, can be an important integral part of fresh and brackish water ecosystems forming extensive blooms during phytoplankton succession worldwide (e.g., Trigueros 2000; Ki and Han 2005; Mac Donagh et al. 2005; Hansen and Flaim 2007; Takano et al. 2008).

To date, the records of dinoflagellates in Iraq are scarcely documented (Kell and Saad 1975; Maulood et al. 1993, 2013; Salman et al. 2013). Meanwhile, according to the last checklist of algal flora in Iraq (Maulood et al. 2013) the occurrence of *Peridiniopsis* species has never been previously recorded in any Iraqi water environment.

Recently, a dense population of a previously unreported small peridinioid dinoflagellate has been detected during the phytoplankton survey of the Shatt Al-Arab River in the vicinity of Basrah city, Iraq. Based on its thecal plate pattern, this taxon was attributed to the genus *Peridiniopsis*. In the present paper, the general morphology and the thecal plate arrangement of this species is described using light, epifluorescence, and scanning electron microscopy. New data on the distribution of this dinoflagellate within Iraqi waters are provided.

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Methods

Sampling sites

The Shatt Al-Arab River is the result of Tigris and Euphrates rivers conjunction north of Basrah city at Qurna. The river flows southwardly through the city and enter the Arabian Gulf at Fao (Fig. 1). The total length of the river is 193 km, the width is nearly 400 m at Basrah city and 1500 m at river inflow into the Gulf. River depth ranges from 7.5 m at upstream to 12.5 m at Fao, and is affected by diurnal tide without thermal stratification (Huq et al. 1978).

The phytoplankton survey was carried out at three sampling sites in the Shatt Al-Arab River (Fig. 1). The first sampling site is situated 30 km south of Qurna, whereas the second sampling site is located downstream the entrance of Hor Al-Hammar into the river. The third site represents a downstream station positioned just above the inflow of Karun River into the Shatt Al-Arab River.

Sampling

Phytoplankton samples were collected by oblique tows using plankton net with 20 μm mesh size and 0.6 m diameter during November and December 2009 and April 2010. The samples were fixed and preserved with 4 % acidic Lugol's solution. In addition, in situ measurements of environmental variables (water temperature, pH and salinity) were obtained from a portable multiparameter handheld meter (Multi 350i, WTW) (Table 1).

Fig. 1 Map of the southern Iraq showing the locations of the sampling sites

Table 1 Environmental variables at the studied sites during *Peridiniopsis minima* occurrence

Site	Coordinates	Date	Water temperature, ºC	pH	Salinity
1	30º44′53.06″ N 47º41′19.30″ E	November 2009	23.9	7.99	2.0
2	30º34′34.64″ N 47º46′27.30″ E	December 2009	19.3	7.16	7.2
3	30º27′44.04″ N 48º00′49.80″ E	April 2010	21.3	7.50	2.1

Samples processing

The material was preliminary viewed alive with a Zeiss Axiophot 2 imaging microscope (Carl Zeiss AB, Göteborg, Sweden) at 400× to 1000× magnifications. For detailed observation, cells were isolated by micropipetting in preparation for high-magnification photomicroscopy. Cells were examined using a Leica DMLM microscope (Leica, Wetzlar, Germany) equipped with epifluorescence (100 W short arc mercury lamp), and Leica DFC420C digital camera at 1000× magnification. Thecal plates were observed after staining with Calcofluor White (Sigma Chemical Co.) according to the method of Fritz and Triemer (1985). To visualize nuclei, cells were stained for 30 min with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI, Sigma-Aldrich) fluorochrome. Scanning electron microscopy (SEM) was employed for detailed observations of the thecal surface.

For SEM, phytoplankton cells from preserved natural samples were filter mounted by a Swinnex filter-holder, rinsed twice with distilled water and subsequently dehydrated in a series of increasing ethanol concentrations (15, 30, 50, 70, 90, 95 and 100 %). The filters were critical point dried, sputter-coated with gold-palladium, and examined either by a Quanta 200 (FEI, Eindhoven, the Netherlands) or by CarryScope JCM-5700 (JEOL, USA) scanning electron microscope.

Morphometric measurements were made either from the calibrated digital light microscope (LM) images using Leica Application Suite v. 3.7 software (Leica Microsystems Ltd., Switzerland) or were calculated from scanning electron micrographs. Dimensions are given as the mean ± standard deviation. SEM photographs were presented on a black background using Adobe Photoshop CS2, v. 9.0.2 (Adobe Systems, San Jose, CA, USA). The material collected during this survey is deposited in the regional collection of microalgae at Marine Science Centre, University of Basrah, Iraq.

Results

Species identification of small bloom-forming Iraqi *Peridiniopsis* was based on observations of its morphological characters using light, epifluorescence, and scanning electron microscopy. Both alive and preserved cells from field samples were examined. Based on the main diagnostic features of the genus *Peridiniopsis* (Bourrelly 1968; Popovský and Pfiester 1990) including cell shape and dimensions, thecal surface ornamentation, and the size, shape, and position of the anterior intercalary plate, the small *Peridiniopsis* taxon from the Shatt Al-Arab River was assigned to *P. minima* Zhang, Liu & Hu, a species originally described recently from the Jiulongjiang River, China (Zhang et al. 2014).

Systematics

Class DINOPHYCEAE West & Fritch, 1927
Order PERIDINIALES Haeckel, 1894
Family PERIDINIACEAE Ehrenberg, 1830
Genus *Peridiniopsis* Lemmermann, 1904
Peridiniopsis minima Zhang, Liu & Hu, 2014
Figs. 2 and 3

Description

Cells were oval to almost rhombic in shape and slightly dorso-ventrally compressed, with nearly equal in size conical epitheca and smoothly round hypotheca (Figs. 2a; 3a, c, e). Cells measured 13.5–19.04 μm in length (mean 16.27 ± 1.66, $n = 14$), 12.8-17.9 μm in width (mean 15.05 ± 1.65, $n = 11$), and 12.3–15.3 μm in depth (mean 13.71 ± 1.04, $n = 7$), with length to width ratio of 1.01–1.1. The cingulum was nearly equatorial, excavated, and descending with a displacement of about half of its own width. The sulcus was excavated and extended from the cingulum to the antapex (Figs. 2c, e, f and 3a-c, h). Numerous small, golden-brown disc-shaped chloroplasts were peripherally arranged (Fig. 2a-c). Relatively large round dinokaryon was centrally located. Endosymbiont nucleus was present (Fig. 2b-d). Distinct red eyespot was located in the sulcal area (Fig. 2a, b). No pyrenoid was observed.

The plate tabulation was Po, X, 3′, 1a, 6″, 5c, 5 s, 5′′′, 2′′′′. The epitheca consisted of twelve symmetrically arranged plates. The apical pore plate (Po) was elongate and possessed slit-like and slightly curved apical pore. The canal plate (X) was small, rectangular, and situated between Po, 1′, 2′ and 3′ on the ventral side of the cell (Figs. 2i, j and 3f). The Po plate was encircled by three apical plates (1′–3′), of which plate 1′ was in contact with the sulcus and connected to the apical pore plate by the canal plate (Figs. 2h-j and 3a, e). The first apical plate (1′) was large, rhombic; the plates 2′ and 3′ were large, irregularly six-sided, and mainly located dorsally.

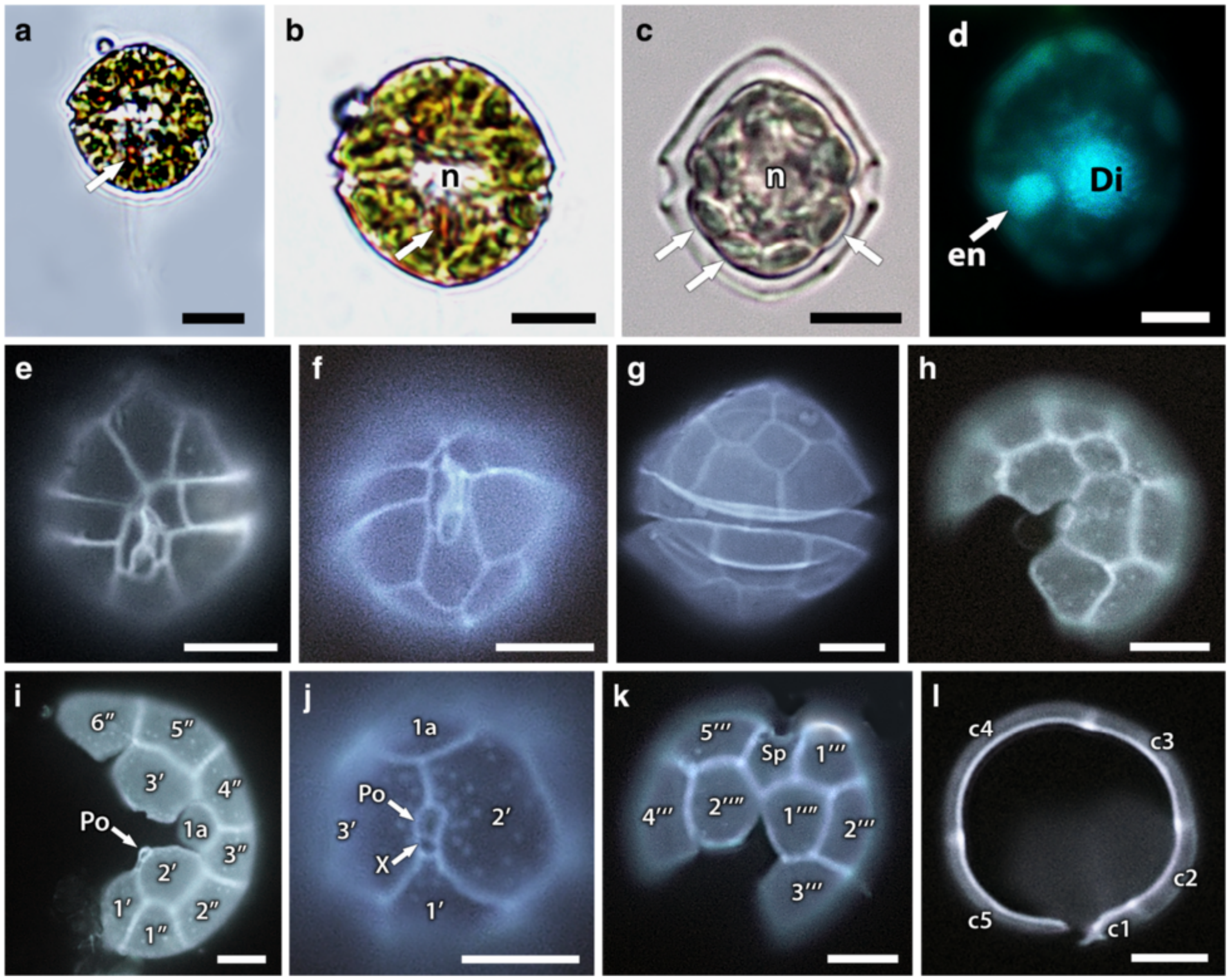

Fig. 2 Light micrographs of *Peridiniopsis minima* from Iraq. (**a**, **b**) cell from freshly collected sample in ventral view showing general appearance, nucleus (n) and chloroplasts arrangement, *arrows* point to eyespot; (**c**) preserved cell showing central nucleus (n) and peripherally arranged chloroplasts (*arrows*); (**d**) DAPI stained cell showing dinokaryotic (Di) and endosymbiont (en, *arrow*) nuclei; (**e**-**g**) calcofluor stained cells in ventral (**e**, **f**) and dorsal (**g**) view showing the thecal plate tabulation; (**h**, **i**) split calcofluor stained epitheca in apical view showing the epithecal plate pattern; (**j**) detail of the apical pore complex and surrounded apical plates; (**k**) split calcofluor stained hypotheca in antapical view showing the hypothecal plate pattern; (**l**) cingular plate pattern. Scale bars: 5 μm

The single anterior intercalary plate (1a) was relatively small, rhombic, and located symmetricaly on dorsal cell side between 2′, 3′, 3′′and 4′′ epithecal plates (Figs. 2g-i and 3b, e). There were six precingular plates, among them the plates 1′′, 2′′ and 5′′ were four-sided, whereas the plates 3′′, 4′′ and 6′′ were pentagonal.

The hypotheca possessed rather symmetrically arranged five postcingular (1′′′-5′′′) and two antapical (1′′′′ and 2′′′′) plates. The first (1′′′) and fifth (5′′′) postcingular plates were large and covered most of the ventral side of the hypotheca, whereas the 2′′′-4′′′ plates together with the two antapical plates cover the dorsal side of the hypotheca (Figs. 2f, k and 3a, b).

The cingulum consisted of five unequal plates (c1–c5), among them the first cingular plate was smallest in the cingular series (Figs. 2l and 3a). In the sulcus, which consisted of five plates, the small anterior sulcal plate (Sa) is contiguous with the first cingular plate at the left end of the cingulum and touched the plates 1′, 6′′ and c6. The large right sulcal plate (Sd) was relatively narrow, elongate, and had a thin internal sulcal list (sl) on its left margin. The left sulcal plate (Ss) was elongate and extended along the right side of the first cingular plate c1 and the first postcingular plate 1′′′. The median sulcal plate (Sm) was small and located between the lower parts of Sd and Ss. The posterior sulcal plate (Sp) was the largest of the sulcal series, wider toward the posterior, and elongated to the antapex between the first and fifth postcingular plates (Figs. 2e, f and 3h, i).

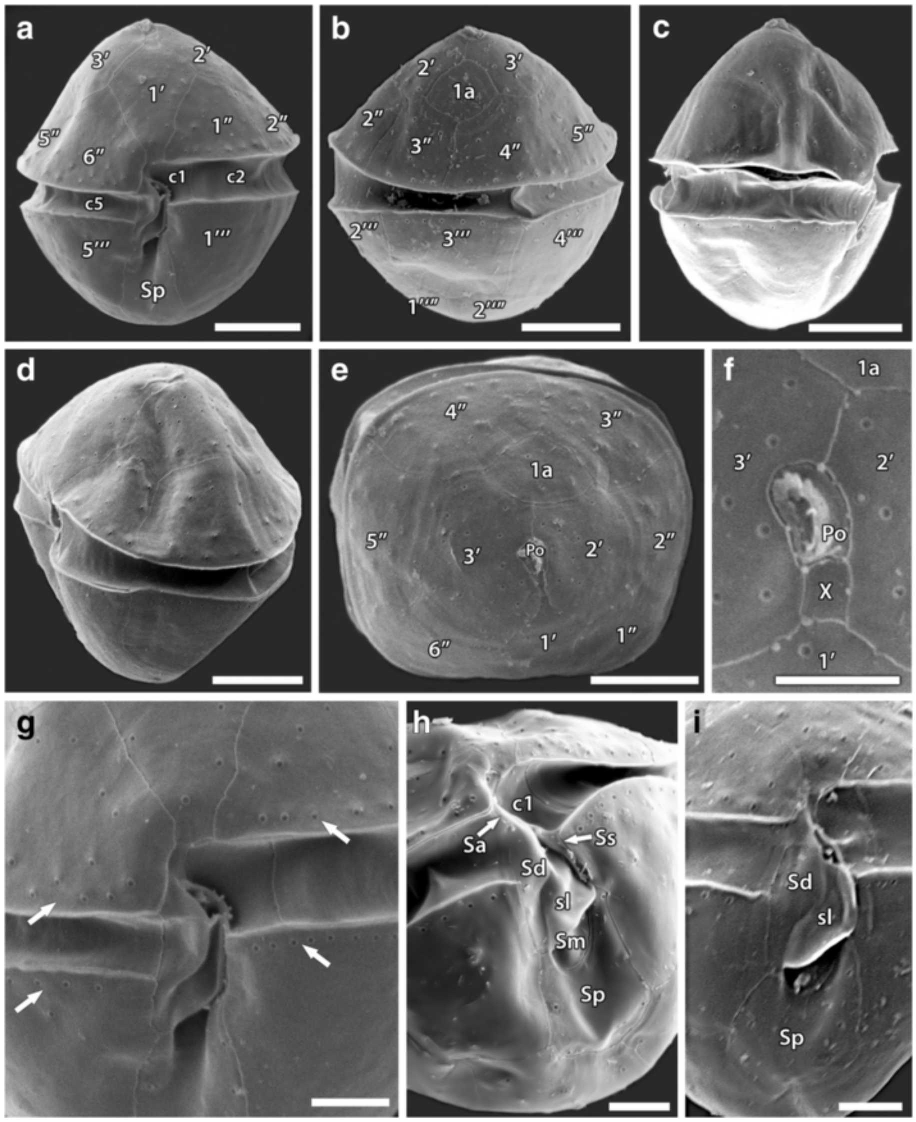

Fig. 3 Scanning electron micrographs of *Peridiniopsis minima* from Iraq. (**a**) ventral view; (**b**) dorsal view; (**c**) oblique *right* lateral view; (**d**) oblique *left* lateral view; (**e**) apical view; (**f**) detail of the apical view showing the apical pore complex with surrounded apical plates; (**g**-**i**) detail of theca in ventral view showing marginal rows of densely lying pores (**G**, *arrows*) and plate pattern of the sulcal series (**h**, **i**). Scale bars: A-E, 5 μm; F-I, 2 μm

The thecal surface was smooth with small rimmed pores (0.15–0.16 μm in diameter) scattered sparsely over the plates (Figs. 2h, j and 3e-g). Densely lying pores formed distinct marginal rows along the upper and lower edges of the pre- and postcingular plates bordering the cingulum (Fig. 3g). The cingular and sulcal plates were lacking pores, except for the Sp plate (Fig. 3h, i).

Known distribution and occurrence

Peridiniopsis minima has been originally described from the freshwater phytoplankton assemblage in the Jiulongjiang River, Fujian Province of China as bloom-forming species (Zhang et al. 2014). Similarly, in the Shatt Al-Arab River system this species represented a major proportion of the overall phytoplankton assemblage at sites 1 and 2 during November and December 2009, and to a lesser extent at site 3 in April 2010. The occurrence of *P. minima* was recorded along the Shatt Al-Arab River at temperatures ranging from 19.3 °C to 23.9 °C and salinity range from 2 to 7.2 (Table 1).

Discussion

The genus *Peridiniopsis* was originally described by Lemmermann in 1904 and later was revised by the addition of species formerly assigned to *Glenodinium* Ehrenberg and *Peridinium* Ehrenberg (Bourrelly 1968). The current classification of *Peridiniopsis* holds together species with varying numbers of plates in epithecal and cingular Kofoidian series, and restricted to species with no or one anterior intercalary plate (Bourrelly 1968). The plate formula of the genus *Peridiniopsis* is defined as 3–5′, 0-1a, 6–8″, 5-6c, 3-5 s, 5‴, 2⁗ (Bourrelly 1968, 1985; Popovský and Pfiester 1990). In the present study, based on analysis of thecal plate pattern, the small peridinioid dinoflagellate from the Shatt Al-Arab River, Iraq was attributed to the genus *Peridiniopsis*.

Except for its size, the *Peridiniopsis* species that we collected in Iraq agreed well with the original description of *P. minima* (Zhang et al. 2014) in respect of cell shape, cytoplasm content, and thecal arrangement including the characteristic shape and position of small anterior intercalary plate (Table 2). The cell size of the Iraqi specimens was slightly larger (13.5–19.04 μm in length and 12.8–17.9 μm in width) than the ones of the originally described *P. minima* (8-15 μm in length, 6–12 μm in width; Zhang et al. 2014) but their size ranges overlap in length and can be considered as intraspecific variability.

The original description of *P. minima* was based on LM observations without details of the thecal surface structure, and a molecular characterization of ribosomal genes and ITS regions (Zhang et al. 2014). Our new observations of cells from Iraq add previously unreported details to the description of this species regarding the APC and sulcus structure and the thecal surface ornamentation. Based on specimens examined by SEM, the slit-like shape of the apical pore, the thecal pores size and its arrangement were documented. Additionally, the presence of a fifth small median sulcal plate was confirmed in the composition of the sulcal area as well as the small internal sulcal list was revealed in the right sulcal plate. In order to confirm its identity with *P. minima* and evaluate its phylogenetic position among other freshwater and marine dinoflagellates, we made several attempts to amplify DNA and get a sequence of this species during this study. However, all stayed unsuccessful and only contaminant (fungi) sequences could be amplified from fixed samples. Hence, obtaining a sequence of this species remains to be done and further analyses will be carried out in the future.

The present work brings new data on the occurrence of the bloom-forming *P. minima* for the first time in the waters of Shatt Al-Arab River, which is considered an important contribution to the aquatic flora of Iraq. Moreover, our observations of freshwater *P. minima* in brackish waters of the Shatt Al-Arab River system in appreciable concentrations extend the known range of salinity tolerance for this species. From an ecological point of view, only a few *Peridiniopsis* species have been described to cope with salty waters. Actually, species in the

Table 2 Morphological comparison of the brackish water *Peridiniopsis* species

	P. minima[a]	*P. minima*[b]	*P. salina*[c]	*P. cristata* var. *cristata*[d,e,f,g]	*P. cristata* var. *boliviensis*[h]	*P. cristata* var. *tubulifera*[h]
Cell shape	oval to rhombic	pentagonal to rhombic	oval to spherical	pyriform	ovoid to subspherical	pyriform
Length (μm)	13.5–19.04	8–15	9.1–14.5	(37) 43–61	32–40	(27) 30–40
Width (μm)	12.8–17.9	6–12	7.3–12.9	29–41	30–38	26–32
Theca ornamentation	smooth	smooth	smooth	verrucose to reticulate	finely reticulated	smooth
Epitheca	broadly conical, equal to hypotheca	broadly conical, equal to hypotheca	broadly conical, equal to hypotheca	helmet-shaped, higher than hypotheca	broadly conical, equal to hypotheca	helmet-shaped, higher than hypotheca
Plate tabulation	Po, X, 3′, 1a, 6″, 5c, 5 s, 5‴, 2⁗	Po, X, 3′, 1a, 6″, 5c, 4 s?, 5‴, 2⁗	Po, X, 4′, 1a, 6″, 6c, 5 s, 5‴, 2⁗	Po, X?, 3′, 1a, 6″, 6c, 4-5 s?, 5‴, 2⁗	Po, X?, 3′, 1a, 6″, ?c, ?s, 5‴, 2⁗	Po, X, 3′, 1a, 6″, 6c, 5 s, 5‴, 2⁗
Intercalary plate	small, rhombic	small, rhombic	small, rhombic	large, hexagonal	large, hexagonal	large, hexagonal
Intercalary plate location	dorsally between 2′, 3′, 3″and 4″	dorsally between 2′, 3′, 3″and 4″	laterally between 2′, 3′, 2″and 3″	dorsally between 2′, 3′, 2″, 3″, 4″ and 5″	dorsally between 2′, 3′, 2″, 3″, 4″ and 5″	dorsally between 2′, 3′, 2″, 3″, 4″ and 5″
Apical spine	no	no	no	small dorsal membranous crest	absent or tiny dorsal membranous crest	no
Antapical spine	no	no	no	membranous crest-shaped extension	membranous crest-shaped extension	no
Eyespot	yes	yes	no	no	no	yes
Endosymbiont	yes	yes	?	?	?	?
Habitat (salinity range)	brackish waters (2-7.2)	fresh water rivers	brackish waters (2.2-16.2)	brackish waters (4.3[5]-5.7[6]; 3-7[7])	brackish waters (7.5)	brackish waters (5.7)

[a] – this study; [b] – Zhang et al. 2014; [c] – Trigueros 2000; [d] – Balech 1961; [e] – Ricard and Bourrelly 1982; [f] – Couté et al. 2012; [g] – Mendoza & Baylon 2014; [h] – Iltis and Couté 1984; ? – no information

genus *Peridiniopsis* are generally typical of freshwater environments (Bourrelly 1985) and absent from salty waters. To date, two species were reported from brackish waters so far, *Peridiniopsis salina* Trigueros (Trigueros 2000) and *Peridiniopsis cristata* (Balech) Bourrelly (Balech 1961; Couté et al. 2012; Mendoza and Baylon 2014). A comparison between Iraqi *P. minima* and other brackish water *Peridiniopsis* species is made in Table 2. In the case of *P. salina*, this small species from the Atlantic Ocean reached highest concentrations at salinities between 2.2 and 16.2 (Trigueros 2000), but it might be related to another heterotrophic genus of pfiesteriacean dinoflagellates (Moestrup, pers. comm.). The three different varieties of *P. cristata* are, however, typical of slightly brackish waters of Clipperton lagoon in the eastern Pacific Ocean (Couté et al. 2012), but also of saline lake in Bolivia (Iltis and Couté 1984) and of saline Encantada lagoon in Peru (Mendoza and Baylon 2014). *P. cristata* can tolerate salinities up to 7.5, which is remarkably similar with our observations on *P. minima* during this study. Among brackish water *Peridiniopsis* taxa, *P. minima* is clearly distinguishable from small-sized *P. salina* by thecal arrangement of the epitheca, and from *P. cristata* by smaller size, shape, and size of the anterior intercalary plate (in case of *P. cristata* var. *tubulifera* Couté, Perrette & Chomérat), and by the absence of apical and antapical crests and thecal ornamentation (in case of *P. cristata* var. *cristata* and *P. cristata* var. *boliviensis* Iltis & Couté) (Table 2).

Conclusion

The outbreaks of dinoflagellates in different aquatic environments signify a real ecological threat to these systems. Blooming of these microorganisms has been linked to anthropogenic pollution, human-mediated dispersal of species and climate changes (e.g., Hallegraeff 1993; Anderson et al. 2002). The discovery of bloom-forming dinoflagellate species in Shatt Al-Arab River system makes evident the necessity of comprehensive monitoring program of phytoplankton as microalgal blooms could be a major problem for aquatic biota and for human living in Basrah city because the river serves as drinking water supply. Therefore, more studies on the ecology and physiology of this species and other dinoflagellates are required to grasp processes that lead to their outgrowth and to decide an appropriate management plan for the river ecosystem in case such event recurs.

Abbreviations

1 - 2'''', antapical plate series; 1–3', apical plate series; 1–5''', postcingular plate series; 1–6'', precingular plate series; 1a, anterior intercalary plate; c1–c5, cingular plate series; Po, apical pore plate; Sa, anterior sulcal plate; Sd, right sulcal plate; Sl, sulcal list; Sm, median sulcal plate; Sp, posterior sulcal plate; Ss, left sulcal plate; X, canal plate

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Authors' contributions

HAH: conceived of the study, carried out the sampling design and field work, participated in the acquisition of biological data and species identification, measured the environmental parameters and helped to draft the manuscript. MS: participated in the identification of the species and LM and SEM examinations, processed the illustrations and drafted the manuscript. NC: participated in the identification of the species and SEM examinations, supporting literatures and contributed to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors are declaring that there are no competing interests.

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