Acta Oceanol. Sin., 2018, Vol. 37, No. 10, P. 28–32
DOI: 10.1007/s13131-018-1294-1
http://www.hyxb.org.cn
E-mail: hyxbe@263.net

The first record of *Pavlova pinguis* (Pavlovophyceae, Haptophyta) in China seas

ZHANG Xiaodong[1, 2], YANG Shuang[1, 2], SUN Jun[1, 2]*, QIAO Yanlong[3], WANG Jing[1, 2], LIU Haijiao[4]

[1] Tianjin Key Laboratory of Marine Resources and Chemistry, Tianjin University of Science and Technology, Tianjin 300457, China

[2] College of Marine and Environmental Sciences, Tianjin University of Science and Technology, Tianjin 300457, China

[3] Tianjin Marine Research and Consulting Centre, Tianjin 300480, China

[4] Institute of Marine Science and Technology, Shandong University, Jinan 250110, China

Received 13 July 2017; accepted 23 June 2018

Abstract

One strain of unicellular flagellated yellow-green algae was successfully isolated from the coastal area near Tianjin in the Bohai Sea in May 2015. The strain ranged from round to elongated in shape. Most of the cells possessed active motility, and some cells formed non-motile aggregation. Based on evidences from morphology, ultrastructure and molecular analysis, we identified the strain as *Pavlova pinguis* which belonged to Pavlovophyceae, Haptophyta. For Pavlovophyceae, only *Pavlova viridis* (*Diacronema viridis*) was reported in China seas prior to this study and it played an important role in aquaculture. This is the first record of *Pavlova pinguis* in Chinese waters.

Key words: *Pavlova*, Pavlovophyceae, Haptophyta, morphology, 18S rRNA gene, China seas

Citation: Zhang Xiaodong, Yang Shuang, Sun Jun, Qiao Yanlong, Wang Jing, Liu Haijiao. 2018. The first record of *Pavlova pinguis* (Pavlovophyceae, Haptophyta) in China seas. Acta Oceanologica Sinica, 37(10): 28–32, doi: 10.1007/s13131-018-1294-1

1 Introduction

The Haptophyta comprises more than 400 species which form a monophyletic group not closely related to any other algae (Edvardsen and Medlin, 2007). Two classes (Prymnesiophyceae, Pavlovophyceae) were included in Haptophyta, with many species having very important ecological significance, especially members in Prymnesiophyceae. Some well-known taxa, such as *Phaeocystis* and coccolithophores, can even cause blooms in costal or oceanic area. Coccolithophores are globally distributed and contribute up to 10% of the global phytoplankton biomass (Holligan et al., 1983; Sadeghi et al., 2012; Hagino and Young, 2015; Oviedo et al., 2015). This calcareous nanoflora plays a vital role in global carbon cycle (Sun et al., 2014).

More than 400 species in Haptophyta were classified in Prymnesiophyceae. Unlike Prymnesiophyceae, the Pavlovophyceae contains only one order (Pavlovales) and one family (Pavlovaceae) comprising four genera (*Pavlova*, *Diacronema*, *Exanthemachrysis* and *Rebecca*). Although members in Pavlovophyceae are very common in global coastal areas, only 13 species was described in Pavlovophyceae (Bendif et al., 2011). For all or most members of the Pavlovophyceae, common structural features discriminating them from the Prymnesiophyceae include the markedly anisokont nature of the heterodynamic flagella, simple arrangement of microtubular as well as fibrous roots of flagellar-haptonematal basal complex (Green and Hori, 1994). It was suggested that the Pavlovophyceae were generally representative of the primitive state and possessed some characteristics related with the ancestral haptophyte (Bendif et al., 2011).

Butcher (1952) erected the genus *Pavlova* with the description of *P. gyrans* as the type species. After that, several species were described (Green, 1973, 1975, 1976). In 2000, Edvardsen et al. (2000) transferred two species of *Pavlova* into a newly described genus *Rebecca* based on the evidence of molecular data. Besides, based on evidences of molecular analysis and ultralstructure information, Bendif et al. (2011) changed five species of *Pavlova* to the genus *Diacronema* (*Pavlova ennorea*, *Pavlova lutheri*, *Pavlova noctivaga*, *Pavlova virescens* and *Pavlova viridis*). Till now, at least four species was included in the genus *Pavlova*: *Pavlova calceolata* van der Veer 1976, *Pavlova granifera* (Mack) Green, 1973, *Pavlova gyrans* (Butcher) Green and Manton, 1970, and *Pavlova pinguis* Green, 1967. All of these species share some common features including the presence of motile and the palmelloid cell of somewhat reduced haptonema associated with two unequal flagella (Green, 1976). Phylogenetic relationship, pigments composition and ultrastructure of these species have already been described, however, some evidence showed that there were still some species undescribed (Bendif et al., 2011), indicating that diversity of this genus may be underestimated. Some species could serve as food of bivalves, crustaceans and fish since they can synthesize long chain polyunsaturated fatty acids such as docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids (Gayral, 1980; Green, 1975; Meireles et

---

Foundation item: The National Natural Science Foundation of China under contract Nos 41876134, 41676112 and 41276124; the National Natural Science Foundation of Tianjin under contract No. 17JCZDJC40000; the University Innovation Team Training Program for Tianjin under contract No. TD12-5003; the Exploration Program of Ocean with Science and Technology of Tianjin under contract No. KJXH2013-22; the Changjiang Scholar Program of Chinese Ministry of Education of China to Jun Sun.

*Corresponding author, E-mail: phytoplankton@163.com

al., 2003; Ponis et al., 2006). Hence, it is very important to study them not only because of taxonomic significance but also economic essentiality.

In May 2015, one strain of motile yellow-green algae was successfully isolated from costal area of Tianjin Harbor. Microscopic observation, ultrastructure features and genetic (18S rRNA) analysis confirmed that the strain was closely related to *Pavlova*. We identified it as *Pavlova pinguis* and named it Strain TJ-B02. This is the first record of this species in China seas.

2 Materials and methods

2.1 *Isolation and culture*

A total of 250 mL water sample was collected from coastal water near Tianjin, Bohai Sea in May 2015. Sample was brought to the lab and single cell was isolated in the lab with series dilution method and was grown in the f/2 medium (Guillard and Ryther, 1962) under a 12 h:12 h light:dark cycle at 2 500 lx with YZ21RR16/G cool-white fluorescent light tubes (Sunshine 117 Company, China) at 25°C. The vegetative cells were transferred into freshly prepared medium every two weeks. All the required chemicals were obtained from Sigma Company unless otherwise stated. Strain TJ-B02 was successful established.

2.2 *Molecular identification and phylogeny analysis*

The vegetative cells of Strain TJ-B02 were collected from exponential growing stage. Genomic DNA was extracted with a plant DNA extraction kit (Sangon, China), following the manufacturer's protocol. The primers and PCR programs amplifying SSU rRNA gene followed Medlin et al. (1988). PCR products were purified with a gel purification kit (Axygen, USA).The purified PCR products were sequenced from both directions in the Beijing Genomics Institute with the same primers as described above. The 18S rRNA gene sequence of Strain TJ-B02 has been submitted to NCBI with accession number MH916633. Closely related sequences were selected from BLAST results. Sequence alignments were run by Bioedit and phylogenetic constructions (Neighbor-joining distance trees) were performed with MEGA 5.1. Bootstrap values were based on 1 000 replicated trees.

2.3 *Morphology observation*

The vegetative cells of Strain TJ-B02 in exponential stage were collected and fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer solution (PBS, pH 7.4) for 4 h. The fixed cells were then concentrated by centrifugation at a speed of 4 000 *g* for 5 min and the concentrated cells were washed with 0.1 mol/L PBS buffer (pH 7.4) twice for 5 min before centrifugation. The cell pellet was then pre-embedded in relatively cool, liquid agar (1%) and the agar was allowed to solidify. The pre-embedded cells with agar were post-fixed with 1% osmium tetroxide solution (OsO_4) overnight after being rinsed in 0.1 mol/L PBS buffer (pH 7.4). The pre-embedded cells were then dehydrated through a gradual ethanol series (10%, 30%, 50%, 70%, 90% and 100%, 10 min at each step) followed by 100% acetone. Spurr's resin (Spurr, 1969) was applied to embed the dehydrated cells which was then sectioned. The ultrathin sections were stained with uranyl acetate and lead citrate, and examined under a HITACHI 7000 Electron Microscope (Hitachi, Japan).

3 Results

3.1 *Mophology observation*

The cells of strain TJ-B02 were yellow-green, solitary with very active motility. The shapes of the algae were variable and most of cells were oval, spherical or elongated (Figs 1a, b, c). They could also form non-motile aggregation embedded in mucilage after the exponential growth phase (Fig. 1d). Size of this strain was very small, ranging from 4 to 8 μm. Two flagella were observed clearly, arising from a concave on the side (Fig. 1c).

The ultrastructure of the strain based on TEM observation was simple. One or two parietal plasmid with parallel thylakoid lamellae in the cell was observed and a bulging pyrenoid was also

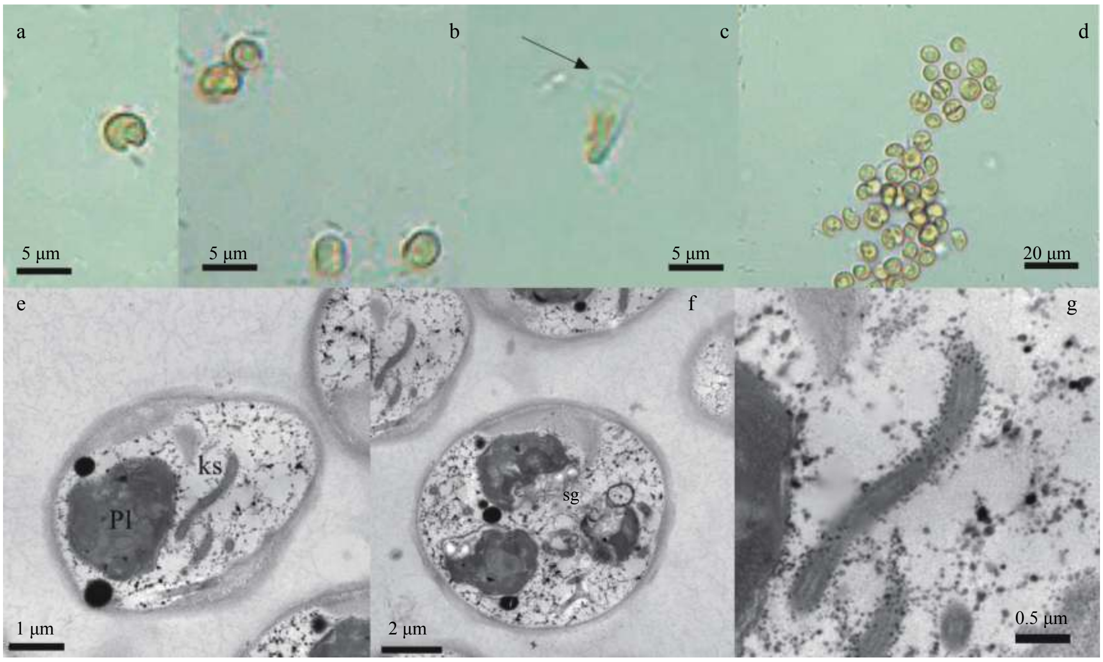

Fig. 1. Morphology and ultrastructure of Strain TJ-B02. Abbreviation ks represents knob-like scales, Pl plasmid, and sg storage granule.

present (Figs 1e, f). Haptonema was not observed in vegetative cells under light microscopic and ultrastructural observation. Knob-like scales and some storage granules were observed in the cells (Figs 1f, g), however, we did not find the eyespot no matter under light microscopic or ultrastructure observation.

3.2 *Phylogenetic analysis*

Phylogenetic trees based on SSU rRNA gene sequence (1 709 bp) were constructed using the maximum likehood method. Phylogenetic analysis confirmed that the isolated Strain TJ-B02 was closely related with *P. pinguis*. The results of phylogeny showed that these species in Pavlovophyceae formed paraphyletic groups with five clades in the phylogeny. *Pavlova pinguis* sequences formed a single clade suggesting its far phylogenetic relationship with other species (Fig. 2). Five species of *Diacronema* (*Diacronema ennorea*, *Diacronema lutheri*, *Diacronema virescens*, *Diacronema viridis* and *Diacronema vlkianum*) grouped together within one clade which is the sister group with *P. pinguis*. *Pavlova gyrans* possessed a far relationship with other *Pavlova* species and *Diacronema*, indicating from its single lineage in the phylogenetic tree. Both genus *Rebecca* and *Exanthemachrysis* formed monophyletic clades, suggesting that these genera represented two single evolutionary lineages. This phylogenic analysis supported the classification system of class Pavlovophyceae very well.

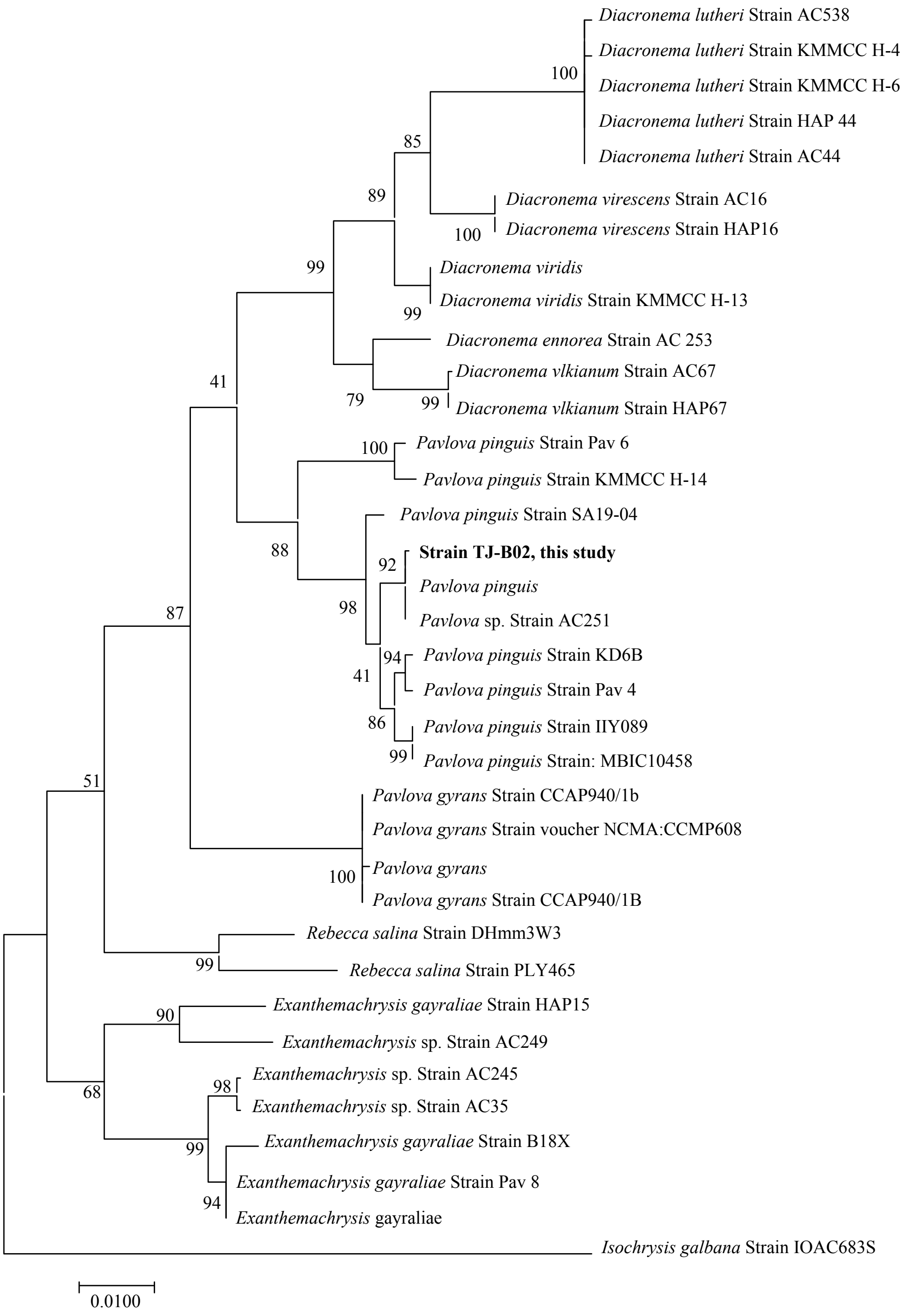

Fig. 2. A maximum likehood phylogenetic tree showing the phylogenetic associations of *Pavlova pinguis* Strain TJ-B02 (highlighted in bold) with closely related sequences based on the 18S rRNA gene sequences. Bootstrap numbers were based on 1 000 replicated trees.

4 Discussion

The common structural feature of Haptophyta is the haptonema located between the flagella (Edvardsen et al., 2000). Traditionally, the haptophytes can be envisioned as a single class Prymnesiophyceae Hibberd or Haptophyceae Christensen ex Silva (Hibberd, 1976; Christensen, 1980; Chrétiennot-Dinet et al., 1993; Jordan and Green, 1994). Two subclasses Prymnesiophycidae and Pavlovophycidae (Cavalier-Smith, 1986, 1989; Jordan and Green, 1994) were included within this class until Cavalier-Smith (1993) revised the classification system and raised them to the class Patelliferea and Pavlovea respectively. Then Cavalier-Smith renamed Pavlovea to Pavlovophyceae and changed the definition of Prymnesiophyceae to include two subclasses: Flavoretophycidae and Prymnesiophycidae which is a synonym of Patelliferea (Cavalier-Smith et al., 1996). Multiple evidences including molecular analysis and ultrastructural information all confirmed this classification. Cavalier-Smith et al. (1996) indicated that the amount of divergence in the rRNA gene differentiating the two classes of haptophytes is up to 6%, which is the equal amount found in class-level divisions of other algae groups. Some structural features including the markedly anisokont nature of the heterodynamic flagella, the relatively simple arrangement of microtubular and fibrous roots of the flagellar-haptonematal basal complex could distinguish Pavlovophyceae from Prymnesiophyceae (Green and Hori, 1994). Besides, characteristics of swimming movement and scales are also different between the two classes. For Pavlovophyceae the scales-knob scales, if present, locate on the flagellum (Bendif et al., 2011). The knob scales are actually knob like dense bodies (Edvardsen et al., 2000) which are different from the calcareous scales (coccoliths) of Prymnesiophyceae. According to molecular clock, divergence of the Pavlovaceae from the Prymnesiophyceae occurred in pre-Cambrian or Early Palaeozoic, (ca. 800 Ma) (Medlin et al., 2008). Pavlovophyceae are generally perceived to possess some primitive characteristics likely to be related to ancestral haptophyte (Bendif et al., 2011) which is very important for the research on evolution of haptophyte.

All of these species share some common features including the presence of motile and the palmelloid cell of somewhat reduced haptonema associated with two unequal flagella (Green, 1976). Pigment type and morphological differences between species of this genus were summarized by Bendif et al. (2011) including habitat, cell size, length of flagellum and haptonema, morphology of plasmid and so on. However, it is difficult to distinguish various species in this genus without enough morphological and phylogenetic information, especially *Pavlova gyrans* and *Pavlova pinguis*. Bendif et al. (2011) concluded that the *Pavlova gyrans* and *Pavlova pinguis* were identical to each other and possible formed a single cryptic species. Another possibility is that *Pavlova gyrans* and *Pavlova pinguis* could be different forms within a common life cycle of one species. However, our phylogenetic tree differentiated these two species very well (Fig. 2), suggesting they are completely different species.

Although Pavlovophyceae is a very common component of costal phytoplankton communities with widespread distributions in the world (Bendif et al., 2011), little is known about the diversity of Pavlovophyceae in China seas. Chen et al. (1985) reported one strain of yellowish green algae with flagella isolated from the coastal area of Haiyang County, Shandong Province in March 1982 and they identified it as *Pavlova lutheri* (Chen et al., 1985). However, Tseng et al. (1992) reexamined the strain and classified it as a newly described species *Pavlova viridis*. Based on evidences of molecular analysis and ultralstructure information, Bendif et al. (2011) changed both *Pavlova lutheri* and *Pavlova viridis* to the genus *Diacronema*. No other species of Pavlovophyceae was recorded in China seas till now. This study was the first report of the species *Pavlova pinguis* and second record of class Pavlovophyceae in China seas.

Due to the ability to synthesize long chain polyunsaturated fatty acides, most of previous study on *Pavlova* in China focused on the optimal culture conditions, the value for food in aquaculture and effects of feeding, especially for *Pavlova viridis* (Chen et al., 1985; Lu and Zhang, 1994; Liu, 1995; Liu and Liang, 1999; Zhao and Sun, 2004). Further study was needed to explore the value of *Pavlova pinguis* in aquaculture of China although there were some reports about the nutrient value of this species (Brown et al., 1998, 1999; McCausland et al., 1999; Ponis et al., 2006). Besides, Bendif et al. (2011) showed that the diversity within the Pavlovophyceae may be much higher than previously thought based on the molecular genetic evidence, although this group is a relatively very small group compared to Prymnesiophyceae and other microalgal classes. Deep research on species diversity of Pavlovophyceae combing multiple methods is very necessary for the research of this group in future.

References

Bendif E M, Probert I, Hervé A, et al. 2011. Integrative taxonomy of the Pavlovophyceae (Haptophyta): a reassessment. Protist, 162(5): 738–761, doi: 10.1016/j.protis.2011.05.001

Brown M R, McCausland M A, Kowalski K. 1998. The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. Aquaculture, 165(3–4): 281–293, doi: 10.1016/S0044-8486(98)00256-7

Brown M R, Mular M, Miller I, et al. 1999. The vitamin content of microalgae used in aquaculture. Journal of Applied Phycology, 11(3): 247–255, doi: 10.1023/A:1008075903578

Butcher R W. 1952. Contributions to our knowledge of the smaller marine algae. Journal of the Marine Biological Association of the United Kingdom, 31(1): 175–191, doi: 10.1017/S0025315400003751

Cavalier-Smith T. 1986. The Kingdom Chromista: origin and systematics. Progress in Phycological Research, 4: 309–347

Cavalier-Smith T. 1989. The Kingdom Chromista. In: The Chromophyte algae: Problems and Perspectives. Oxford: Clarendon, 381–407

Cavalier-Smith T. 1993. Kingdom Protozoa and its 18 phyla. Microbiological Reviews, 57(4): 953–994

Cavalier-Smith T, Allsopp M T E P, Häuber M M, et al. 1996. Chromobiote phylogeny: the enigmatic alga *Reticulosphaera japonensis* is an aberrant haptophyte, not a heterokont. European Journal of Phycology, 31(3): 255–263, doi: 10.1080/09670269600651461

Chen Jiaofen, He Yichao, Tan Guiying, et al. 1985. Two newly isolated marine chrysophyta and its food values to *Mytilus edulis* larva. Transaction of Oceanology and Limnology (in Chinese), (2): 44–46

Chrétiennot-Dinet M J, Sournia A, Ricard M, et al. 1993. A classification of the marine phytoplankton of the world from class to genus. Phycologia, 32(3): 159–179, doi: 10.2216/i0031-8884-32-3-159.1

Christensen T A. 1980. Algae. A Taxonomic Survey. Odense: AiO Trykas, 216

Edvardsen B, Eikrem W, Green J C, et al. 2000. Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data. Phycologia, 39(1): 19–35, doi: 10.2216/i0031-8884-39-1-19.1

Edvardsen B, Medlin L K. 2007. Molecular systematics of Haptophyta. In: Lewis J, Brodie J, eds. Unravelling the Algae–the Past, Present and Future of Algal Molecular Systematics. Boca Raton, Florida: CRC Press, 183–196

Gayral P. 1980. Particularités et intérêt subséquent de la famille des Pavlovophycées (Chrysophycophytes, Prymnesiophycées). 105e Congrès national des Sociétés savantes III: 201–212

Green J C. 1973. Studies in the fine structure and taxonomy of flagellates in the genus Pavlova: II. A freshwater representative, *Pavlova granifera* (Mack) comb. nov. British Phycological Journal, 8(1): 1–12, doi: 10.1080/00071617300650011

Green J C. 1975. The fine-structure and taxonomy of the haptophycean flagellate *Pavlova lutheri* (Droop) comb. nov. (= *Monochrysis lutheri* Droop). Journal of the Marine Biological Association of the United Kingdom, 55(4): 785–793, doi: 10.1017/S0025315400017707

Green J C. 1976. Notes on the flagellar apparatus and taxonomy of *Pavlova mesolychnon* Van der Veer, and on the status of *Pavlova* Butcher and related genera within the Haptophyceae. Journal of the Marine Biological Association of the United Kingdom, 56(3): 595–602, doi: 10.1017/S0025315400020671

Green J C, Hori T. 1994. Flagella and flagellar roots. In: Green J C, Leadbeater B S C, eds. The Haptophyte Algae. The Systematics Association Special Volume 51. Oxford: Oxford University Press, 47–71

Guillard R R L, Ryther J H. 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (CLEVE) Gran. Canadian Journal of Microbiology, 8(2): 229–239, doi: 10.1139/m62-029

Hagino K, Young J R. 2015. Biology and paleontology of Coccolithophores (Haptophytes). In: Ohtsuka S, Suzaki T, Horiguchi T, et al, eds. Marine Protists. Tokyo: Springer, 311–330

Hibberd D J. 1976. The ultrastructure and taxonomy of the Chrysophyceae and Prymnesiophyceae (Haptophyceae): a survey with some new observations on the ultrastructure of the Chrysophyceae. Botanical Journal of the Linnean Society, 72(2): 55–80, doi: 10.1111/boj.1976.72.issue-2

Holligan P M, Viollier M, Harbour D S, et al. 1983. Satellite and ship studies of coccolithophore production along a continental shelf edge. Nature, 304(5924): 339–342, doi: 10.1038/304339a0

Jordan R W, Kleijne A. 1994. A classification system for living coccolithophores. In: Winter A, Siesser W G, eds. Coccolithophores. Cambridge: Cambridge University Press, 83–105

Liu Dongchao, Liang Feilong. 1999. The feeding effects of *Pavlova viridis* to *Brachionus plicatilis*. Journal of Fujian Fisheries (in Chinese), (1): 9–13

Liu Luwei. 1995. Studies on nutrient requirements of *Pavlova viridis* and its productive culture. Journal of Hebei Fisheries (in Chinese), (5): 3–7

Lu Jun, Zhang Daonan. 1994. *Pavlova viridis* as bait for artificial propagation of *Macrobrachium rosenbergii* in imitating ecological system. Journal of Shanghai Fisheries University (in Chinese), 3(1): 63–68

McCausland M A, Brown M R, Barrett S M, et al. 1999. Evaluation of live microalgae and microalgal pastes as supplementary food for juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture, 174(3–4): 323–342, doi: 10.1016/S0044-8486(99)00018-6

Medlin L, Elwood H J, Stickel S, et al. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene, 71(2): 491–499, doi: 10.1016/0378-1119(88)90066-2

Medlin L K, Sáez A G, Young J R. 2008. A molecular clock for coccolithophores and implications for selectivity of phytoplankton extinctions across the K/T boundary. Marine Micropaleontology, 67(1–2): 69–86, doi: 10.1016/j.marmicro.2007.08.007

Meireles L A, Guedes A C, Malcata F X. 2003. Lipid class composition of the microalga *Pavlova lutheri*: Eicosapentaenoic and docosahexaenoic acids. Journal of Agricultural and Food Chemistry, 51(8): 2237–2241, doi: 10.1021/jf025952y

Oviedo A, Ziveri P, Álvarez M, et al. 2015. Is coccolithophore distribution in the Mediterranean Sea related to seawater carbonate chemistry?. Ocean Science, 11(1): 13–32, doi: 10.5194/os-11-13-2015

Ponis E, Probert I, Véron B, et al. 2006. Nutritional value of six Pavlovophyceae for *Crassostrea gigas* and *Pecten maximus* larvae. Aquaculture, 254(1–4): 544–553, doi: 10.1016/j.aquaculture.2005.11.017

Sadeghi A, Dinter T, Vountas M, et al. 2012. Remote sensing of coccolithophore blooms in selected oceanic regions using the PhytoDOAS method applied to hyper-spectral satellite data. Biogeosciences, 9(6): 2127–2143, doi: 10.5194/bg-9-2127-2012

Sun Jun, Gu Xiaoyao, Feng Yuanyuan, et al. 2014. Summer and winter living coccolithophores in the Yellow Sea and the East China Sea. Biogeosciences, 11(3): 779–806, doi: 10.5194/bg-11-779-2014

Spurr A R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research, 26(1–2): 31–43, doi: 10.1016/S0022-5320(69)90033-1

Tseng C K, Chen Jiaofen, Zhang Zhefu. 1992. On a new species of *Pavlova* (Prymnesiophyceae) from China. Chinese Journal of Oceanology and Limnology, 10(1): 23–30, doi: 10.1007/BF02844296

Zhao Sufen, Sun Huiqiang. 2004. Studies on the ecological factors to the growth of Pavlova viridis. Fisheries Science (in Chinese), 23(2): 9–11