

MORPHOLOGY OF A NEW *HETEROCAPSA* SPECIES (PERIDINIALES, DINOPHYCEAE) OCCURRED IN HUE, VIETNAM

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ABSTRACT

To investigate the distribution of *Heterocapsa* including a harmful species *H. circularisquama*, cells were surveyed in three Vietnamese coasts, Ha Long Bay, Hue and Phu Quoc Island in 2006. Cells of *Heterocapsa* were detected from Lang Co Lagoon and adjacent coast in Hue. Cell of the species possesses thecal plates corresponding to the typical of *Heterocapsa*. Under TEM, body scales approximately 500 nm in diameter are observed. The scale structure is similar to that of *H. illdefina*, however, number of the marginal spine is different. We concluded it is an undescribed *Heterocapsa* species possessing a new body scale ultrastructure.

Keywords: Body scale, Dinoflagellate, *Heterocapsa*, Morphology, Thecal plates

INTRODUCTION

An armored dinoflagellate *Heterocapsa circularisquama* Horiguchi is one of the most harmful red tide forming species, which specifically affects shellfishes such as oysters and pearl oysters (Horiguchi, 1995; Matsuyama, 1999). This species was first found in 1988 at Uranouchi Inlet, Kochi Prefecture, Japan and subsequently its distribution has spread along the coasts of western Japan involving mass mortalities of shellfish cultures (Matsuyama *et al.*, 1995, 1997; Matsuyama, 1999; Nagai *et al.*, 2000). Since such a species of which red tide specifically affected on the shellfish have never been reported, detailed morphology of the species was investigated and described as a new species *H. circularisquama* based especially on the shape of the body scale with the circular basal plate (Horiguchi, 1995). Considering the abrupt occurrence in Japanese coasts and its relatively higher optimum temperature for growth (30°C, Yamaguchi *et al.*, 1997), this species was supposed to be distributed also in the subtropical to tropical areas (Honjo *et al.*, 1998, Iwataki *et al.*, 2002b). The presence of this species has been actually detected from

preserved samples of red tides occurred at Hong Kong in 1986-1987 (Iwataki *et al.*, 2002b), prior to the first report in Japan. Nevertheless, there is scarce information on distribution of *H. circularisquama* in the East and Southeast Asian coasts.

Presence of *Heterocapsa* species including a harmful red tide forming species *H. circularisquama* was surveyed along the coast of Vietnam in 2006. As a result of samplings from three Vietnamese coasts, Ha Long Bay, Hue and Phu Quoc Island, cells of *Heterocapsa* were found from two sampling stations in Hue and culture strains were established. For identification of the *Heterocapsa* species, observation using light, fluorescence and electron microscopy were carried out. In the present paper, cellular and body scale morphologies of this *Heterocapsa* species are shown, and the taxonomic position is discussed.

MATERIAL AND METHODS

Survey of *Heterocapsa* species was performed in three locations in Vietnam, Ha Long Bay, Hue and Phu Quoc Island during 25 June to 2 July 2006 (Fig. 1). Of these locations, cells of

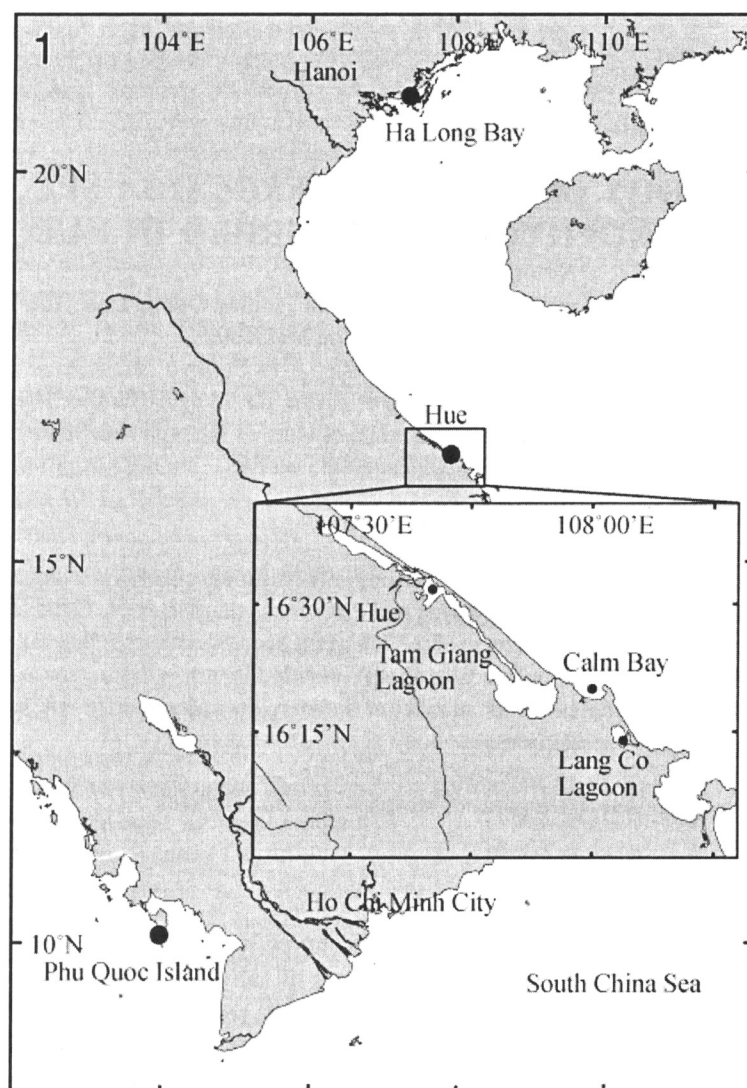


Figure 1. Sampling locations in Vietnam. Cells of *Heterocapsa* sp. were found from Lang Co Lagoon and Calm Bay near Hue City.

Heterocapsa could be detected from two stations in Hue, namely, Lang Co Lagoon and Calm Bay on 26 June 2006. Seawater and plankton net samples were transferred to laboratory and isolated for culture strain establishment. Cultures of *Heterocapsa* were maintained using ESM medium (Watanabe *et al.*, 2000), under condition of 25°C with normal cool fluorescent tube irradiance.

Cells of *Heterocapsa* were observed under a light microscope (Olympus BX51, Tokyo, Japan) equipped with a differential interference contrast device. Shape and position of chloroplasts were observed by their autofluorescence under a blue excitation of epifluorescence of the same microscope. For thecal plate observation, cells

were stained with Fluorescent Brightener 28 (Sigma, St Louis, MO, USA) according to Fritz and Triemer (1985), and observed with a filter arrangement for violet excitation. Photographs were taken by an Olympus DP50 digital camera.

For scanning electron microscopy (SEM), a drop of suspended cells was fixed with the same volume of 4% osmium tetroxide for 30 min on a poly-L-lysine-coated glass plate. Cells were dehydrated through an ethanol series and finally dried in a Hitachi HCP-2 critical point drier. They were coated with platinum-palladium and observed under a JEOL JSM-6390 (Tokyo, Japan) scanning electron microscope at 10 kV accelerating voltage.

For body scale observation, whole mounts for transmission electron microscopy (TEM) were

prepared according to Iwataki *et al.* (2002a) or Hansen (1989). Fixed cells with 2.5% glutaraldehyde were placed on a Formvar-coated mesh grid, stained with 2% aqueous uranyl acetate for 1.5 min, then rinsed with distilled water three times. The microscope used was a JEOL JEM-1010 operated at an accelerating voltage of 80 kV.

RESULTS

Among three sampling locations conducted in Vietnam, *Heterocapsa* species could be found only from coastal seawaters of Hue (Fig. 1). Cells of *Heterocapsa* were collected in Lang Co Lagoon and Calm Bay in Hue. *Heterocapsa* and similar armored dinoflagellates were not detected from other locations in Vietnam during this investigation.

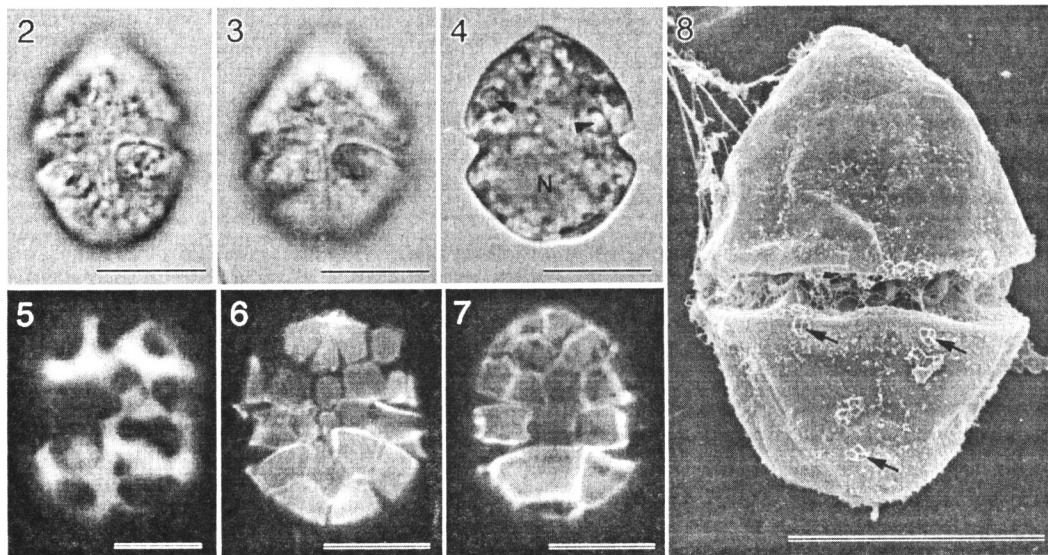
Motile cells of *Heterocapsa* collected at Hue, Vietnam are ellipsoid with the conical epitheca almost same to the hemispherical hypotheca in size (Figs 2, 3), approximately 15–18 μm in length. The cingulum is located on the equator of the cell, displaced about 0.5–1 times its own width on the ventral side (Figs 2, 3). The sulcus is relatively wide, particularly towards the antapex. A large dinokaryotic nucleus is located in the hypotheca (Fig. 4). Several amorphous pyrenoids, surrounded by starch sheaths, are situated above the nucleus,

usually near the cingulum (Fig. 4). The chloroplast is reticulated and located along the periphery of the cell (Fig. 5). Thecal plate arrangement of this species is Po, cp, 5', 3a, 7'', 6c, 5s (as, rs, las, lps, ps), 5''', 2'''' (Figs 6, 7), which is congruent with the typical thecal plate number of the genus *Heterocapsa*. Under scanning electron microscopy, numbers of small body scale triangular in outline, were observed on the cell surface (Fig. 8).

The body scale of this *Heterocapsa* species has a triangular basal plate, a central spine and six peripheral spines (Fig. 9). No intraspecific variation in scale ultrastructure was observed. The scale morphology of this *Heterocapsa* strongly resembles that of *H. illdefina*, but the number of peripheral spines is different. Body scales of this *Heterocapsa* possess two peripheral spines on each triangular end of the basal plate, while those of *H. illdefina* have three of each.

DISCUSSION

An armored dinoflagellate collected in Lang Co Lagoon and the adjacent coast has a typical thecal plate arrangement of the genus *Heterocapsa*; Po, cp, 5', 3a, 7'', 6c, 5s, 5''', 2'''' . Nucleus of this species is located in the hypotheca



Figures 2-8. Light, fluorescence and scanning electron microscopy of *Heterocapsa* sp. collected from Hue, Vietnam. Figs 2-3. Cells of *Heterocapsa*, ventral view. Figure 4. Optical sections of a living cell showing a dinokaryotic nucleus (N) located in the hypotheca and pyrenoids (arrowheads) positioned above the nucleus. Figure 5. Autofluorescence of reticulate chloroplasts peripherally located under fluorescence microscope. Figure 6. Stained thecal plates, ventral view. Figure 7. Thecal plates, dorsal view. Figure 8. SEM, dorsal view, showing small body scales located on the cell surface (arrows). Scale bars = 10 μm .

of the cell and this characteristic has so far been reported from five *Heterocapsa* species, *H. minima*, *H. niei*, *H. orientalis*, *H. psammophila* and *H. pygmaea* (Loeblich III, 1968; Loeblich III *et al.*, 1981; Pomroy, 1989, Iwataki *et al.*, 2003; Tamura *et al.*, 2006). Most of these species have the pyrenoid immediately above the nucleus, usually single and located in the axile of the cell. Of these five species, *H. pygmaea* has cellular morphological characters corresponding to *Heterocapsa* from Hue due to having several numbers of pyrenoid near the cingulum as well as the cell size.

Since the cellular morphology of *Heterocapsa* species strongly resemble each other and sometimes variable, body scale ultrastructure is mainly used for certain species identification (e.g. Iwataki *et al.*, 2004). Fine structures of body scale have been reported from thirteen *Heterocapsa* species until now (Pennick and Clarke, 1977; Morrill and Loeblich III, 1981, 1983; Hansen, 1989; Horiguchi, 1995, 1997; Iwataki *et al.*, 2002a, 2003, 2004; Tamura *et al.*, 2006), by which almost all species can be distinguished. Body scale ultrastructure of this *Heterocapsa* species collected from Hue is ordinary of the genus, namely composed of a triradiate basal plate with fine reticulation and three-dimensional ornamentation. Because of the triangular basal plate, body scale structure is rather different from that of *H. circularisquama* composed of a circular basal plate. Compared to scale structures of other *Heterocapsa* species previously reported, that of *H. illdefina* has a close resemblance to this species especially in the shape of the basal plate.

However, the number of the marginal spines is dissimilar between these two species, i.e. scale of the species collected Hue has two spines on each rounded corner of the triangle while that of *H. illdefina* has three of each (Iwataki *et al.* 2004).

In consequence of the morphological comparison, *Heterocapsa* species occurred in Hue, Vietnam has cellular characteristics congruent with *H. pygmaea*, and the body scale similar to that of *H. illdefina* but incongruent to any of *Heterocapsa* body scale structures previously reported. We concluded that the armored dinoflagellate occurred in the lagoon and adjacent coastal waters are an undescribed *Heterocapsa* species. Presence of the *Heterocapsa* species has been confirmed at least since 2005 in Lang Co Lagoon. The present morphological examination revealed that the species is not a famous harmful red tide species *H. circularisquama* but an undescribed species newly found in the lagoon of Hue, Vietnam, which should be described after the detailed morphological observation and molecular comparison especially to similar species, *H. illdefina* and *H. pygmaea*.

Acknowledgements. We thank Dr. Dang Diem Hong of Vietnamese Academy of Science and Technology, Ms Dao Viet Ha of National Institute of Oceanography, and Le Thanh Tung of Research Institute for Marine Fisheries, Vietnam, for their assistance with the sampling carried out at the Vietnamese coasts. Dr. Toshifumi Yamatogi of Nagasaki Prefectural Institute of Fisheries, Japan, provided TEM facilities for body scale observation.

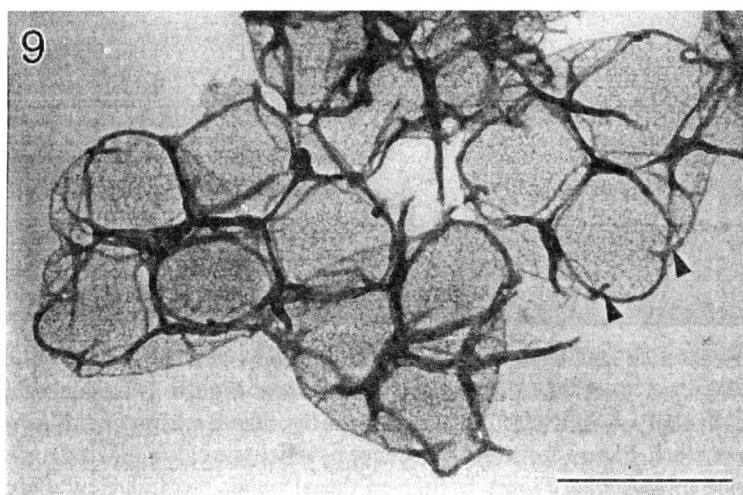


Figure 9. Transmission electron microscopy, whole mount preparation of body scales. Arrowheads indicate two spines located in triangular end. Scale bar = 400 nm

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