

Taxonomic study on *Heterocapsa* with special reference to their body scale ultrastructure

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INTRODUCTION

The genus *Heterocapsa* is an armored dinoflagellate often observed worldwide in coastal waters. It has been characterized by the thecal plate arrangement; Po, cp, 5', 3a, 7'', 6c, 5s, 5''', 2'''. This genus is also known to possess organic body scales on the cell surface, the fine structure of which is expected as the main identifying character for *Heterocapsa* species. Indeed the fine structure of the body scale was mentioned in taxonomic description of some species^{1,2)}. Among 10 *Heterocapsa* species, body scales of 7 species have been observed so far, 4 of which were reported in detail.

Several unknown species of this genus were confirmed during the red tide monitoring of *H. circularisquama*, a causative species associated with mass mortality of bivalves in the western Japan. We investigated the body scale fine structure as well as cellular morphology and thecal plate arrangement to 1) clarify the taxonomic positions of these unknown *Heterocapsa* species, and 2) evaluate the utility of the body scale ultrastructure as a taxonomic criterion at species level.

MATERIALS AND METHODS

Unialgal culture strains used in the present study were collected from coastal waters at Iwate, Niigata, Tokyo, Mie, Hiroshima, and Nagasaki Prefectures during 1998 - 2001. Culture strains were also obtained from the following culture collections; CCCM (Canada), CCMP (USA), NIES (Japan), Plymouth Culture Collection (United Kingdom) and SCCAP (Denmark). All unialgal strains were maintained in ESM medium.

Observations were made by light, fluorescence and transmission electron microscopy. Thecal plates were stained with Fluorescent Brightener 28 (Sigma) and observed under an Olympus BX-60 fluorescence microscope. For observations of body scale ultrastructure, whole mounts were prepared as follows: A drop of

suspended cells was put on the Formvar-coated grid, and fixed using OsO₄ vapor for 30 sec. It was allowed to dry, and then rinsed three times with distilled water to remove salt crystals. Subsequently cells were stained with 2% aqueous uranyl acetate for 90 sec, and rinsed again with distilled water. Body scales were observed under a JEOL JEM 1010 transmission electron microscope.

RESULTS

Cells of *Heterocapsa* species were generally less than 35 μm and surrounded by thin thecal plates, the arrangement of which is difficult to determine by light microscopy. Only the size, shape, and positions of nucleus and pyrenoid could be observed at the light microscopic level. Using these characters, eleven morphotypes attributed to the genus *Heterocapsa* were differentiated. These morphotypes consisted of seven previously known species, viz. *H. arctica*, *H. circularisquama*, *H. illedefina*, *H. niei*, *H. pygmaea*, *H. rotundata*, *H. triquetra*, and four undescribed ones. These species have a spherical or ellipsoidal pyrenoid surrounded by starch sheaths, and a chloroplast connected with the pyrenoid.

Thecal plate arrangements of all morphotypes were determined as Po, cp, 5', 3a, 7'', 6c, 5s, 5''', 2'''. This plate arrangement seemed to be a stable character in the genus, despite some intraspecific variations due to the culture conditions.

The organic body scales were found on the surface of the plasma membrane of all specimens. Body scales of *Heterocapsa* were basically composed of a reticular basal plate and spine-like ornamentations. It exhibited triradial symmetry and superficially resembled each other. However, detailed observation revealed differences in the size of basal plate, presence or absence of the central hole, and number of the uprights and bars. These detailed structures were stable in each specimen. The body scale structures of described species that we observed were consistent with

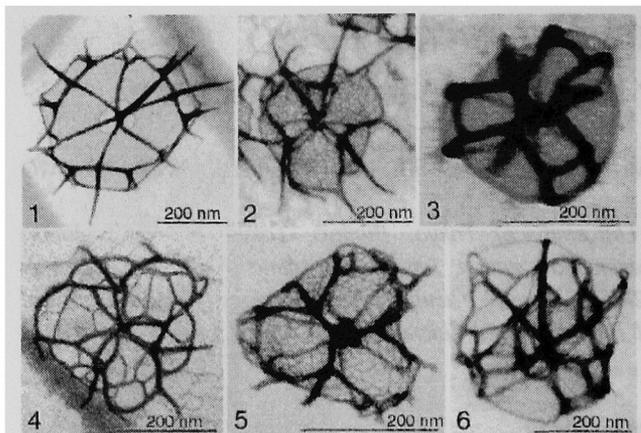


Fig. 1-6. Body scales of undescribed *Heterocapsa* species collected from coastal Japan.

- Fig. 1: *Heterocapsa* sp. 1 from Tokyo Bay, Tokyo.
 Fig. 2: *Heterocapsa* sp. 2 from Fukuyama, Hiroshima.
 Fig. 3: *Heterocapsa* sp. 3 from Kashiwazaki, Niigata.
 Fig. 4: *Heterocapsa* sp. 4 from Kashiwazaki, Niigata.
 Fig. 5: *Heterocapsa* sp. 5 from Tsushima, Nagasaki.
 Fig. 6: *Heterocapsa* sp. 6 from Miyako Bay, Iwate.

previous reports. TEM studies of four unknown morphotypes showed six different types of body scales (Figs 1-6). Fine structures of these six body scales differed from the seven types previously reported. We have tentatively assigned these six scale types to undescribed species of *Heterocapsa* (Figs 1-6).

DISCUSSION

To identify *Heterocapsa* species, cell size, shape and tubular invaginations in pyrenoid matrix, as well as the thecal plate arrangement have been used. In recent years, also body scale ultrastructure has been used as a taxonomic character at species level.^{1,2)} Since the first finding of body scales in *H. triquetra*,³⁾ six others, viz. *H. niei*,^{4, 5)} *H. pygmaea*,⁴⁾ *H. illdefina*,⁴⁾ *H. rotundata*,¹⁾ *H. circularisquama*²⁾ and *H. arctica*⁶⁾ have been reported so far.

Under the light microscope, *H. arctica*, *H. rotundata*, *Heterocapsa* sp.1 and *H. triquetra* could be easily identified from their distinctive cell shape and size. The former three species possess the epitheca obviously larger than the hypotheca. Among them, cells of *H. arctica* are elongated, *H. rotundata* are relatively small (ca. 10 μm in length) and *Heterocapsa* sp.1 has a pointed antapex. *H. triquetra* has conical epitheca and hypotheca. *H. niei*, *H. pygmaea* and *Heterocapsa* sp.6 have the nucleus located in the hypotheca, which differentiates these species from other *Heterocapsa* species. Using the position of this organelle together with cellular morphology, *Heterocapsa* sp.6 is easily identified. On the contrary the other six species, *H. circularisquama*, *H. illdefina*, *Heterocapsa* sp.2, sp.3, sp.4 and sp.5 possess a hemispherical epitheca and hypotheca of almost the same in

size. Considering their cell size, the pairs of *H. circularisquama* and *H. illdefina*, *Heterocapsa* sp.2 and sp.4, *Heterocapsa* sp.3 and sp.5 are practically impossible to differentiate from each other by light microscopical observations of motile cells. Cell shape thus seems to be a useful specific character for some *Heterocapsa* species.

Each culture strain exhibits some variations in thecal plate arrangement. But the typical arrangement is common in all the strains, i.e. Po, cp, 5', 3a, 7'', 6c, 5s, 5''', 2'''. The plate arrangement is a common feature of the genus, and cannot be used for species identification.

The body scales have a triaxial symmetry and are more or less triangular, circular or hexagonal in outline. Possessing these scales seem to be a significant characteristic of the genus. Moreover, the fine structure of the scale varies among species. It means that the structure is a useful feature for specific identification. Six new *Heterocapsa* species are distinguishable based on the fine structure of scales. Especially this character can be applied to morphological similar species, such as *H. circularisquama* and *H. illdefina*, and *Heterocapsa* sp.3 and sp.5. Since *Heterocapsa* sp.2 and sp.4 are distinguished only by fineness of the basal plate texture of scale, further information will be required to prove their speciation. In *H. triquetra* and *Heterocapsa* sp.5, their distinctive cell shapes indicate the speciation, whereas their body scale ultrastructure is almost identical. This may imply a phylogenetic affinity between them. In spite of all these exceptions, the scale ultrastructure is still utilized as a character for species identification. At least five new species were confirmed from Japanese coastal waters during this study.

REFERENCES

- Hansen G. Ultrastructure and morphogenesis of scales in *Katodinium rotundatum* (Lohmann) Loeblich (Dinophyceae). *Phycologia* 1989; 28: 385-394.
- Horiguchi T. *Heterocapsa circularisquama* sp. nov. (Peridinales, Dinophyceae): A new marine dinoflagellate causing mass mortality of bivalves in Japan. *Phycol. Res.* 1995; 43: 129-136.
- Pennick NC, Clarke KJ. The occurrence of scales in the peridinium dinoflagellate *Heterocapsa triquetra* (Ehrenb.) Stein. *Br. Phycol. J* 1977; 12: 63-66.
- Morrill LC, Loeblich AR III. A survey for body scales in dinoflagellates and a revision of *Cachonina* and *Heterocapsa* (Pyrrophyta). *J. Plankton. Res.* 1981; 3: 53-65.
- Morrill LC, Loeblich AR III. Formation and release of body scales in the dinoflagellate genus *Heterocapsa*. *J. Mar. Biol. Ass. UK* 1983; 63: 905-913.
- Horiguchi T. *Heterocapsa arctica* sp. nov. (Peridinales, Dinophyceae), a new marine dinoflagellate from the arctic. *Phycologia* 1997; 36: 488-491.