大阪湾における襟鞭毛虫類(Protozoa: Choanoflagellida)の電子顕微鏡学的研究(1)

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An Investigation with Electron Microscope of Marine Choanoflagellates (Protozoa: Choanoflagellida) from Osaka Bay, Japan

I. Re-investigation of Bicosta spinifera, B. minor and Crucispina cruciformis

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Abstract

The choanoflagellates, Bicosta spinifera, B. minor and Crucispina cruciformis were reexamined. In B. spinifera: (1) the lorica is longer than 55 µm, (2) the posterior spine is longer than the anterior ones, and (3) the longitudinal costae twist in "sinistrorum externe visus" and cross at the level of the costal strips of the anterior lorica chamber. In B. minor: (1) the lorica is smaller than 45 µm, (2) the posterior spine is shorter than the anterior ones, and (3) the longitudinal costae either do not twist at all or twist in "dextrorsum externe visus" and cross at the level of the costal strips of the posterior lorica chamber. The twisting of longitudinal costae itself is not the species specific characteristic of B. spinifera. The process of replication of the lorica was also observed in B. minor. There is a preordinated sequence of formation of costal strips: the costal strips of the anterior lorica chamber are formed first and followed by the posterior ones, next the anterior spines and the posterior spine at last. These costal strips in the mother protoplast are oriented in the opposite direction to the corresponding costal strips of the mother lorica. In Crucispina cruciformis, the lengths of the costal strips forming the anterior and posterior spines vary correlatively to the water density, whereas the length of the costal strip forming the heavy longitudinal costa is constant.

The collared flagellates form a well-defined group of bacterio-phagotrophic protozoa in aquatic environments (LEADBEATER & MORTON 1974). They may play an important role in the marine detritus food chain as the major bacteriofeeder which connects the bacterial biomass to the animals of the higher levels (HARA & TANOUÉ 1984, TANOUÉ & HARA 1986). In Osaka Bay, the majority of the choanoflagellate populations is made up of members of the Acanthoeicidea, microorganisms with loricae composed of siliceous costal strips (HARA unpubl.). The size and arrangement of these costal strips are usually so precise as to constitute the most effective known basis for the classification of these organisms (LEADBEATER & MANTON 1974, THOMSEN & MOESTRUP 1983, HARA & TAKAHASHI 1984).

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On the other hand, it has been shown by analysis of the materials collected from biogeographically separated areas that there are morphological and/or dimensional variations in the loricae of some particular species, such as *Bicosta spinifera* (MANTON et al. 1980), *Polyfibula caudata* (Syn. *Pleurasiga caudata*) (THOMSEN 1982) and *Parvicorbicula socialis* (HARA & TANOUÉ 1984). In order to provide more definite information about the morphological variability of a particular species, much more material collected at various seasons at a fixed location must be examined, because the geographical variability mentioned above indicate a possible temporal variability in an area where the environmental conditions vary widely within a year. Our studies are based on the long-term investigations and report the seasonal variation of lorica morphology in Osaka Bay. The studies also avail in increasing the knowledge of the geographical distribution of choanoflagellate species over the coastal waters of the western Pacific region. In this part of the studies we examined species of *Bicosta* and of *Crucispina*.

**Materials and Methods**

Surface seawater samples were collected by polyethylene bottles (1,000 ml) from the Shioya Coast of Osaka Bay, which faces to the inland sea, Setonaikai. Samples were taken weekly from 26 April 1979 to 17 January 1981, and then monthly through August 1982. Environmental factors varied throughout the sampling period: 7.5-27.3°C for water temperature; 8.1-8.9 for pH; and 30-33 ppt for salinity. The seawater sample was brought to the laboratory within 1 hr of collection. An aliquot of the sample was immediately concentrated by centrifugation (1,400 g, 10 min) and processed for whole mounts for a scanning and transmission electron microscopy (STEM). The whole mounts were prepared by transferring a small drop of the concentrated material onto carbon-collodion coated grids. Samples were fixed in osmium tetroxide vapour and then dried. The grids were rinsed with distilled water three times, shadowed with Pt-Pd alloy and examined with a JEM-100B analytical electron microscope.

The length of the lorica is meant the shortest distance from tip to tip of the anterior and posterior spines in the previous investigations (MANTON & OATES 1979). The costal structure of the lorica of Acanthoecidae, however, is easily damaged by handling of the cells during processing. For this reason, the length of the lorica is calculated as subtracting the length of costal strip connections from the sum of the lengths of the costal strips that form the anterior spine, longitudinal costa, and posterior appendage in our investigation. The length of lorica calculated by our method is larger than the value calculated by the previous method. The factor is up to 1.20 in the organisms of *Bicosta minor* of the present materials.

By our method, different values for the length of the lorica were obtained from a single lorica because individual costal strips are not always equal in length. In such cases, the mean length is used to express the length of a particular lorica. In some species, such as *Bicosta spinifera*, having anterior spines of very different lengths, the length of the lorica is defined as the largest calculated value.


Results and Discussion

*Bicosta spinifera* (Thronsden)

(Pl. I, 1–3)

*Salpingoeca spinifera* Thronsden, 1970, p. 87, fig. 1A.


The “Y” shaped lorica (Pl. I, 1), 57–66 µm in length (mean: 61 µm), is composed of only seven costal strips, arranged as two longitudinal costae and a posterior spine. No transverse costa is present. Each longitudinal costa contains three successive costal strips and the apical costal strip, 11–19 µm in length, attenuates to a pointed tip anteriorly and acts as a spine. The anterior spines are unequal in length. The lorica chamber, 13.5–17.0 µm in length (mean: 15.4 µm), is made up of the posterior pairs of the longitudinal costae, each of them are composed of two costal strips. These longitudinal costae are twisted. The spirals rise from lower right to upper left and cross at the level of the anterior costal strips to form the lorica chamber (Pl. I, 2–3). The costal strips of the anterior lorica chamber, 7.4–8.3 µm in length, are usually a little shorter than the posterior ones, which are 7.4–10.0 µm in length. The posterior spine, 18–32 µm in length (mean: 24.5 µm), attenuates to a fine point. It is longer than the anterior spines and ends in a characteristic S-shaped distal extremity.

![Graph showing seasonal occurrence](image)

Fig. 1. Seasonal occurrence of *Bicosta spinifera* (solid triangle), non-twisted (solid circle) and twisted (open circle) specimens of *B. minor*, and *Crucispina cruciformis* (open triangle). Occurrence at 20.6°C of *B. minor* on 16 October 1981 is also indicated in upper right.

The lorica structures of five specimens, collected from Osaka Bay on 12 May 1980 when the water temperature was 16.4°C (Fig. 1), were exactly identical with previous descriptions (e.g., MANTON et al. 1980). SEM observation of the specimens from Osaka Bay reveals that the spiral of the longitudinal costae invariably rises from lower right to upper left or “sinistorsum externe visus” (Pl. I, 2–3). The direction of twisting has not been mentioned previously, but the schematic drawing in Fig. 10 of REYNOLDS (1976) and the transmission electron microscopy (TEM) photograph in Fig. 28 of MANTON et al. (1980) indicate the same direction as shown...
The range of the lorica dimensions found by us corresponds to those reported by MANTON et al. (1980) for the South African population (Cape, water temperature 10°C) and the South Alaskan population (Homer, water temperature 10°C). These are the localities with the highest water temperatures recorded prior to our study. This species has been rarely encountered outside the cold-water realm and has been thought to be absent from water at temperatures above 16°C (MANTON et al. 1980). Nevertheless, the material was collected from water at 16.4°C in May, 1980, when the water temperature was increasing steadily, but was absent when the water was colder in Osaka Bay. Temperature is not the only determinative environmental factor in the distributions of B. spinifera. This species has been recorded in the sub-polar regions, off South Africa and off Japan (MANTON et al. 1980; this study).

Bicosta minor (Reynolds)  
(Pl. I, 4-13)  
Salpingoeca minor Reynolds, 1976, p. 13, fig. 11.  

The lorica, 26-41 µm in length (mean: 31 µm), is composed of seven costal strips arranged as two longitudinal costae and a posterior spine. There are no transverse costa. Each longitudinal costa contains three successive costal strips and the uppermost costal strip attenuates to a pointed tip and acts as a spine anteriorly. The two anterior spines are almost equal in length and range between 9.5-17.5 µm in length (mean: 12.7 µm). The lorica chamber, 9.3-13.1 µm in length (mean: 10.9 µm), is made up of the posterior pairs of costal strips that form the two longitudinal costae. These costae are either not twisted (Pl. I, 4, 12) or twisted (Pl. I, 5, 9). The spiral rises from lower left to upper right or "dextrorsum externe visus", and the costae cross at the level of the posterior costal strip to form the lorica chamber (Pl. I, 5-7, 9). In the two successive costal strips forming the lorica chamber, the anterior one, 4.8-6.6 µm in length (mean: 5.4 µm), is usually a little shorter than the posterior one, 5.3-7.1 µm in length (mean: 6.1 µm). The posterior spine is almost straight or simply curved and attenuates to a pointed end. The posterior spine, 7.7-13.6 µm in length (mean: 10.0 µm), is shorter than the anterior ones.

The presence of a certain twisting of the longitudinal costae has been regarded as an important specific characteristic of Bicosta spinifera, and B. minor has been attributed to a non-twisted species (MANTON et al. 1980). In Osaka Bay, however, the longitudinal costae of some small organisms which were referable to B. minor were twisted like those of B. spinifera (Fig. 2, Pl. I, 5, 9).

There was not essential difference in the size ranges of the anterior spines among B. minor and B. spinifera (Fig. 2b). On the other hand, in sizes of the lorica chamber, the small twisted organisms are identical with B. minor, but B. spinifera is a little larger than B. minor (Fig. 2c). This trend is very apparent in lengths of the posterior spines (Fig. 2d). In conclusion, the dimensional ranges of the small twisted organisms correspond to those of B. minor, and
Fig. 2. Changes in length with water temperature of (a) whole lorica, (b) anterior spine, (c) lorica chamber and (d) posterior spine of Bicosta spinifera (solid triangle), non-twisted (solid circle) and twisted (open circle) specimens of B. minor. Mean water temperature over five days before each sampling was taken.

not to B. spinifera.

The small twisted organisms have straight or simply curved posterior spines which are shorter than its two equally long anterior spines (Fig. 2, Pl. I, 4, 5). These characteristics are similar to those of the typical B. minor. The posterior spine of B. spinifera is characteristic S-shaped at its distal end and substantially longer than either of its two unequally long anterior spines (Pl. I, 1). Furthermore, the twisting modes are different in the small twisted organisms and B. spinifera. In B. spinifera, the longitudinal costae twist in the direction from lower right to upper left and cross at the level of the anterior longitudinal costal strips that form the lorica chamber (Pl. I, 1-3). In the small twisted organisms, however, the spiral rises from lower left to upper right and the longitudinal costae cross at the level of the posterior costal strips of the lorica chamber (Pl. I, 6, 7). The intersection point itself moves from the apical end (Pl. I, 9) to the posterior end (Pl. I, 5) of the posterior costal strip of the lorica chamber.

Photographs in Pl. I illustrate the variation of the mode of the twist from non-twisted to twisted states; i.e., a clearly non-twisted lorica (Pl. I, 4, 8), a lorica with a narrow posterior end of the lorica chamber (Pl. I, 12), a lorica twisted only at the posterior end of the lorica chamber (Pl. I, 5, 13) and a clearly twisted lorica (Pl. I, 9). The small twisted organisms and untwisted B. minor share common and/or continuous morphological and morphometrical characteristics. On the other hand, clear differences between the small twisted organisms and B. spinifera are observed in the twisting direction and the crossing levels of the costae. At this stage, we include the small twisted organisms within the specific category of B. minor, instead of establishing a separate taxon.

Gathering all specimens of Bicosta minor that we took from Osaka Bay, total lengths of loricae ranged from 26 to 41 µm (mean: 31 µm). The size distribution of specimens of B. minor, collected in different seasons, rules out any simple interpretation in terms of direct influence by temperature (Fig. 2). MANTON et al. (1980) reached the same conclusion from
data collected in geographically separate localities. The ratios of the anterior and posterior spine lengths to the lorica chamber length were constant among the organisms of *B. minor* with differently sized loricae, which were collected from water at various temperatures. In general, anterior spines were longer than the lorica chamber by a factor of approximately 1.2. The posterior spine was shorter than the lorica chamber by a factor of 0.9. Extremely long or short anterior and/or posterior spines were also observed occasionally.

The size is apparently uniform over different temperatures (Fig. 2). This seems to suggest that the population of *Bicosta minor* in Osaka Bay is of a single biotype with respect to size. According to MANTON et al. (1980) who used materials collected from several localities of the world, the most typical range of whole lorica length of *B. minor* was between 20-30 µm. As the lorica length which was used by MANTON et al. (1980) was meant the shortest distance from tip to tip of the anterior and posterior spines, the length calculated by their method was smaller than the value calculated by our method by the factor 0.83 in minimum for *Bicosta minor*. The range reported by MANTON et al. (1980) (20-30 µm), however, is smaller than that of our materials (26-41 µm), even if the bias of the value may be calibrated. The range of the whole lorica length, based on the specimens collected from the type locality in the Norwegian water, is 30-45 µm (REYNOLDS 1976), and then larger than our specimens. The specimens in Osaka Bay are intermediate in size range between those reported by REYNOLDS (1976) and MANTON et al. (1980). In addition to size, the existence of twisted organisms in our materials indicates that the population of *B. minor* in Osaka Bay may be included in a separate biotype to the other populations in other localities.

There is an obvious discontinuity in whole lorica length between *Bicosta spinifera* and *B. minor* (Fig. 2a). These two species have the same or the continuous range in the anterior spine length and the lorica chamber length (Fig. 2b, c). The obvious discontinuity in the whole lorica length, therefore, is ascribed to different length of the posterior spines, which is short in *B. minor* and long in *B. spinifera* (Fig. 2d).

Direct evidence on replication of the lorica is, unfortunately, scarce, but the organisms shown in Plate I (Pl. I, 8-13) illustrate the possible stages of replication in *Bicosta minor* so far encountered in this studies. It should be noted that the development of the lorica interpreted here has been pieced together from observation of shadowcast whole mounts under the electron microscope.

Arrowheads in the photo 8 in Pl. I indicate the two nascent costal strips, one as thick as the complete one but the other thinner, and neither liberated to the exterior, are still present within the cytoplasmic area. From their curved shape, we hypothesize that these two costal strips may be the strips that will be forming the lorica chamber. Because of the short and curved morphology, and the fact that the ends are obliquely cut and bent backwards (Pl. I, 9), we can guess that these two new costal strips represented are the two anterior costal strips that will form the lorica chamber. Since the three (or four) new costal strips shown in photo 10 in Pl. I are short and slightly curved, they will be the anterior two and the posterior one (or two) costal strips of the lorica chamber.
Besides these strips forming the lorica chamber, photo 11 in Pl. I depicts two long attenuated costal strips with pointed ends, which project backward from the posterior end of the lorica chamber of the mother cell. This pair of long costal strips will be of two anterior spines of the new cell. All the seven costal strips for the whole lorica have already been completely formed in the region previously shared by the mother cell (Pl. I, 12).

It is now obvious that the order of formation of costal strips is predetermined for these species. The anterior coastal strips of the lorica chamber are formed first, followed by the posterior costal strips of the lorica chamber, then anterior spines and finally the posterior spine costal strip. The preceding formation of the costal strips of the lorica chamber over the formation of spine strips was suggested also by MANTON et al. (1980) for Bicosta spinifera, the species most closely related to B. minor.

The apical ends of the newly formed costal strips of the lorica chamber in photos 9 and 11 in Pl. I point posteriorly. The attenuated tips of the newly formed anterior spines in photos 11–13 in Pl. I also point posteriorly, while the new posterior spine points anteriorly in photos 12 and 13 in Pl. I. In addition to the order of formation, the direction of the newly formed costal strips is also predetermined. As a whole, the new costal strips that are synthesized and stored in the mother protoplast are oriented in the opposite direction to the corresponding costal strips of the mother lorica (Pl. I, 11, 13).

In the previous investigations (THOMSEN 1977, LEADBEATER 1979), it has been observed in Acanthoecidae that the newly formed costal strips are released outside and are accumulated in the front of the mother protoplast before cell division. In B. minor, these costal strips may be neither released nor accumulated outside the mother protoplast before cell division (Pl. I, 11, 13).

The water temperature in Osaka Bay ranged from 8.2 to 26.2°C throughout the period investigated, and Bicosta minor was abundant and common in December and January when the water temperature ranged around 10°C. They were found, though in less abundance, at all other seasons as well (Fig. 1). This species has been found almost all over the world, wherever temperature is in a range from –1 to 26.2°C (MANTON et al. 1980, this study), and seems to occur at lower frequencies at temperature below 4°C (MANTON et al. 1980) but the most abundant at moderate temperatures.

**Crucispina cruciformis** (Leadbeater)

(Plate II)

_Salpingoeca cruciformis_ Leadbeater, 1974, p. 184, pl. Ic, d, fig. 1A.


The morphological characteristics of the organisms collected from Osaka Bay (Plate II) were basically identical to those of the Norwegian organisms (ESPELAND & THRONDSEN 1986), but some differences were found in the structure of the lorica chamber. (1) No variation in numbers of the costal strips were indicated by ESPELAND & THRONDSEN (1986), i.e. the number of strips forming the lorica chamber was 10 and that of the transverse costa was 4 in the Norwegian materials. We observed that these numbers vary respectively from 8 to 12
and from 4 to 5 in the materials collected from Osaka Bay. (2) The delicate longitudinal costae attached to the center of the costal strips forming the transverse costa have been described in Norwegian materials (ESPELAND & THRONSEN 1986). In our materials, some transverse costal strips, e.g., the strips which connect the two heavier longitudinal costae (arrowheads in Pl. II, 3-4, 6-7), have no attachment of the delicate longitudinal costae. (3) The loria chamber is basically asymmetrical in our material (Pl. II, 2, 5), while it is symmetrical in the Norwegian material (ESPELAND & THRONSEN 1986).

Despite the difference of the loria structure, the essential similarity of the loria morphology makes it justify to include the organisms collected from Osaka Bay into the specific category of *Crucispina cruciformis*, and the difference just mentioned may be of an intraspecific variation.

Figure 3 suggests that there may be a relationship between water density and the sizes of specimens of *Crucispina cruciformis*. Since the seawater density at the time of costal strip formation is probably the most determinative, the density used in Fig. 3 was the mean water density taken over five days before each sampling (the data were cited from the data pool reported by the Hyogo Prefectural Fisheries Experimental Station, which had been measured ca. 6 km west from the present sampling point).

![Figure 3](image)

Fig. 3. Changes in length with water density of (a) anterior spine, (b) posterior spine and (c) thick longitudinal costa of *Crucispina cruciformis*. The density averaged over 5 days before each sampling was taken.

Characteristic feature can be found in the relationship between the water density and the length of the costal strips. The lengths of the anterior and posterior spines vary correlatively to the density (Fig. 3a, b), whereas the length of the heavy longitudinal costal strip of the loria chamber is almost constant during the periods investigated (Fig. 3c). These facts indicate the seasonal variability of this species, which is controlled by the water density. Although the biological function of the silicified loria of *Crucispina cruciformis* has not been revealed, the
seasonal variability observed here suggests that the length of the ornamental spine, which is made of a heavy costal strip, has strong relation to floating or the planktonic habitat for this species. On the other hand, water temperature itself, which may be a physiologically important environmental factor, shows no correlation to the dimensional factor of the lorica.

The seasonal distribution of *C. cruciformis* is shown in Fig. 1. This species was found from December to May, when the water temperature ranged from 8.6 to 17.2°C. The range of water temperature previously reported for this species was from 7.0°C (Norway: ESPELAND & THRONSEN 1986) to 25°C (Gulf of Elat: THOMSEN 1978). This species may be a eurythermal species, although it occurs in the cold-water season in Osaka Bay.

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**Explanation of Plate I**

**Electron microscopy of *Bicosta spinifera***.

1. A whole lorica structure. Scale: 10 µm.
2. SEM image of the lorica chamber in the specimen of Photograph 1. Scale: 5 µm.
3. More highly magnified view of the crossing clearly show the twist rises from the lower right to upper left. Scale: 1 µm.

**Electron microscopy of *Bicosta minor***.

4. A whole lorica structure. Scale: 10 µm.
5. A cell with protoplast, flagellum and collar. The longitudinal costae of the lorica are twisting. Scale: 10 µm.
6. SEM image of the base of the lorica chamber shown in Photograph 5. Posterior end of the chamber twists from lower left to upper right. Scale: 1 µm.
7. SEM image of the lorica chamber which is shown in Photograph 9. Posterior lorica chamber costal strips twist at the middle part of the lorica chamber. Scale: 2 µm.
8. A lorica with two nascent lorica chamber costal strips within the cytoplasmic area (arrowheads). Scale: 10 µm.
9. A lorica, with longitudinal costae twisted, with two supplementary costal strips. Scale: 10 µm.
10. A lorica with a bundle of lorica chamber costal strips within the cytoplasmic area. Scale: 10 µm.
11. A lorica chamber with lorica chamber costal strips plus anterior spine costal strips (large arrowheads). Small arrowhead indicates the anterior end of the anterior lorica chamber costal strip formed within the mother protoplast. Scale: 5 µm.
12. A complete set of the costal strips for a new lorica within the cytoplasmic area is arranged in the opposite direction of the corresponding mother lorica costal strips. Scale: 10 µm.
13. A mother protoplast projecting posteriorly the pointed tips of the two new anterior spines (arrowheads) and anteriorly the posterior end of the new posterior spine. Scale: 5 µm.
PLATE I
Explanation of Plate II

Electron microscopy of *Crucispina cruciformis*.

1. Cell with protoplast flagellum, collar tentacles, and posterior peduncle. Scale: 1 µm.

2–4. A lorica. Whole lorica structure (2) and the chamber of the lorica, TEM (3) and SEM (4). Arrowheads in (3) and (4) indicate a transverse costal strip connecting the anterior ends of thick longitudinal costae. Scales: 1 µm.

5–7. Another lorica. The lorica chamber comprises 12 delicate costal strips and 2 heavy costal strips. Arrowheads in (6) and (7) indicate that single transverse costal strip connects the two thick longitudinal costae. Scales: 1 µm.