# Taxonomic study on the genus *Heterocapsa* (Peridiniales, Dinophyceae)

# 有殻渦鞭毛藻 *Heterocapsa* 属の分類学的研究

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**March, 2002**

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**ABSTRACT (in Japanese)**

渦鞭毛藻類は沿岸域において比較的出現頻度の高い藻群であり、生態的・形態的に多様な種を含むとともに、麻痺性貝毒や下痢性貝毒そして魚毒の原因種としても知られている。近年では有殻渦鞭毛藻 *Heterocapsa circularisquama* が、西日本沿岸域において赤潮による二枚貝の大量斃死を引き起こすことが知られ、有害プランクトンの一種として注目されている。

有殻渦鞭毛藻 *Heterocapsa* 属は沿岸性の小型種からなる藻群で、渦鞭毛藻としては特徴的な細胞鱗片を持つことが知られている。同属は有殻類であるが、多くの種の鎧板は極めて薄いため外見上無殻類とも酷似しており、光学顕微鏡下における同定が困難である。また近年の二枚貝大量斃死に対して同種の出現や分布の把握や被害対策研究も行われてきているが、同定の困難さに加え *H. circularisquama* 類似種も確認されてきている。

本研究は、1）日本沿岸に出現する未同定の *Heterocapsa* の記載を行うこと、2）本属構成種の包括的な形態比較を行うことで共有形質を示し、本属の特徴を明確にすること、3）それぞれの形態比較から派生形質を示し、種レベルの分類形質を明らかにすることを目的として行った。

研究には世界各地から採集した 84 の *Heterocapsa* 属藻類の単藻培養株と固定試料を供した。形態観察には、ノマルスキー型微分干渉顕微鏡による細胞外形とサイズ、細胞内における核とピレノイドの位置の観察、蛍光顕微鏡を用いた鎧板配列の観察を行った。透過型電子顕微鏡を用いた細胞内微細構造の観察には超薄切片を、細胞鱗片の微細構造にはホールマウント試料を用いた。そして SSU rRNA 遺伝子の塩基配列を用いた系統解析により渦鞭毛藻中の *Heterocapsa* 属の系統的位置を推定し、さらに ITS1、5.8S rRNA、ITS2、と周辺の一部を含む領域（ITS 領域）を用いた分子系統解析により *Heterocapsa* 属内の系統関係の解明を試みた。最終的に形態形質と分子系統解析の結果を照らし合わせることで、属内における各形態形質の進化を想定し、分類形質としての評価を行った。

**これまでの *Heterocapsa* 属の分類とその問題点**

*Heterocapsa* 属は Stein (1883)により上殻のみに鎧板をもつ藻群として設立された。最初に *Glenodinium triquetrum* を新組み合わせ *H. triquetra* として提唱したため、同種がタイプ種となっている。この属の基準は下殻にも鎧板を持つことが知られる現在では適用することはできないが、*H. triquetra* は世界各地の沿岸域における普遍種であり、その特徴的な細胞外形から現存する同種が *H. triquetra* であることは一般的に受け入れられている。1977 年に同種から細胞鱗片が発見され、1981 年には *H. triquetra* の全鎧板配列が表された。そして同種と似た鎧板配列と細胞鱗片を持つ *Cachonina illdefina* と *C. niei* が同属に移され、共通の鎧板枚数と細胞鱗片を持

つ渦鞭毛藻が*Heterocapsa*と認識されるようになった。さらに近年では、細胞外形、鎧板配列、ピレノイドの内部構造の他に細胞鱗片の微細構造を示すことで*H. rotundata*や*H. circularisquama*が記載されており、細胞鱗片の形態の違いも種レベルの分類形質として認識されるようになっている。

このような分類学的経緯により、*Heterocapsa* 属はタイプ種 *H. triquetra* と共通の鎧板枚数と細胞鱗片を持つ有殻渦鞭毛藻の一群と見なされている。しかし同属に対する包括的な分類研究はなく、種レベルでは様々な研究者が各自の分類基準により同属に種を帰属させてきたため、各種がどの形態形質により他種と識別されるのかは明確でない。例えば、有用な種レベルの分類形質として期待されている細胞鱗片の微細構造に関しても *Heterocapsa* 全種では観察されてはおらず、比較が困難な状況となっていた。

**形態観察と分子系統解析の結果**

形態観察の結果、全ての種は基本的に同一の鎧板枚数、ピレノイド、細胞鱗片を持っていた。これらの形態形質中、ピレノイドと核の位置、ピレノイド内の管状陥入の有無、細胞鱗片の微細構造等に違いが見られ、これらを組み合わせることで試料中から 7 既知種 *H. arctica*、*H. circularisquama*、*H. illdefina*、*H. niei*、*H. pygmaea*、*H. rotundata*、*H. triquetra*、そして明らかに既記載種と異なる形態形質を持つ 5 つの形態型が識別された。これら 5 新種を、*H. lanceolata*、*H. horiguchii*、*H. ovata*、*H. pseudotriquetra*、*H. orientalis* として記載した。

SSU rRNA 分子系統解析の結果、*Heterocapsa* 属は単系統群を形成した。しかしどの外群とも類縁が示されることはなく、同属の姉妹群は明らかにできなかった。ITS 領域を用いた系統解析も同様に *Heterocapsa* 属の単系統性が示され、本属が自然分類群であることが支持された。属内でそれぞれの種は他種と混ざり合うことはなく、今回提唱した 5 新種も含めた各種は遺伝的にも分化していることが示された。そして構成種は 3 つのクレード、すなわち *H. horiguchii* と *H. ovata*（クレード 1）、*H. pseudotriquetra* と *H. triquetra*（クレード 2）、*H. arctica* と *H. lanceolata* と *H. rotundata*（クレード 3）を形成し、*H. circularisquama*、*H. illdefina*、*H. pygmaea* はそれぞれ独立して分枝していた。

**各分類形質の評価**

(1) 細胞外形

*Heterocapsa* 属の細胞外形はそれぞれの種ごとに安定しており、楕円形、球形、菱形、そして上殻が大きい種の 4 つに大別することができる。このうち菱形と上殻の大きい *H. triquetra* と *H. lanceolata* は後角を持つことから、その他の上殻の大きな種である *H. arctica* と *H. rotundata* を含めたこれらは細胞外形のみにより識別された。しかも上殻の大きな 3 種は系統樹においても

クレード 3 を形成することで類縁性を示しており、この形質は保存性が高く属内でも一度だけ獲得していることがわかった。逆に楕円形と球形の細胞外形は他のクレード内に混在しており、細胞幅は進化的にも複数回の変化を経てきた比較的変わりやすい形質であることが分かった。

(2) 鎧板配列

属内における外部形態の多様性にかかわらず、全種は Po, cp, 5', 3a, 7", 6c, 5s, 5"', 2""と表される共通の鎧板枚数を持っていた。また配列においても前縦溝板が上殻に深く入り込み、上殻中央付近で第一頂板と接する特徴的な配列も全種にみられた。鎧板配列は属内でほぼ共通しており、種を識別することはできなかった。唯一の種間での違いは、背面観において *H. triquetra* の第一前挿間板は 3 枚の前帯板と接するが、他種では 2 枚のみの点であった。

(3) 細胞内におけるピレノイドの位置と管状陥入の有無

*Heterocapsa* の細胞核は球形か楕円形であり、球形の場合には上殻か下殻のどちらかに偏って存在していた。核が上殻にある場合にはピレノイドは核の下部に、下殻にあるときにはピレノイドは上部にあるという位置関係がみとめられ、これは種内で安定していた。楕円形の核の場合には、核は細胞内でピレノイドとほぼ同じ高さに並ぶために位置関係を把握することはできなかった。以上のように球形の核を持つ種に限り、核とピレノイドの位置関係が種の識別形質となりうることが分かった。系統的にもそれぞれのタイプは同じクレードに含まれることから、この形質は進化的にも安定した形質であることが示唆された。

また、ピレノイド基質中の多数の管状陥入は約半数の種に存在し、種識別のための形態形質として使用することができた。しかしクレード 1 と 3 には管状陥入を持つ種と持たない種が含まれており、これらのピレノイド構造の多様性は必ずしも系統を反映しているとは限らない形質であることが示唆された。この形質は比較的細胞サイズの大きい種に偏って存在することから、細胞が大型化するに伴い細胞内輸送の効率を高めるために属内のそれぞれの系統で獲得した（もしくは細胞の小型化にともない平行的に失われた）形質なのかもしれない。

(4) 細胞鱗片

細胞鱗片は三部からなる放射相称の基盤と、上部の立体的な骨組みでできた装飾構造で構成されている。この構造は全ての *Heterocapsa* に共通した形質であり、他の渦鞭毛藻に限らずプラシノ藻やハプト藻の鱗片とも識別される形態形質と考えることができる。また、鱗片の直径、基盤の形態、肋線や柱の数が種間で異なっており、これら微細構造を比較することで各種を識別することができた。*H. arctica*、*H. circularisquama*、*H. niei* の 3 種からは 2 種類の鱗片が同じ試料中から観察されたが、周縁部の柱など基本的な構造に変化がないため、構造的に小さな鱗片は未成熟のものであると考えられた。また、種内では基盤の網目の粗さの違いが *H. horiguchii*、*H. rotundata* において観察された。この変異は株毎に安定しており、培養株が維持されてから 10 年以上たった株のみに粗い網目が観察され、しかも天然試料ではすべて細かい網目を持ってい

たことから、長年の継代培養による人為的な変異であると判断した。従って基盤の網目の相違は、分類基準としての価値を評価しなかった。また、唯一同じ鱗片を持つ種に *H. triquetra* と *H. pseudotriquetra* が挙げられる。これらはクレード 2 を形成しており近縁な種と考えられた。そしてクレード 1 と 2 においてもそれぞれの構成種の鱗片構造は比較的似ていることから、鱗片は細胞外形と比べ進化的に安定した構造であることが示唆された。従って、鱗片の微細構造はこれら 2 種を除く他の全種を明確に識別できる有用な分類形質となることが分かった。

本研究により、日本沿岸域より見いだされた 5 新種が記載された。また、構成種の包括的な形態比較により、現在まで曖昧であった *Heterocapsa* 属の共有形質を明確化した。さらに同属各種は細胞形態、ピレノイドと核の位置関係、ピレノイド内の管状陥入の有無、そして細胞鱗片の微細構造の組み合わせより識別されることが明らかとなった。中でも鱗片構造は、これのみによりほとんどの種が識別される最も有用な分類形質であることが示された。現在までに 4 種の鱗片構造が模式化されていたが、本研究からさらに 8 つの鱗片と 3 つの未成熟な鱗片が図示されたことにより、鱗片構造比較による *Heterocapsa* の種レベルでの同定が可能となった。この鱗片観察技術は透過電顕観察法の中でも比較的簡便であり、固定天然試料にも使用できることから、*Heterocapsa* 属の分類研究はもとより分布・広域化研究への応用が今後期待される。

## INTRODUCTION

Dinoflagellates are an extremely diversified microalgal group that includes planktonic, benthic and symbiotic species. Half of them are known to be autotrophic while others are heterotrophic in nature. Dinoflagellates are also recognized as causative organisms for red tides and shellfish poisonings. Red tides are beneficial for aquaculture in most cases, however, in some situations red tides often have a negative effect, causing severe economic losses to aquaculture (Hallegraeff 1993). Some dinoflagellates produce potent toxins that can affect the human food chain especially through shellfish. Toxins of dinoflagellates cause a variety of gastrointestinal and neurological illnesses, such as paralytic shellfish poisoning (PSP) and diarrhetic shellfish poisoning (DSP). Therefore, the ecological, toxicological and taxonomical characters of PSP and DSP causative dinoflagellates, e.g. *Alexandrium* spp. and *Pyrodinium bahamense* Plate, have been investigated in detail.

The thecate dinoflagellates *Heterocapsa* Stein has been known as a cosmopolitan phytoplankton especially in the coastal waters. Among the species of this genus, *H. triquetra* (Ehrenberg) Stein and *H. rotundata* (Lohmann) Hansen have been reported frequently (Steicinger & Tangen 1996; Fukuyo *et al.* 1997; Konovalova 1998), and others like, *H. niei* (Loeblich III) Morrill & Loeblich III and *H. pygmaea* Loeblich III, Schmidt & Sherley. have been used as model dinoflagellates for cytological investigations (Dodge & Crawford 1971; Morrill & Loeblich III 1981; Morrill 1984; Bullman and Roberts 1986; Roberts *et al.* 1987; Höhfeld & Melkonian 1992). This genus has been also well known to bear small organic scales around the cell body (Pennick & Clarke 1977, Morrill & Loeblich III 1983). However, the genus *Heterocapsa* itself had not received particular attention until the occurrence of the harmful species *H. circularisquama* Horiguchi, the first species in the genus *Heterocapsa* that make harmful algal blooms.

*H. circularisquama* was first found at Uranouchi Bay, Kochi Prefecture in 1988, when 1,560 tons of short-necked clams *Ruditapes philippinarum* were dead (Matsuyama 1999). Subsequently, red tides of *H. circularisquama* were also found at Fukuoka Bay, Fukuoka Prefecture in 1989 (Yamamoto & Tanaka 1990) and at Ago Bay, Mie Prefecture in 1992 (Matsuyama *et al.* 1995), when shellfish such as the oyster *Crassostrea gigas* and pearl oyster *Pinctada fucata* were killed due to these red tides. Using the sample collected from the red tide at Ago Bay, *H. circularisquama* was officially described as a new species (Horiguchi 1995). Within a decade of its first record of the occurrence, this species has spread extensively and has been confirmed from more than coastal 20 areas along western Japan. Consequential economic loss of shellfish aquaculture by death of marketable fishery products was estimated to reach around 10 billion yen so far (Matsuyama 1999). Of these bivalve mass mortalities, economic loss by red tide at Hiroshima Bay alone in 1998 was approximately 3.8 billion yen, which is one of the worst recorded economic losses suffered by just one red tide in Japan. Red tide events of *H. circularisquama* and the affected organisms were reviewed by Matsuyama (1999).

The toxic material of *H. circularisquama* affects only shellfish, and records indicate that it was non toxic to other commercial organisms such as finfish, or to human beings (Matsuyama *et al.* 1997). It is well known that some dinoflagellates produce toxin which is harmful to finfish and crustacean directly, or to the vertebrates via the shell fish. Therefore, bloom caused by *H. circularisquama* is markedly different from PSP, DSP, amnesic shellfish poisoning (ASP), ciguatera poisoning and ichthyotoxicity (Matsuyama 1999), hence some authors call it "novel red tide" (e.g. Tarutani *et al.* 2001; Yamaguchi *et al.* 2001).

Since this noxious species was not recorded from Japan or any other country until the first record at Uranouchi Bay in 1988, biogeographical characteristics of this dinoflagellate could not be determined. This species grows at more than 15 °C and the optimal growth temperature in culture condition is 30 °C (Yamaguchi *et al.* 1997; Yamaguchi *et al.* 2001). Therefore the

original habitat of *H. circularisquama* is considered to be tropical or subtropical area rather than temperate or cold regions. Moreover, this supposition could be supported by evidences of these red tides in Hong Kong during 1986 and 1987 were found from the preserved samples (Iwataki *et al.* in press). Thus it is supposed that *H. circularisquama* is distributed not only in the western Japan but also in some bays and inlets as well as inland seas along the coast of South China Sea and East China Sea. A special characteristic of the species is that it caused red tide and mass mortality of bivalves only in the inner bay regions. According to Honjo *et al.* (1998), temporary cyst of *H. circularisquama* is able to survive at least 24 hours in the bivalve shell, and the spread of this species is predicted to be due to the transportation of shellfish and aquaculture activities (Honjo *et al.* 1998).

*H. circularisquama* is now recognized as one of the most popular harmful species in Japan, and ecological and physiological information of *H. circularisquama* have been accumulated (e.g. Yamaguchi *et al.* 1997). To avoid economic losses by red tides of this species, monitoring of occurrence is periodically conducted by each Prefectural Fisheries Station in the western Japan. Immunological techniques using antibody for fluorescent *in situ* hybridization is now developed and purified to detect cells of *H. circularisquama*. For termination of red tides, several laboratories are surveying virus and bacteria, which can infect species-specific to causative organisms (e.g. Kitaguchi *et al.* 2001). However several *Heterocapsa* species similar to *H. circularisquama* were detected during the red tide monitoring and these are tentatively named as *Heterocapsa* sp. 1 and *Heterocapsa* sp. 2 (Iwataki *et al.* 2001). These species were different from *H. circularisquama* by cell shape, cell size and swimming behavior under light microscopy. Taxonomic conclusions for these species and accurate taxonomic criteria for *Heterocapsa* species by which each species could be clearly discerned is an essential requirement.

## CHAPTER 1

## HISTORICAL BACKGROUND OF TAXONOMY OF THE GENUS *HETEROCAPSA*

### 1-1 Taxonomic position

Since the genus *Heterocapsa* was originally established by Stein (1883), taxonomic position of the genus in dinoflagellates based on morphological comparison has been discussed by several authors (e.g. Tappan 1980, Loeblich III 1982, Dodge 1984, Sournia 1986, Taylor 1987, Fensome *et al.* 1993). These classifications are basically consistent with the validity of the genus *Heterocapsa*, and all of them assigned it to the order Peridiniales. However treatment of the family were more or less different between these classification systems. In 1993, Fensome *et al.* summarized the classification of all dinoflagellates compiling living and fossil dinoflagellates. According to his classification system, *Heterocapsa* was assigned to the following position.

| | |
|---|---|
| Class | DINOPHYCEAE Pascher 1914 |
| Subclass | PERIDINIPHYCIDAE Fensome *et al.* 1993 |
| Order | PERIDINIALES Haeckel 1894 |
| Suborder | HETEROCAPSINEAE Fensome *et al.* 1993 |
| Family | HETEROCAPSACEAE Fensome *et al.* 1993 |
| Genus | *HETEROCAPSA* Stein 1883 |

The suborder Heterocapsineae Fensome *et al.* comprised the single family Heterocapsaceae Fensome *et al.* These new hierarchies were established based on their common thecal plate arrangements. The family consisted of *Heterocapsa* and eight fossil genera, therefore related extant genera to *Heterocapsa* is not sure.

## 1-2 General features

*Heterocapsa* is a unicellular thecate dinoflagellate, consisting of relatively small sized, free-living marine species. As the most *Heterocapsa* species possess rather thin and tiny thecal plates, they superficially resemble the gymnodinioid dinoflagellates under light microscope. One *Heterocapsa* species, *H. rotundata* (Lohmann) Hansen (= *Katodinium rotundatum* (Lohmann) Loeblich III), has been indeed treated as unarmored dinoflagellates previously. Typical cell shape is elliptical in ventral view, with cingulum located on the equatorial plane. There are some specific variations, for instance, elongated cell with antapical horn and larger epitheca. All species are autotrophic, containing yellowish brown parietal chloroplast without an eyespot. Sexual reproduction is unknown.

This genus has been generally characterized by its thecal plate arrangement (Po, cp, 5', 3a, 7", 6c, 5s, 5"', 2"") similar to other peridinioid dinoflagellates, and some variations (Po, cp, 5', 3a, 7-8", 6c, 5-8s, 5"', 0-1p, 2"") have been reported in the genus (Loeblich III *et al.* 1981; Morrill & Loeblich III 1981; Hansen 1995; Horiguchi 1995, 1997). Body scales and pyrenoid are also known as common morphological characters of *Heterocapsa*. The body scale is especially a characteristic feature of this genus. Scaly dinoflagellates have been reported only from three genera; *Oxyrrhis*, *O. marina* Dujardin (Clarke & Pennick 1976), *Lepidodinium*, *L. viride* Watanabe *et al.* (Watanabe *et al.* 1987; Watanabe *et al.* 1990) and *Heterocapsa*. (Pennick & Clarke 1977; Loeblich III, Schmidt & Sherley 1981; Morrill & Loeblich 1981, 1983; Hansen 1995; Horiguchi 1995; Horiguchi 1997). Of these, *O. marina* has body and flagellar scales comprising only of a basal plate, while the body scale of *L. viride* consists of three-dimensional, basket-like architecture. Body scales of *Heterocapsa* spp. are easily distinguished from these two dinoflagellates by showing three-dimensional, triradiate structure (see Section 5-2-5).

Moreover, another characteristic of the swimming behavior has been observed in some *Heterocapsa* species. The harmful species *H. circularisquama* does not swim in constant velocity. The motile cell can quickly move backward and forward, then change its own cell orientation by inches and swim small distances. This peculiar behavior has sometimes been used for identification of *H. circularisquama* in the red tide monitoring program by Prefectural fisheries experimental stations. Dr. H. Takayama, Hiroshima Fisheries Experimental Station, compares this oscillating behavior to woodpecker-like movement (Takayama per. com.), on the other hand Dr. P. J. Hansen, University of Copenhagen, calls the quick slide to "jumping" (Per Juel Hansen per. com.).

Although many species of *Heterocapsa* often occurs in coastal areas and sometimes make red-tides, only one species, *H. circularisquama* is known to be responsible for shellfish mass mortality so far. Red tides due to *Heterocapsa* species such as *H. triquetra* and *H. pygmaea* are recognized as beneficial for fisheries rather than harmful for fishery activities.

### 1-3 History of taxonomic study

The genus *Heterocapsa* was originally established by Stein in 1883 by making a new combination of the type species *H. triquetra* (Ehrenberg) Stein from *Glenodinium triquetrum* Ehrenberg (Fig. 1-1. a-e). The other two species, *H. umbilicata* Stein (Fig. 1-1, f, g) and *H. quadridentata* Stein (Fig. 1-1, h), were simultaneously described as new species (Stein 1883). In the contemporary systematics of thecate dinoflagellates, the two thecate genera, *Peridinium* Ehrenberg (Ehrenberg 1830) and *Glenodinium* Ehrenberg (Ehrenberg 1840), had been distinguished based on whether or not the thecal plates can be counted under the microscope. In the state of affair, Stain (1883) could have observed the sutures only on the epitheca of *G. triquetrum*. As a result, he tentatively established a new genus *Heterocapsa*, which had

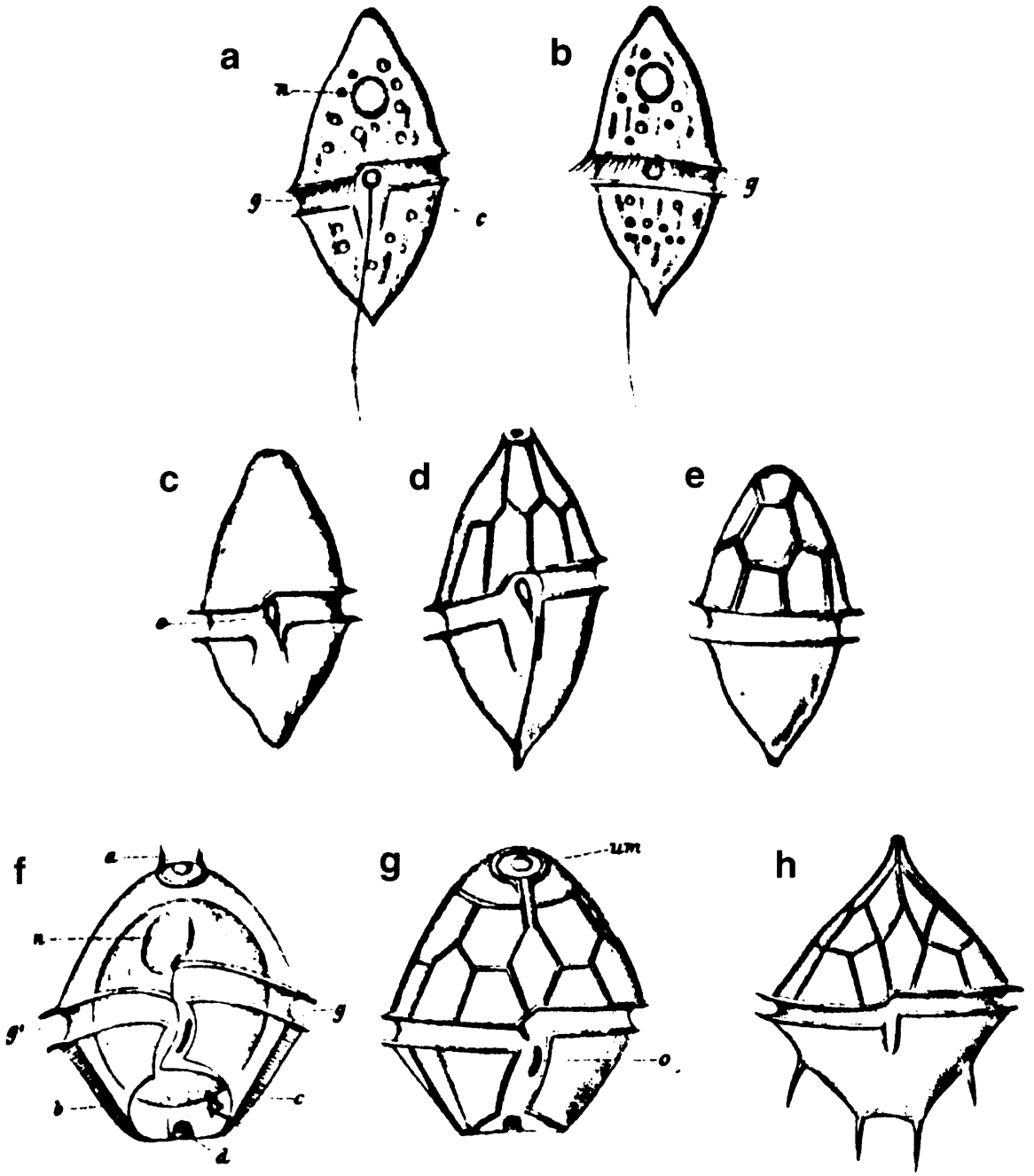


**Figure 1-1.** Original line drawings of type species *Heterocapsa triquetra* and other two *Heterocapsa* species. **a-e**, *H. triquetra*; **f, g**, *H. umbilicata*; **h**, *H. quadridentata* (Stein 1883).

sutured epitheca and flimsy hypotheca. The original etymology of the genus *Heterocapsa*, therefore, had denoted different types of the hemitheca. These three species were indeed illustrated to possess "peridinioid" epitheca and "glenodinioid" hypotheca in his figure plates (Fig. 1-1). However, their cell shapes and thecal plate arrangements of the epitheca were entirely different from each other. These morphological and thecal plates variances precisely indicate the generic difference.

The next description of *Heterocapsa* species was *H. pacifica* Kofoid, which was described by using plankton sample collected from offshore of San Diego, California (Kofoid 1907). The cell shape of this species was somewhat similar to *H. triquetra,* but it differed in points of broader cell width, position of nucleus and prominent antapical spine. However, it contradicted from the original description of *Heterocapsa*, as thecal plates were not illustrated at all (Fig. 3-2-13, h, i). Subsequently, Massart (1920) reported a new species *H. quinquecuspidata* Massart. The cell of *H. quinquecuspidata* superficially resembled *H. quadridentata* Stein with regard to possessing five spines on the hypotheca (Fig. 1-2. a-e). Only the thecal plates of the dorsal epitheca were then illustrated. In those days, generic criteria among *Glenodinium, Heterocapsa* and *Peridinium* seemed to be confused, because either of the two characters, viz. presence of partial thecal plates and cell shape, had been simultaneously used for generic criteria. These four *Heterocapsa* species *H. umbilicata, H. quadridentata, H. pacifica* and *H. quinquecuspidata* have not been reported since then, however, only *H. triquetra* has been reported by several authors (Schütt 1895; Paulsen 1908; Meunier 1910; Lindemann 1924). The type species *H. triquetra* was re-observed and sutures were found also in hypotheca (Lindemann 1924), but this plate tabulation still differed from later reports that recently used. During that period, cell shape may have been the most fundamental character for *H. triquetra*.

In 1960s, all thecal plates of *Heterocapsa* could be observed and their arrangements were adopted as a criterion of the genus. Loeblich III (1968) described a new species *Cachonina*

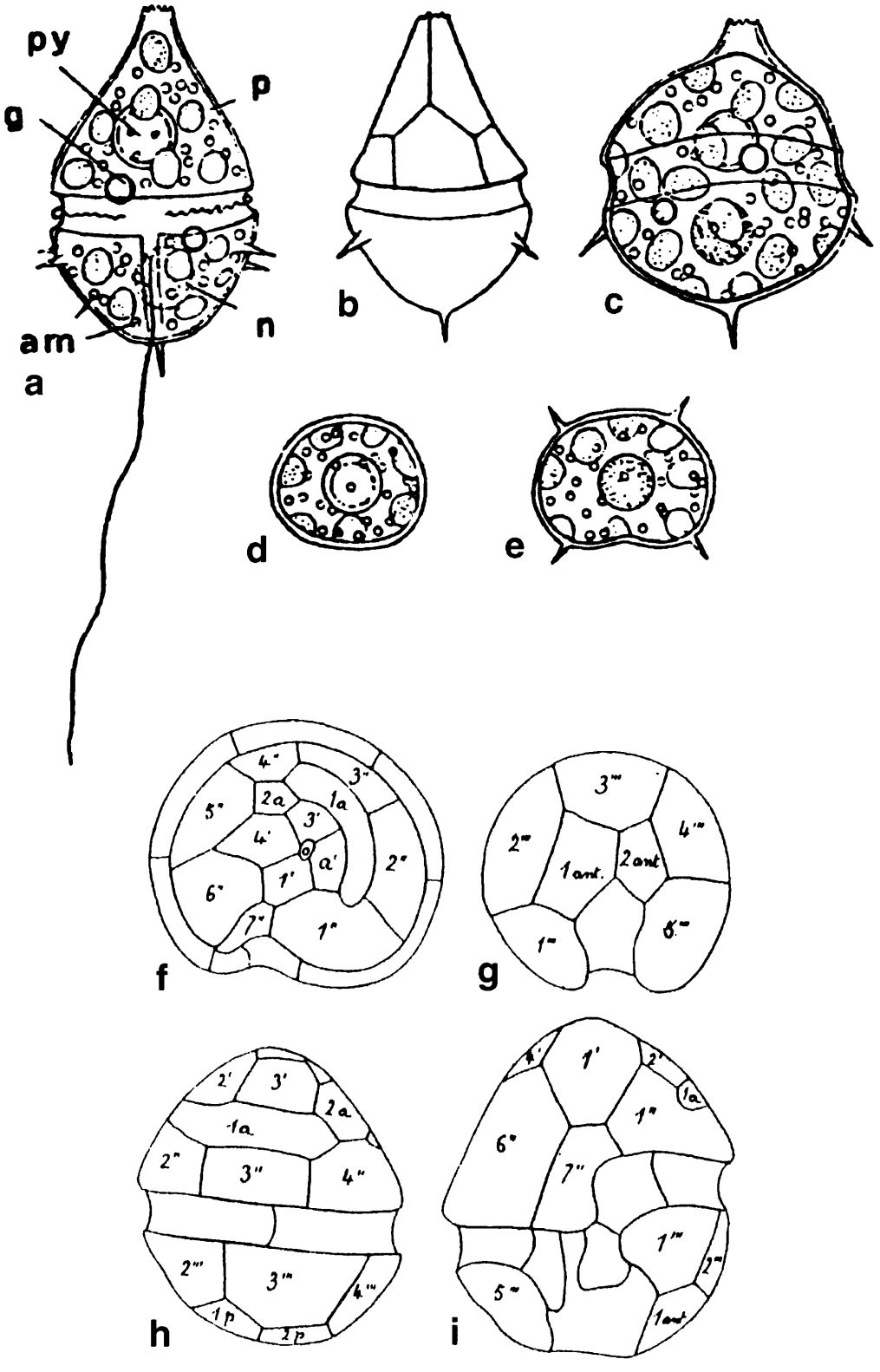


**Figure 1-2.** Original line drawings of *Heterocapsa quinquecuspidata* Massart (**a-e**; Massart 1920) and *Peridnium chattonii* Biecheler (**f-i**; Biecheler 1952) .

*niei* Loeblich III as type species of a new genus *Cachonina* (*Cachonina* = *Heterocapsa*) with thecal plate arrangement, and Stosch (1969) reanalyzed the plate arrangement of *C. niei*. Generic affiliation of each species was then discussed based on its thecal plate arrangement.

In 1970, nutritional, physiological and morphological characters of *Heterocapsa kollmeriana* Swift & McLaughlin were reported using sample of a bloom in Phosphorescent Bay, Puerto Rico (Swift & McLaughlin 1970). This species was a thecate dinoflagellate smaller than 10 μm in length, and sometimes formed an ellipsoidal cyst. Thecal plate arrangements and position of nucleus were unclear, because detailed species description with a Latin diagnosis was not provided in the paper.

Campbell (1973) transferred *Peridinium chattonii* Biecheler to the genus *Heterocapsa* in his Ph.D. thesis, proposing a new combination *H. chattonii* (Biecheler) Campbell (Fig. 1-2, f-i). He also observed *H. triquetra*, and considered its short first apical plate 1' as an important taxonomic character of *Heterocapsa*.

In 1968, new genus and species *Cachonina niei* Loeblich III was described using red tide sample collected from the Salton Sea, California in 1966 (Loeblich III 1968). The name *Cachonina niei* was dedicated after Dr. Jean Cachon and Dr. Dashu Nie. All thecal plates of this species were then investigated in details and illustrated as p.p., 5', 3a, 8", 6c, 4s, 5"', 2"". In the year following the original description, more detailed thecal plate arrangement of *C. niei* was determined by von Stosch (1969), and it was represented as; po, 6', 3a, 7", 6c, 4s (s.a., t, s.l., s.l. s.p.), 5"', 2"". The canal plate was then discovered for the first time and mentioned as the sixth apical plate 6' (Stosch 1969). This was the first report of the complete thecal plate tabulation to be worked out in detail for the member of the *Heterocapsa* (Loeblich III, Schmidt & Sherley 1981), and it is almost equivalent to the plate arrangement recognized at present. The next *Cachonina* species, *C. illdefina* Herman & Sweeny was described using a red tide sample from the coastal area of California (Herman & Sweeney 1976). This species was named from "ill-define" in English, because of the frustration for its classification. Thecal

plate tabulation of *C. illdefina* was identical to *C. niei*, however, the configuration of sulcal series and cell size were different. TEM photomicrograph then revealed that the former species had tubular cytoplasmic invaginations within the pyrenoid matrix. This kind of pyrenoid was alike with that of *H. triquetra* reported by Dodge & Crawford (1971). In 1977, Balech analyzed the thecal plates of *C. illdefina* in detail, and considered it to *Cachonina niei*, based on their similarity of thecal plate arrangements. Against the opinion of Balech (1977), Morrill & Loeblich III contradicted the view with the indication of difference in cell size range between *C. niei* and *C. illdefina* (Morrill & Loeblich III 1981). They also re-observed complete thecal plate arrangement of *H. triquetra*, 2pr, 5', 3a, 7", 6c, 7s, 5"', 1p, 2"", and regarded that the arrangements of *Heterocapsa* and *Cachonina* were almost identical. Moreover they indicated the presence of organic body scales in these *Cachonina* species. Body scale was a character, which has already been found out from *H. triquetra* (Pennick & Clarke 1977). They transferred *C. niei* and *C. illdefina* to the genus *Heterocapsa* based on these common characters with two new nomenclatural combinations, *H. niei* (Loeblich III) Morrill & Loeblich III, and *H. illdefina* (Herman & Sweeney) Morrill & Loeblich III. The genus *Cachonina* has been considered to be a junior synonym of *Heterocapsa* (Morrill & Loeblich III 1981; Fensome *et al.* 1993; Hansen 1995; Horiguchi 1995, 1997). In the same year, a new species *H. pygmaea* Loeblich III, Schmidt & Sherley was described (Loeblich III, Schmidt & Sherley 1981). The thecal plate arrangement of *H. pygmaea* was mentioned as; apical pore plate, canal plate, 5', 3a, 7", 5-7c (a.s., r.s., l.a.s., l.p.s., [? a.a.s. and p.a.s. p.s.), 5"', 2"". They firstly regarded the eighth precingular plate (8") as the anterior sulcal plate (as), which is a commonly accepted interpretation in recent years. In the same paper they also mentioned that the presence of body scale was a generic feature of *Heterocapsa*, and the scale sizes were significant characteristic at the species level (Morrill & Loeblich III 1981).

Organic body scales, the distinctive feature of the genus *Heterocapsa*, was first found from the type species *H. triquetra* (Pennick & Clarke 1977). These were observed in detail

using both thin sections and whole mounts for TEM, and illustrated its three dimensional structure. Thereafter several workers have reported the presence of the delicate organic body scales on the outer cell surface of *Heterocapsa* species (Morrill & Loeblich III 1981a; Morrill & Loeblich III 1983; Bullman & Roberts 1986; Dodge 1987). Of these, Morrill & Loeblich III (1981a), presence of the body scales was used as a decisive character of new combinations *H. niei* and *H. illdefina*. Since then, the body scale has been recognized as a common character of the genus. Morrill & Loeblich III (1983), moreover, investigated detailed structure of the scale of *H. niei*, and clearly illustrated the difference from *H. triquetra*. They mentioned that the body scales were not only common character of the genus *Heterocapsa* but also that detailed differences might exist between the scales of each species.

Another species of the genus, *H. minima* Pomroy, was reported from Celtic Sea, England (Pomroy 1989). This species was described mainly based on its small cell size and thecal plate arrangement using SEM, although body scales were not shown (Fig. 3-2-13, a – g). It is a rare species of *Heterocapsa* reported from offshore.

During the stay in New Zealand in 1979-1980, Moestrup of University of Copenhagen discovered that the unarmored dinoflagellate *Katodinium rotundatum* (Lohmann) Loeblich III was also furnished with a surface layer of scales similar to those in *Heterocapsa* (Hansen 1989). The scale structure was not identical to previously described *Heterocapsa* scales, *H. triquetra* or *H. niei*. Hansen (1995) observed the thecal plates of *K. rotundatum* and showed that their arrangement was similar to that of *Heterocapsa*. He made the new combination *H. rotundata* (Lohmann) Hansen. He then also examined a culture of *Heterocapsa* cf. *minima* isolated from the southern part of Kattegat, Denmark and disclosed the body scale, and indicated distinction among them from cell shape and body scale ultrastructure. Thus the morphology of body scale has been recognized as the most important species character in the genus *Heterocapsa*. In the same year, causative species of shellfish mass mortalities, *H. circularisquama* Horiguchi, was described from the coastal area of the western Japan (Horiguchi 1995). Cell shape, size and

theca! plate arrangement of *H. circularisquama* were quite similar to those of *H. illdefina*, although the body scales clearly differed between these species. The species name of *H. circularisquama* means circular scale, and it was the first species established based on the body scale ultrastructure. Recently, *Heterocapsa arctica* Horiguchi was described from Arctic Sea (Horiguchi 1997). The elongated cell and the large epitheca was rather characteristic, however the body scale is triangular resembling that of *H. triquetra*. Those studies mainly recognized theca! plate arrangements and presence of body scale as common characters of the genus *Heterocapsa*.

Until the latest description of *Heterocapsa* species, *H. arctica* (Horiguchi 1997), fourteen *Heterocapsa* species have been reported so far; *H. triquetra* Stein, *H. umbilicata* Stein, *H. quadridentata* Stein, *H. pacifica* Kofoid, *H. quinquecuspidata* Massart, *H. kollmeriana* Swift & McLaughlin, *H. chattonii* (Biecheler) Campbell, *H. niei* (Loeblich III) Morrill & Loeblich III, *H. illdefina* (Herman & Sweeney) Morrill & Loeblich III, *H. pygmaea* Loeblich III, Schmidt & Sherley, *H. minima* Pomroy, *H. rotundata* (Lohmann) Hansen, *H. circularisquama* Horiguchi, and *H. arctica* Horiguchi. Of these, ten species, *H. triquetra, H. umbilicata, H. pacifica, H. niei, H. illdefina, H. pygmaea, H. minima, H. rotundata, H. circularisquama* and *H. arctica* have recently agreed with valid *Heterocapsa* species (Hansen 1995, Horiguchi 1995).

**1-4 Rarely cited species**

Fourteen species of *Heterocapsa* have been reported so far, although several of them have been cited rarely, *H. umbilicata, H. quadridentata, H. pacifica, H. quinquecuspidata, H. kollmeriana, H. chattonii* and *H. minima*. Some of these species unsuited to the genus *Heterocapsa* have already been transferred to other genera. Others are difficult to refer incompleteness in the original description and rarity in successive findings and observation.

For *H. quinquecuspidata* Massart, Schiller (1937) regarded the number of spines projected from the hypotheca as intraspecific variation, and treated it as one of the synonyms of *Glenodinium quadridens* (Stein) Schiller. On the other hand, Popovsky & Pfiester (1990) regarded the species as one of the synonym of *Peridiniopsis cunningtonii* Lemmermann. In either case, *H. quinquecuspidata* was not treated as *Heterocapsa* species.

The other species possessing short spines on the hypotheca is *H. quadridentata* (Stein 1883). Hansen (1995) pointed out that *H. quadridentata*, possessing antapical spines, was undoubtedly conspecific with *Peridinium quinquecorne* Abé, and made new combination *Peridinium quadridentata* (Stein) Hansen. These two species, *H. quinquecuspidata* and *H. quadridentata*, differ from *Heterocapsa*, on the basis of cell shapes, thecal plate arrangements and minute spines (not horns) on their hypotheca.

The species *H. chattonii* (Biecheler) Campbell possesses the first apical plate 1' which stopped at the anterior end of seventh precingular plate 7" and did not reach the cingulum (Fig. 1-2, 1 - i). Number of apical, anterior intercalary and precingular plate series of *H. chattonii* was originally illustrated as 4', 2a and 7" (including anterior sulcal plate of *Heterocapsa*) respectively. These plate numbers are not identical to any of the presently known *Heterocapsa* species. Morrill & Loeblich III (1981) have discouraged this new combination on the ground of these discrepancy of plate number and default of detailed research such as cell division and body scale. Therefore this species utterly not referred as *Heterocapsa* in recent studies.

Whole plate arrangement of *H. umbilicata* Stein, one of the first members described as *Heterocapsa*, is not clear, although ventral thecal plate arrangement on epitheca was illustrated (Fig. 1-1, f, g). It possesses the apical pore plate Po, the canal plate (X plate) and several anterior intercalaries in the ventral side. Plate numbers are uncertain, but the positions of the Po plate, the canal plate and the first precingular plate 1" are clearly designated. Anterior part of the 1' plate is bordered with the canal plate, it never contacts with the Po plate. This arrangement looks similar to the species of *Scrippsiella* and some species of *Peridinium*, and is

discrepant with *Heterocapsa*. Therefore, this species should be transferred to one of these genera, but the selection of the best genus for settlement is rather abstruse, because of availability of insufficient information. Many species of *Peridinium* are usually found in freshwater environment, whereas *Scrippsiella* is regarded as marine and tide pool dinoflagellates. Since *H. umbilicata* inhabit the marine environment, it may be a relative species of *Scrippsiella* rather than *Peridinium*. At least it is obvious that this ambiguous species is not *Heterocapsa* species.

Other two rarely cited species, *H. pacifica* Kofoid and *H. minima* Pomroy, will be discussed as *Heterocapsa* species in Section 3-2-13 and 3-2-14, respectively.

Although the genus *Cachonina* Loeblich III is now treated as a synonym of *Heterocapsa*, several authors have still cited the nomenclatural combination of *Cachonina hallii* (Freudenthal & Lee) (e.g. Rhodes *et al.* 1995; Walsh *et al.* 1998). The name is also used in DDBJ/EMBL/Genbank. accession numbers AF033867 and AF033865. All these names are ascribed to same culture strain collected at Bream Bay, New Zealand. Probably this species was originally described as *Glenodinium halli* Freudenthal & Lee from Long Island, New York (Freudenthal & Lee 1963). Plate number of *G. halli* were originally reported as 3', 5a, 6", 3c, 3"', 2"", although ventral view of the species, especially on the plate 1', was rather similar to *Heterocapsa/Cachonina* species. If the combination were settled with valid description, the species should be moved into *Heterocapsa* with type species of *Cachonina, C. niei* Loeblich III. However, I could not find the original report of this combination, thus its validity is still unclear. According to Loeblich III *et al.* (1981), cell of *G. hallii* appears similar to *H. pygmaea* in cell size and shape. Moreover they referred Wilson's suggestion that either *G. hallii* is a very unusual dinoflagellate or that original published pattern is in error in all series with exception of antapical series. Original culture of *G. hallii* has been lost hence it could not be re-observed any more. However, there were some reports of internal ultrastructure of the strain (Dodge 1971; Dodge & Crawford 1971; Dodge 1975). Loeblich III *et al.* (1981) compared

ultrastructural illustrations of *G. hallii* and *H. pygmaea* (as *Glenodinium* sp. = Texan *H. pygmaea*, isolate 7), and discussed that the former species possessed bulged pyrenoid in contrast to the stalked pyrenoid of the latter species. The species was distinguished only from this reason. Therefore it is possibly conspecific to *H. pygmaea*. In either case, the species should be treated as *G. hallii* Freudenthal & Lee until validly transferred to another genus using extra information.

In the present study, I treat the following nine as valid species of the genus *Heterocapsa*; *H. arctica* Horiguchi, *H. circularisquama* Horiguchi, *H. illdefina* (Herman & Sweeney) Morrill & Loeblich III, *H. minima* Pomroy, *H. niei* (Loeblich III) Morrill & Loeblich III, *H. pacifica* Kofoid, *H. pygmaea* Loeblich III, Schmidt & Sherley, *H. rotundata* (Lohmann) Hansen and *H. triquetra* Stein.

**1-5 Definition of the genus**

The genus *Heterocapsa* had been originally established as a group of species with sutures only in epitheca (see Section 1-3). Since the original description could be read that new combination of *H. triquetra* firstly performed and following two species were added, *H. triquetra* could be regarded as the type species of the genus. At present, the genus *Heterocapsa* is actually recognized as an assemblage of thecate dinoflagellates, which have characters in common with *H. triquetra* such as thecal plate arrangement, pyrenoid and extracellular organic body scales. From these morphological characters, the genus is now strictly supposed to be a natural group in peridinioid dinoflagellates. For this assemblage, the original criterion is not applicable any more. The new suitable criteria for the genus *Heterocapsa* based on present knowledge should be settled.

## 1-6 Taxonomic problems of the genus

The most crucial taxonomic problem of the genus *Heterocapsa* is the ambiguity of the generic criteria as mentioned above (see Section 1-4).

Another is associated with the species level. This problem is deeply related with history of taxonomic confusion. Since the election of the genus *Heterocapsa*, more than hundred years have been past, and its taxonomic criteria have so far undergone considerable change. Until 1950 since the generic establishment, the cell shape has mainly been treated as the diagnostic character of the genus. Two species, *H. pacifica* Kofoid and *H. quinquecuspidata* Massart have been described on the ground of superficial similarity to *H. triquetra* Stein and *H. quadridentata* Stein, respectively. During the subsequent period in 1960s-1980s, complete thecal plate number has been used as a criterion for the genus. The species possessing identical plate number with type species *H. triquetra* were considered as *Heterocapsa* species. These species include *H. niei* (Loeblich III) Morrill & Loeblich III, *H. illdefina* (Herman & Sweeney) Morrill & Loeblich III and *H. pygmaea* Loeblich III, Schmidt & Sherley. Ultrastructural characters such as tubular cytoplasmic invaginations in pyrenoid matrix and size of body scale have also been considered. In addition to the characters described above, detailed structure of body scale is recognized to be specific character in recent years. Using this character, *H. rotundata* (Lohmann) Hansen and *H. circularisquama* Horiguchi were described. All these characters, viz. cell sizes, cell shapes, thecal plate arrangements, tubular invaginations into pyrenoid matrix, positions of nucleus and pyrenoid and ultrastructure of the body scales, are used for decretive features at present. However, complete data sets for each species were rarely employed. Although fine structure of body scale is widely recognized as a specific criterion, it has not investigated from all *Heterocapsa* species. To solve these problems and make unequivocal systematics of the genus, morphological re-investigation of all

the *Heterocapsa* species is needed.

Another taxonomic problem is in the treatments of *Heterocapsa* and *Cachonina* species, which were rarely cited since their original descriptions, such as *H. umbilicata* Stein, *H. quadridentata* Stein, *H. pacifica* Kofoid, *H. quinquecuspidata* Massart, *H. kollmeriana* Swift & McLaughlin, *H. chattonii* (Biecheler) Campbell, *H. minima* Pomroy and *Cachonina hallii* (Freudenthal & Lee). It is possible to be deficient registration or nomenclatural invalidity. Causation of this problem was overviewed in the Section 1-3.

## 1-7 Objectives of the present study

The present study was carried out to clarify the taxonomic problems of the genus *Heterocapsa* (Peridiniales, Dinophyceae) at specific and generic levels. The definitive purposes are summarized as follows:

1) To re-investigate the described species of *Heterocapsa* using the culture strains maintained at the culture collections and originally isolated in this study, basically depending on their morphological characters, in particular, thecal plate arrangement and body scale ultrastructure.

2) Based on the results obtained, to clarify the generic and specific definitions, including the emendation of the genus and descriptions for the new species.

3) To infer the phylogenetic position of the genus *Heterocapsa* in the Dinophyceae using SSU rRNA gene sequences.

4) To infer the phylogenetic relationships among *Heterocapsa* species using ITS regions.

5) To evaluate morphological characters which used to be used and newly introduced as the specific and generic criteria on the basis of the molecular phylogenetic information.

6) Finally, to propose the better understanding taxonomic system and to establish the monographic base for the genus *Heterocapsa*.

Of these, 1) and 2) are treated in Chapter 3, 3) and 4) are shown in Chapter 4, and 5) and 6) are discussed in Chapter 5.

## CHAPTER 2

## MATERIALS AND METHODS

### 2-1 Localities

*Heterocapsa* species investigated in the present study included unialgal cultures and preserved samples which consisted of the provided cultures from several culture collections such as Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP), North East Pacific Culture Collection at University of British Colombia (NEPCC = Canadian Centre for the Culture of Microorganism, CCCM), Microbial Culture Collection of National Institute for Environmental Studies (NIES), Scandinavian Culture Centre for Algae and Protozoa (SCCAP) and Plymouth Marine Laboratory (PLY), as well as cultures originally established. These samples were collected from coastal waters of Arctic Sea, Canada, Denmark, Hong Kong, Italy, Japan, U.K. and U.S.A (Figs. 2-1 and 2-2). Details of the collection dates, localities and isolators of all samples are given in Table 2-1.

### 2-2 Collection, isolation and culture

Unialgal cultures were established and maintained by the following procedure. Water and plankton-net samples collected at each site were immediately transferred in plastic bottles to the laboratory. For precultures, a 3 - 5 ml aliquot was inoculated into a plastic cup previously filled with 100 ml of ESM medium (Okaichi *et al.* 1982) containing 2.5 mg/ml germanium dioxide for preventing the growth of diatoms. The plastic cups were placed for several weeks in an incubator at 20°C, and 15 - 20 μmol photon $m^{-2}$ $s^{-1}$ light intensity provided by white

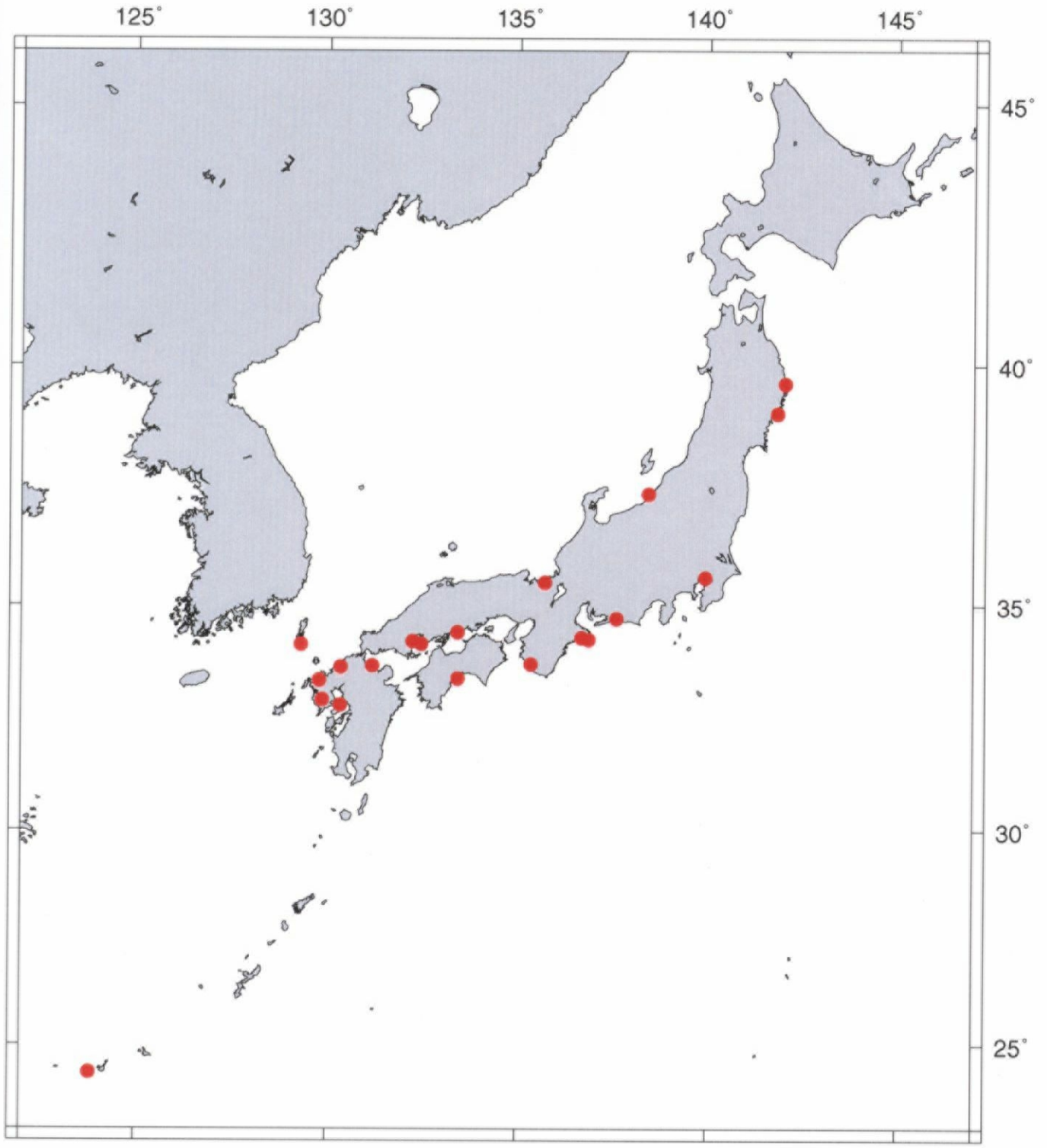


**Figure 2-1.** A map showing locations to collect samples of *Heterocapsa* species from Jananese coastal waters.

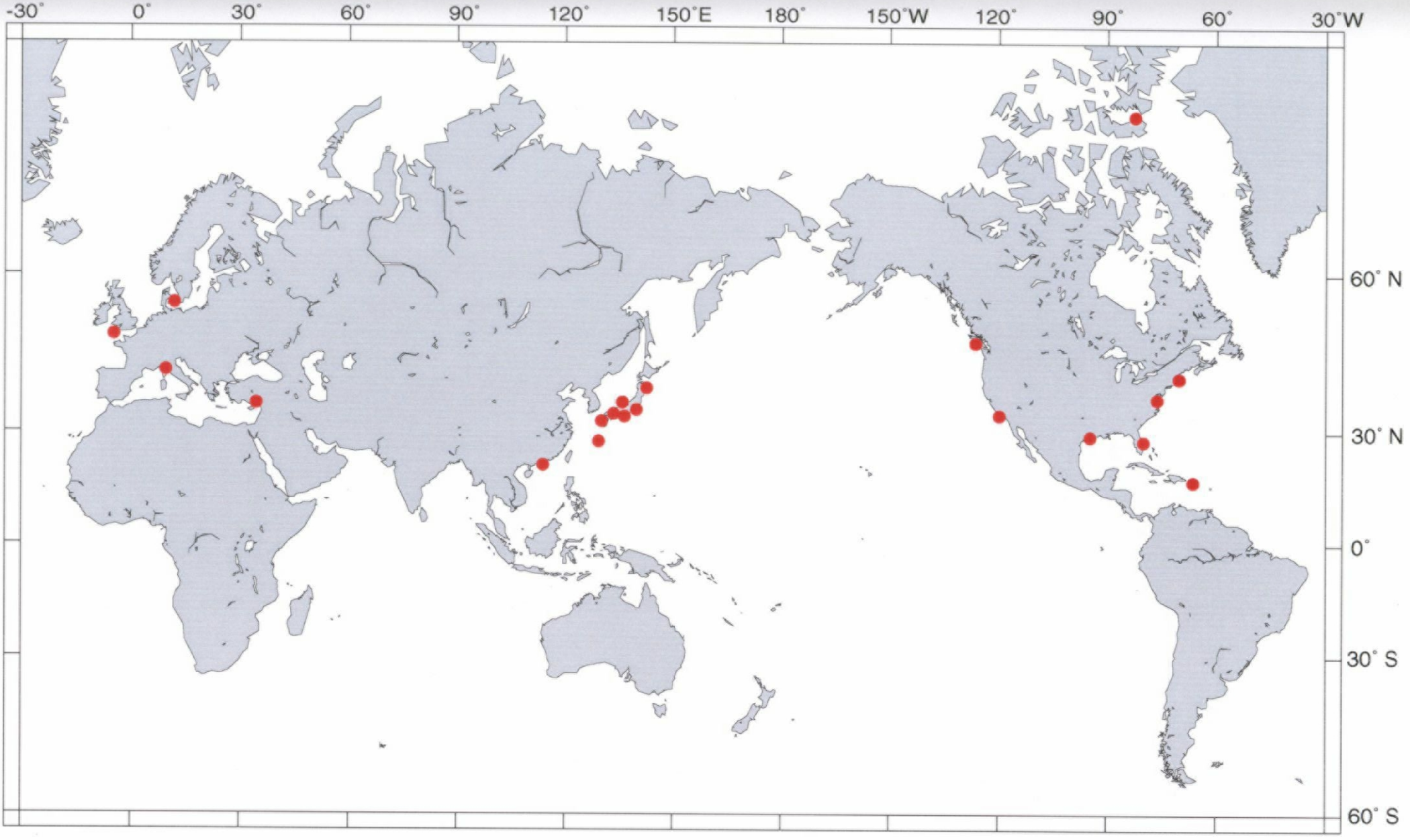


**Figure 2-2.** A map showing to collecting samples of *Heterocapsa* species providing to the present study.

**Table 2-1.** A list of culture strains and preserved samples examined in the present study.

| Species | Strain | Locality | Date | Isolator |
|---|---|---|---|---|
| ***H. arctica*** | CCMP445 | Arctic Sea | 1986.6.3 | R. Selvin |
| ***H. circularisquama*** | (fixed) | Tai Po Kau, Hong Kong | 1986.9 | M. W. Wong |
| | (fixed) | Yung Shue Au, Hong Kong | 1987.7 | M. W. Wong |
| | HA92-1 | Ago Bay, Mie | 1992.6 | T. Uchida |
| | (fixed) | Lake Hamana, Shizuoka | 1993.10.10 | K. Okamoto |
| | HCHS95 | Hiroshima Bay, Hiroshima | 1995.11 | H. Takayama |
| | HCLG-1 | Gokasyo Bay, Mie | 1998.8.6 | K. Nagasaki |
| | OK-1 | Obama Bay, Fukui | 1999.8.10 | H. Seto |
| | OK-3 | Obama Bay, Fukui | 1999.8.10 | R. Nakai |
| | FK811-3 | Fukuoka Bay, Fukuoka | 1999.8.11 | S. Itakura |
| | OA-1 | Obama Bay, Fukui | 1999.9.1 | H. Seto |
| | TG627-1 | Ago Bay, Mie | 2000.6.27 | S. Itakura |
| | TG627-2 | Ago Bay, Mie | 2000.6.27 | S. Itakura |
| | TG627-3 | Ago Bay, Mie | 2000.6.27 | S. Itakura |
| | TG710-1 | Ago Bay, Mie | 2000.7.10 | S. Itakura |
| | TG710-2 | Ago Bay, Mie | 2000.7.10 | S. Itakura |
| | TG710-3 | Ago Bay, Mie | 2000.7.10 | S. Itakura |
| | (fixed) | Omura Bay, Nagasaki | 2000.7.21 | H. Maruta |
| | TN830 | Tanabe Bay, Wakayama | 2000.8.30 | S. Itakura |
| | TG925-1 | Ago Bay, Mie | 2000.9.25 | S. Itakura |
| | TG925-2 | Ago Bay, Mie | 2000.9.25 | S. Itakura |
| | TG925-3 | Ago Bay, Mie | 2000.9.25 | S. Itakura |
| | HO-3 | Obama Bay, Fukui | | K. Nagasaki |
| | HU9433 | Uranouchi Bay, Kochi | 1994.3.3 | T. Uchida |
| | HB-5 | Buzen Bay, | | S. Itakura |
| ***H. illdefina*** | CCMP446 | Santa Barbara, CA, U.S.A. | 1973 | Sweeney |
| ***H. niei*** | CCMP447 | Puerto Rico | 1982.6 | L. Loeblich |
| | NIES420 | Iriomote Is., Okinawa | 1986.1 | T. Sawaguchi |
| | TG607-1 | Ago Bay, Mie | 2000.6.7 | S. Itakura |
| | TG607-2 | Ago Bay, Mie | 2000.6.7 | S. Itakura |
| ***H. pygmaea*** | CCMP1322 | Galveston, TX, U.S.A. | 1957 | Wilston |
| | CCMP1490 | Ligrian Sea, Italy | pre-1975 | M. Bernhard |
| | (fixed) | Perdido Bay, FL, U.S.A. | 2000.6.11 | K. Rhew |
| | (fixed) | Mersin Bay, Turkey | 2001.8.22 | Z. Uysal |
| | AK23 | Ariake Sound, Nagasaki | 2001.6.2 | S. Itakura |
| ***H. rotundata*** | K-479 | Isefjord, Denmark | 1984.3.15 | G. Hansen |
| | K-480 | Isefjord, Denmark | | G. Hansen |
| | | Tokyo Bay, Tokyo | 1999.9 | M. Iwataki |
| | (fixed) | Ariake Sound, Nagasaki | 1999.6.28 | H. Maruta |
| | TK12-D44 | Tokyo Bay, Tokyo | 1999.12.16 | M. Iwataki |
| | TK12-D45 | Tokyo Bay, Tokyo | 1999.12.16 | M. Iwataki |
| | TK6-D55 | Tokyo Bay, Tokyo | 2000.6.16 | M. Iwataki |
| | DFLS0102 | Lake Suigetu, Fukui | 2001.4.10 | R. Nakai |

(Continued)

**Table 2-1.**

| Species | Strain | Locality | Date | Isolator |
|---|---|---|---|---|
| ***H. tirquetra*** | PLY169 | Cornwall, U.K. | 1957.6.24 | I. Adams |
| | CCMP448 | Falmouth, MA, U.S.A. | 1979 | L. Brand |
| | K-447 | Denmark | | G. Hansen |
| | HTHS9906 | Hiroshima Bay, Hiroshima | 1999.6 | H. Takayama |
| | TK12-D40 | Tokyo Bay, Tokyo | 1999.12.16 | M. Iwataki |
| | TK12-D41 | Tokyo Bay, Tokyo | 1999.12.16 | M. Iwataki |
| | TK4-D39 | Tokyo Bay, Tokyo | 2000.4.9 | M. Iwataki |
| | CCMP450 | Damariscotta, ME, U.S.A. | | F. Jackoff |
| ***H. lanceolata*** **ms.** | (fixed) | Ariake Sound, Nagasaki | 1999.6.28 | H. Maruta |
| | (fixed) | Tokyo Bay, Tokyo | 1999.10 | K. Miyauchi |
| | TK6-D56 | Tokyo Bay, Tokyo | 2000.6.19 | M. Iwataki |
| | TK6-D57 | Tokyo Bay, Tokyo | 2000.6.19 | M. Iwataki |
| | TK6-D58 | Tokyo Bay, Tokyo | 2000.6.19 | M. Iwataki |
| | TK6-D59 | Tokyo Bay, Tokyo | 2000.6.19 | M. Iwataki |
| | TK6-D60 | Tokyo Bay, Tokyo | 2000.6.20 | M. Iwataki |
| | TK6-D61 | Tokyo Bay, Tokyo | 2000.6.20 | M. Iwataki |
| | TK6-D62 | Tokyo Bay, Tokyo | 2000.6.20 | M. Iwataki |
| | TK6-D63 | Tokyo Bay, Tokyo | 2000.6.20 | M. Iwataki |
| | HR7-D64 | Hiroshima Bay, Hiroshima | 2000.7.3 | M. Iwataki |
| ***H. horiguchii*** **ms.** | NIES614 | Kashiwazaki, Niigata | 1986.8 | T. Sawaguchi |
| | HCL99706-2 | Hiroshima Bay, Hiroshima | 1999.7.6 | K. Nagasaki |
| | FK6-D46 | Fukuyama, Hiroshima | 2000.6.2 | M. Iwataki |
| | FK6-D47 | Fukuyama, Hiroshima | 2000.6.2 | M. Iwataki |
| | FK6-D48 | Fukuyama, Hiroshima | 2000.6.2 | M. Iwataki |
| | FK6-D49 | Fukuyama, Hiroshima | 2000.6.2 | M. Iwataki |
| | DFGK0104 | Gokasho Bay, Mie | 2001.7.10 | R. Nakai |
| ***H. ovata*** **ms.** | NIES472 | Kashiwazaki, Niigata | 1986.8 | T. Sawaguchi |
| | KZHt1 | Kashiwazaki, Niigata | 2000.5.30 | T. Sawaguchi |
| ***H. pseudotriquetra*** **ms.** | CCMP451 | unknown (U.S.A.?) | | L. Brand |
| | NIES473 | Tsushima Is., Nagasaki | 1986.3 | T. Sawaguchi |
| | GH013 | Woods Hole, MA, U.S.A. | 1996.3 | G. Hansen |
| | NEPCC515 | unknown (Canada) | 1983 | K. Grell |
| ***H. orientalis*** **ms.** | D-8-A-2 | Ofunato Bay, Iwate | 1997.6.16 | K. Sekiguchi |
| | D-87-B-3 | Ofunato Bay, Iwate | 2000.1.11 | K. Sekiguchi |
| | D-127-B-3 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-B-4 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-B-5 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-B-6 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-C-1 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-C-2 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-C-3 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-C-4 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |
| | D-127-C-5 | Miyako Bay, Iwate | 2000.11.24 | K. Sekiguchi |

(Fixed) = Fixed natural sample, not clonal culture.

fluorescent tubes. Cells of *Heterocapsa* species in natural and pre-cultured samples were isolated by micropipette and inoculated in multi-well plate (Iwaki Glass), filled with 1 ml culture medium. Subsequently, clonal cultures were established in the test tubes. These unialgal strains were maintained at conditions similar to the preculture. The strains were used for morphological observations by light and fluorescence microscopes, and scanning and transmission electron microscopes, as well as molecular phylogenetic analysis.

**2-3 Light microscopy**

Cell shape, position of nucleus and pyrenoid, and swimming behavior were basically observed under an Olympus BX-60 microscope equipped with Nomarski differential interference contrast attachment. Cell sizes were measured by eyepiece micrometer on a Nikon Optiphot light microscope.

**2-4 Fluorescence microscopy**

For observation of thecal plate, cells were stained with Fluorescent Brightener 28 (Sigma) for 30 min or overnight. Although the dye solution was originally explained as 10 μg/ml (Fritz & Triemer 1985), it was dissolved more than 100 μm/ml at first, and immersion time was adjusted to suitable conditions for each sample in the present study. For several species possessing thin thecal plates such as *H. rotundata* and *H. lanceolata*, cells were prefixed by 1 - 2.5% $OsO_4$ solution for 1 hour to prevent the thecal plates scattering. Stained thecal plates were analyzed under UV excitation using an Olympus BX-60 fluorescence microscope.

## 2-5 Scanning electron microscopy

For scanning electron microscopy, a drop of suspended cells were fixed in the same volume of 4% osmium tetroxide made up with filtered seawater (w/v) for 30 min on a poly-L-lysine coated glass plate. Fixed cells cleaved onto the plate were rinsed twice in distilled water for 30 min. Fixed specimens were dehydrated through an ethanol series, and finally placed in isoamyl acetate. Cells were dried in critical point drier (Hitachi HCP-2), and coated with gold-palladium. Observations were performed with a scanning electron microscope (Hitachi S-800).

## 2-6 Transmission electron microscopy

### 2-6-1 Thin sections

For electron microscopic thin section preparations, cells in the culture strains were harvested by gentle centrifugation, and the pellets of cells were fixed by mixing a 2 ml cell suspension with 2 ml of 5% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) or filtered seawater. After 1 - 4 hours fixation, the cells were centrifuged and rinsed in the same buffer without fixative solutions for 15 min (3 changes of 5 min each). Then the pellets were fixed in 2% osmium tetroxide prepared in filtered sea water for 1 - 2 hours at 4°C, and were rinsed for 15 min (3 changes of 5 min each). The fixed materials were dehydrated in an ethanol series, 15 min in each change of 50%, 75%, 90%, 95% and 99.5% ethanol, and 45 min in absolute ethanol (3 changes of 15 min each). For further dehydration, the specimens were transferred to a 50 : 50 mixture of absolute ethanol and propylene oxide (v/v) for 15 min, followed by 30 min in

propylene oxide (2 changes of 15 min each). Propylene oxide was then replaced by a 50 : 50 mixture of propylene oxide and Spurr's resin (Spurr 1969) at room temperature. After 8 hours, the mixture was replaced with fresh Spurr's resin for 16 h (2 changes of 8 hours each). Specimens were embedded in the polyethylene cup containing fresh resin and polymerized at 70°C for 8 hours. Thin sections cut with an ultramicrotome (Reichert: Super Nova), were mounted on slit grids coated with polyvinyl formal films. Sections were then stained for 20 min in 2% uranyl acetate, and for 5 min in Reynolds' lead citrate (Reynolds 1963). Observations were made with JEM-1010 transmission electron microscope (JEOL).

### 2-6-2 Whole mount preparations

For observation of body scales, whole mounts were prepared by following procedure. A drop of cell suspension was placed on a Formvar-coated mesh grid and fixed with osmium vapor for 30 sec. It was allowed to dry, and then rinsed three times with distilled water. Cells were stained with 2% aqueous uranyl acetate for 1.5 min, and rinsed again. Stained body scales were observed under a JEOL JEM-1010 transmission electron microscope.

## 2-7 Molecular phylogeny based on SSU rRNA gene and ITS region sequence data

Cells were harvested by centrifugation of 1500 rpm for 10 min in 50 ml disposable centrifuge tube (CORNING). After centrifugation, supernatant was removed and the pellet was kept in deep freezer at -85°C until DNA extraction. Frozen pellets were allowed to melt in room temperature, and suspended in 10 times of NET buffer (w/v) in a 15 ml centrifuge tube. Sodium dodecyl sulfate solution (0.1%) and proteinase K (200 mg/ml) were added in the tube

and well mixed, then incubated at 55°C for an hour. Subsequently, same volume of PCI (phenol : chloroform : isoamyl alcohol = 1 : 1: 1) was added to the tube. The solution was pipetted repeatedly by use of a syringe in order to burst the plasma membrane. It was mixed for 30 min, and then centrifuged for 20 min at 3000 rpm, and DNA suspended in the supernatant fluid was transferred to a new tube. Same volume of PCI was added to the suspension and mixed for 10 min. After centrifugation, the supernatant was put to a new tube, one-tenth volume of sodium acetate added to the tube and mixed gently. Then, one-sixth volume of isopropyl alcohol was added. After 10 min, the solution was centrifuged at 3000 rpm for 20 min. The total genomic DNA was extracted as following; 1) the supernatant was removed, 2) 500 μl of 80% cold ethanol was added followed by centrifugation at 15000 rpm for 5 min, 3) the supernatant ethanol was removed, 4) the rest of DNA pellet was washed with cold 80% ethanol, and 5) dried to remove ethanol and redissolved in 100 μm TE buffer (1 mM Tris-HCl, 1 mM EDTA, pH 8.0) and stored at -20°C.

ITS1 and ITS2 regions including 5.8S ribosomal RNA coding regions (ITS region) were amplified with the polymerase chain reaction (PCR) protocol. The oligonucleotide primers used in this study were described by Adachi *et al.* (1994). For amplification of ITS region sequences, Ex Taq (Takara) was employed by following the manufacturer's recommendations. Amplification reactions were performed in an automated cycle as follows: preheating at 10 min at 95°C; twenty five amplification cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 45 - 55°C, and extension 2 min at 72°C; and a final extension for 6 min at 72°C. The reaction volume was 100 μl comprised 300 ng total genomic DNA, 8 μl of dNTP mix, 20 pmol each primer, 10 μl of 10x reaction buffer and 2.5 unit of Taq polymerase. Subsequently, the PCR products were gel-purified to isolate the amplified SSU rRNA gene. Low melting point agarose gels (NuSieve® GTG® Agarose, FMC Bioproducts) were prepared from 1% agarose in TAE buffer (50 mM Tris-HCl, 40 mM sodium acetate, 2 mM EDTA, pH 8.0). Electrophoresis was completed in TAE buffer at 50 V until the products had migrated into a half of the gel slice.

Then, the ITS band region (ca. 680 bp) in gel was cut out and moved to the tube. Same volume of TE buffer was added and incubated at 70°C for 15 min, subsequently β-agarase (5 unit/1 mol gel) was added to the tube and incubated at 45°C for an hour. The tube was centrifuged and the supernatant was transferred to a new tube. The ITS region was extracted with ethanol and ammonium acetate, and was stored with TE buffer by the same methods followed for total genomic DNA as mentioned above.

Double-stranded PCR products were directly sequenced using TAQ DyeDeoxy Terminator Cycle Sequencing Kit according to manufacture's recommendations (Perkin Elmer Cetus). Electrophoresis of sequencing reaction was completed on the ABI model 373A sequencer (Perkin Elmer Cetus).

To polarize the ingroup taxa, following three dinoflagellates ITS region sequences released by DDBJ/EMBL/Genbank databases were used as outgroup (DDBJ/EMBL/Genbank accession numbers are given in parenthesis); *Prorocentrum micans* Ehrenberg (AF208245), *Prorocentrum minimum* (Pavillard) Schiller (AF208244), and *Prorocentrum triestinum* Schiller (AF208246). Names of *Heterocapsa* cultures examined in this analysis are given in Table 2-2.

To determine the phylogenetic position of the genus *Heterocapsa* in dinoflagellates, small subunit ribosomal RNA (SSU rRNA) gene sequences of 72 dinoflagellates including 12 species of outgroup were analyzed. Species names and DDBJ/EMBL/Genbank accession numbers of all dinoflagellates used in this analysis are given in Table 2-3.

The sequences were aligned with these ITS region and SSU rDNA gene sequences using CLUSTAL X 1.8 computer algorithm for multiple sequence alignment (Higgins *et al.* 1995), and obscurely aligned region were removed from subsequent analysis.

Maximum parsimony analysis was performed by using PAUP computer package (version 3.1.1, Swofford 1993) on a Macintosh computer with the following options: Heuristic search sorting by random (10 replicates) sequential addition of taxa (Swofford & Olsen 1990), and branch swapping algorithm (tree bisection reconnection [TBR]). All nucleotide characters

**Table 2-2.** A list of dinoflagellates examined in the phylogenetic analysis of ITS region.

| Species | Strain | DDBJ/EMBL/Genbank accession number |
|---|---|---|
| *Heterocapsa arctica* Horiguchi | CCMP445 | * |
| *Heterocapsa circularisquama* Horiguchi | HA92-1 | * |
| | HB-5 | * |
| | HU | * |
| | OA-1 | * |
| | OK-1 | * |
| | OK-3 | * |
| *Heterocapsa illdefina* (Herman *et al.* ) Morrill *et al.* | CCMP446 | * |
| *Heterocapsa pygmaea* Loeblich III et al. | CCMP1322 | * |
| | CCMP1490 | * |
| *Heterocapsa rotundata* (Lohmann) Hansen | TK12-D44 | * |
| *Heterocapsa triquetra* (Ehrenberg) Stein | CCMP448 | * |
| | NIES7 | * |
| | TK12-D39 | * |
| *Heterocapsa lanceolata* ms. | TK6-D57 | * |
| *Heterocapsa horiguchii* ms. | NIES614 | * |
| | FK6-D47 | * |
| *Heterocapsa ovata* ms. | NIES472 | * |
| *Heterocapsa pseudotriquetra* ms. | NIES473 | * |
| *Prorocentrum micans* Ehrenberg | | AF208245 |
| *Prorocentrum minumumi* (Pavillard) Schiller | | AF208244 |
| *Prorocentrum triestinum* Schiller | | AF208246 |

* Sequences were decided by Ryuichi Nakai of Fukui Prefectural University.

**Table 2-3.** List of dinoflagellates examined in the phylogenetic analysis of SSU gene sequences.

| Species | Strain | DDBJ/EMBL/Genbank accession number |
|---|---|---|
| *Adenoides eludens* (Herdman) Balech | CCCM683 | AF274249 |
| *Alexandrium minutum* Halim | | U27499 |
| *Alexandrium tamarense* (Lebour) Balech | MUCC99 | AF022191 |
| *Amphidinium herdmanii* Kofoid & Swezy | CCCM532 | AF274253 |
| *Amphidinium longum* Lohmann | | AF274254 |
| *Amphidimium semilunatum* Herdmann | | AF274256 |
| *Amyloodinium ocellatum* | | AF080096 |
| *Cachonina hallii* (Freudenthal & Lee) | | AF033865 |
| *Ceratium furca* (Ehrenberg) Claparède & Lachmann | | AJ276699 |
| *Ceratium fusus* (Ehrenberg) Dujardin | CCMP154 | AF022153 |
| *Ceratocorys horrida* Stein | CCMP157 | AF022154 |
| *Cryptoperidonopis brodyi* | | AF080097 |
| *Fragilidium subglobosum* | | AF033869 |
| *Gloeodinium viscum* Banaszak, Iglesias-Prieto & Trench | | L13716 |
| *Glenodinopsis steinii* (Lemmermann) Woloszynska | NIES463 | AF274257 |
| *Gonyaulax spinifera* (Claparède & Lachmann) Diesing | CCMP409 | AF022155 |
| *Gymnodinium beii* Spero | | U37406 |
| *Gymnodinium catenatum* Graham | MUCC273 | AF022193 |
| *Gymnodinium fuscum* (Ehrenberg) Stein | MUCC282D | AF022194 |
| *Gymnodinium galatheanum* Braarud | KT-77B | AF172712 |
| *Gymnodinium sanguineum* Hirasaka | | U41085 |
| *Gymnodinium simplex* (Lohmann) Peters | | U41086 |
| *Gymnodinium* sp. | MUCC284 | AF022196 |
| *Gymnodinium* sp. | | AF274260 |
| *Gyrodinium dorsum* Kofoid & Swezy | UTEX LB2334 | AF274261 |
| *Gyrodinium impudicum* Fraga & Bravo | MUCC276D | AF022197 |
| *Gyrodinium uncatenum* Hulburt | CCCM533 | AF274263 |
| *Gyrodinium* sp. | | AB001438 |
| *Haplozoon axiothellae* Siebert | | AF274264 |
| *Heterocapsa niei* (Loeblich III) Morrill & Loeblich III | CCMP447 | AF274265 |
| *Heterocapsa pygmaea* Loeblich III, Schmidt & Sherley | CCCM681 | AF274266 |
| *Heterocapsa rotundata* (Lohmann) Hansen | CCCM680 | AF274267 |
| *Heterocapsa triquetra* (Ehrenberg) Stein | MUCC285 | AF022198 |
| *Karenia brevis* (Davis) Hansen & Moestrup | CCCM718 | AF274259 |
| *Karenia mikimotoi* (Miyake & Kominami) Hansen & Moestrup | MUCC098 | AF022195 |
| *Karlodinium micrum* (Leadbeater & Dodge) Larsen | CCCM555 | AF274262 |
| *Kryptoperidinium foliaceum* (Stein) Lindemann | UTEX LB1688 | AF274268 |
| *Lepidodinium viride* Watanabe *et al.* | MUCC247D | AF022199 |
| *Lingulodinium polyedrum* (Stein) Dodge | CCCM202 | AF274269 |
| *Pentapharsodinium tyrrhenicum* (Balech) Montresor *et al.* | MUCC097 | AF022201 |
| *Pentapharsodinium* sp. | CCMP771 | AF274270 |
| *Peridinium balticum* | | AF231803 |
| *Peridinium bipes* Stein | | AF231805 |
| *Peridinium foliaceum* (Stein) Biechler | | AF231804 |

(Continued)

**Table 2-3.**

| Species | Strain | DDBJ/EMBL/Genbank accessionnumber |
|---|---|---|
| *Peridinium umbonatum* Stein | UTEX LB2255 | AF274271 |
| *Peridinium willei* Huitfeld-Kaas | NIES304 | AF274272 |
| *Peridinium willei* Huitfeld-Kaas | NIES365 | AF274280 |
| *Peridinium* sp. | field isolate | AF022202 |
| *Pfiesteria piscicida* Glasgow *et al.* | | AF077055 |
| *Pfiesteria piscicida* Glasgow *et al.* | | AF080098 |
| *Polarella glacialis* Montresor *et al.* | | AF099183 |
| *Prorocentrum arenarium* | | Y16234 |
| *Prorocentrum concavum* Fukuyo | | Y16237 |
| *Prorocentrum emarginatum* Fukuyo | | Y16239 |
| *Prorocentrum lima* (Ehrenberg) Dodge | | Y16235 |
| *Prorocentrum maculosum* Faust | | Y16236 |
| *Prorocentrum mexicanum* Tafall | | Y16232 |
| *Prorocentrum micans* Ehrenberg | | M14649 |
| *Prorocentrum minimum* (Pavillard) Schiller | | Y16238 |
| *Prorocentrum panamensis* | | Y16233 |
| *Protoceratium reticulatum* (Claparède & Lachmann) Bütschli | CCCM535 | AF274273 |
| *Pyrocystis lunula* (Schütt) Schütt | CCCM517 | AF274274 |
| *Pyrocystis noctiluca* | CCMP372 | AF022156 |
| *Pyrodinium bahamense* Plate | | AF274275 |
| *Scrippsiella nutricula* | | U52357 |
| *Scrippsiella sweeneyae* Balech ex Loeblich III | CCCM280 | AF274276 |
| *Scrippsiella trochoidea* (Stein) Loeblich III | CCCM602 | AF274277 |
| *Symbiodinium corculorum* | | L13717 |
| *Symbiodinium meandrinae* | | L13718 |
| *Symbiodinium microadriaticum* Freudenthal | | M88521 |
| *Symbiodinium pilosum* | | X62650 |
| *Thoracosphaera heimii* (Lohmann) Kamptner | CCCM670 | AF274278 |

were assigned different weight to transitions versus transversions, that is, twice more weight to transversions than transitions. Alignment gaps were treated as missing data. Stability of groups was assessed with bootstrap analysis (1000 replications, Felsenstein 1985).

To convert to a distance matrix for neighbor joining analysis, the DNAdist algorithm of PHYLIP (Felsenstein 1995) was used. The Kimura two-parameter option was employed to compute evolutionary distances (Kimura 1980) for pairwise comparisons of all taxa in the alignment, and this distance matrix was converted to a phylogenetic tree using neighbor-joining algorithm (Saito & Nei 1987) of PHYLIP. Bootstrap resampling (1000 replications) was completed to estimate the robustness of internal branches.

## CHAPTER 3

## MORPHOLOGY, ULTRASTRUCTURE AND TAXONOMIC DESCRIPTIONS

### 3-1 Characteristics of the genus *Heterocapsa* and its emendation

The genus *Heterocapsa* was originally established as a taxon of dinoflagellates commonly possessing sutured epitheca and unsutured hypotheca (see Section 1-3). At present, however, this generic criterion has lost the diagnostic value for *Heterocapsa* species recently regarded, because it is obvious for the hypotheca to be sutured. Consequently, we need to find out appropriate definition much more closely to the genus, instead of old one proposed by Stein (1883). The thecal plate arrangement of a whole cell and presence of three-dimensional body scales supposed to be potent for generic criteria. In the present study, these assumed morphological characters for generic criteria are reinvestigated by using seven known and five new *Heterocapsa* species with special reference to their taxonomic significance. First of all, the most fruitful results obtained in this study is given as the emendation for the genus.

**The genus *Heterocapsa* Stein emend. Iwataki & Fukuyo**

Unicellular, thecate, photosynthetic dinoflagellate. Typical thecal plate arrangement Po, cp, 5', 3a, 7'', 6c, 5s, 5''', 2''''. Chloroplast parietal, containing peridinin as major carotenoid, with pyrenoid. Eyespot lacking. Three dimensional, triradiate body scale present.

Type species

*Heterocapsa triquetra* (Ehrenberg) Stein 1883

Synonym

*Cachonina* Loeblich III 1969

### 3-1-1 Light microscopy

Compared to other dinoflagellates, cells of *Heterocapsa* species were relatively small, with size ranging from 8.8 μm (*H. rotundata*, TK12-D44 strain) to 37.5 μm (*H. arctica*, CCMP 445 strain) in length, and 7.2 μm (*H. rotundata*, TK12-D44 strain) to 28.0 μm (*H. ovata* ms., KZHt1 strain) in width. With exceptions of some very small species such as *H. rotundata* and *H. pygmaea*, average cell sizes of the most species were between 15 to 30 μm in length (Table 5-1). Cells of *H. rotundata* were clearly smaller than others, but cell sizes of many species overlapped each other and seemed to have few significant differences among species. Therefore, cell size appears to be unsuitable character for distinguishing species.

Cells of *Heterocapsa* exhibited normal dinoflagellate configuration (Fig. 3-1-1, a - j), namely it consisted of the epitheca and hypotheca, and possessed longitudinal and transverse flagella housed in sulcus and cingulum. A cingulum usually displaced about 1/2 – 1/3 of its own width. All species examined were thecate on their whole cells. Thecal plates of almost all species, however, were quite thin and difficult to observe at the light microscope level of resolution. Therefore, many of them superficially appeared to be gymnodinioid dinoflagellates. The plates could be sometimes observed in the cultures alone, because they shed their thecal plates. The cell shapes were spherical or ellipsoidal, the epitheca and hypotheca were hemispherical or conical and almost same in size, but included some variations. These cell shapes seemed to be stable in each species. For example, *H. triquetra* and *H. lanceolata* ms. possessed a horn at the posterior end, and *H. arctica*, *H. rotundata* and *H. lanceolata* had markedly larger epitheca than their hypotheca. These species with characteristic cell shapes could be distinguished from others. Other species exhibited normal dinoflagellate shapes were merely subdivided into two groups; species having ellipsoidal shape such as *H. niei*, *H. illdefina* and *H. circularisquama*, and others having large and somewhat spherical shape such as *H. ovata*

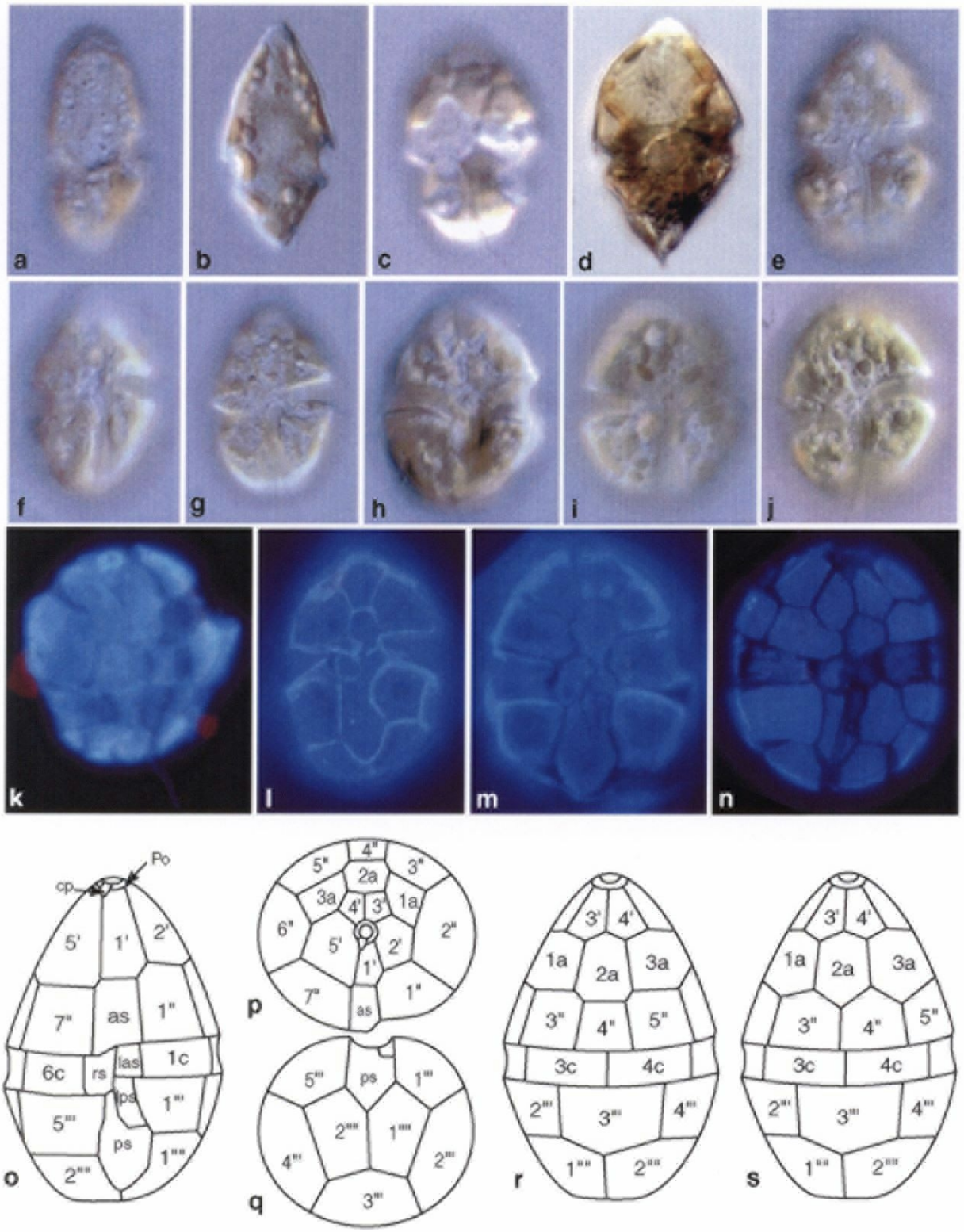


**Figure 3-1-1.** Cell shapes and thecal plate arrangement of *Heterocapsa* species.

**a-j**. Light microscopy showing cell bodies resembling gymnodinioid dinoflagellates.
**k-l**. Thecal plates under fluorescence microscope.
**o-s.** Schematic drawings of typical thecal plate arrangement. o, ventral view; p, apical view; q, antapical view; r, dorsal view with seven-sided 2a; s, dorsal view with six-sided 2a. Numbers of thecal plates are referred for abbreviations of Plates.

ms. and *H. pseudotriquetra* ms.

All *Heterocapsa* species are autotrophic, possessing yellowish brown chloroplast peripnery situated, and an eyespot lacked. A dinokaryotic nucleus and a pyrenoid (rarely two pyrenoids) surrounded by starch sheaths were present. Positions of these organelles in cytoplasm varied in each species.

Swimming behavior of *Heterocapsa* species is quite characteristic. These do not swim at constant speed. The small species, *H. rotundata* frequently stops suddenly during gentle swimming, whereas ellipsoidal species, especially *H. circularisquama*, often repeated backward and forward quickly. Moreover, relatively large species e.g. *H. ovata* swims with vibration. Many *Heterocapsa* species are morphologically quite similar to other dinoflagellates, e.g. *Gymnodinium* and *Scrippsiella*, but they could be often distinguished from these genera by their characteristic swimming behavior.

### 3-1-2 Thecal plate arrangements

Although observation of thecal plates of *Heterocapsa* species under light microscope were rather difficult, it could be carried out by use of fluorescence microscope with ultraviolet excitation after Fluorescent Brightener 28 staining (Fig. 3-1-1, k – n). Thecal plates of *H. rotundata* and *H. lanceolata* ms. were especially thin and fragile, and the plates usually got scattered when the cells died. In such cases, cells were prefixed with osmium tetroxide to analyze thecal plate arrangements. Plate arrangements of other species could be determined without fixation. No ornamentations such as wings or spines were found on the thecal plates. Most common thecal plate number of *Heterocapsa* species is 35, which includes Po, cp (or X), 5', 3a, 7", 6c, 5s (as, rs, las, lps, ps), 5"', 2"" (Fig. 3-1-1, o - s). In spite of morphological variability, plate number seemed to be stable in each species. Plate numbers were slightly

variable in the same culture strain, with extra, lacking or misplaced sutures were often found. The common plate arrangements were regularly observed in all the species, and therefore, could be considered as typical thecal plate arrangement of this genus.

The anterior part of thecal plates consisted of an apical pore plate (Po) and a canal plate (cp, or X plate), both of which were surrounded by five plates of the apical series (Fig. 3-1-1, p). The Po plate was U-shaped and located at apical part of the cell. The cp plate was rather small and rhomboid, located in the opening of the Po plate, which slightly off-centered from ventral to right direction. The cp plate also bordered with two apical plates, 1' and 5'. The plate 1' contacted with not only the cp plate but also the Po plate. Since anterior part of the 1' plate of peridinioid genera such as *Scrippsiella* contacts only with the X plate, thus the suture between the Po plate and the 1' plate of *Heterocapsa* seemed rather characteristic.

As shown in ventral view (Fig. 3-1-1, o), posterior end of the first apical plate 1' of *Heterocapsa* stops in the middle of the epitheca, while the 1' of *Scrippsiella* reaches to the cingulum. Depending on the short 1' plate, the anterior sulcal plate (as) of *Heterocapsa* deeply penetrated into the epitheca. The arrangement of the 1' and the as plates is one of the most distinctive feature of the genus *Heterocapsa*.

In dorsal view (Fig. 3-1-1, r, s), anterior end of the second anterior intercalary plate (2a) of *Heterocapsa* was an obtuse angle and bordered with the apical plates 3' and 4'. That of *Scrippsiella* is flat and borders with only the 3' plate. Therefore, this arrangement should be also one of the plate characteristics of the genus. The plate 2a was usually seven-sided and contacted with three precingulars, 3", 4" and 5". It sometimes changed to six-sided because that the posterior end borders with only two precingular of the 3" and the 4" plates, shifting slightly to left direction.

The cingular plate series consisted of six plates. The number seemed to be stable in the culture strains.

The sulcal plate series was composed of five plates; anterior sulcal (as), right sulcal (rs),

left anterior sulcal (las), left posterior sulcal (lps) and posterior sulcal (ps) plates. The as plate was situated neither in the sulcus nor cingulum, but seemed to be penetrated into the epitheca. The rs and las plates contacted with the 6c and 1c of the cingular series respectively. The lps plate which bordered with the rs, las and ps plates was the smallest in the sulcal series. The ps plate was the largest, and occupied the major part of the sulcus. Another small fragments were sometimes found in the sulcal part, however stability of these plates was not confirmed. Since numbers and arrangements of the sulcal plates could have been observed under the flattened condition, it is possible that these contained artificial fragments.

The hypotheca, consisting of post cingular series and antapical plate series, had 5 and 2 plates, respectively. This pattern is almost the same as those of other marine peridinioid dinoflagellates e.g. *Scrippsiella*, *Ensicurifera* and *Protoperidinium*.

### 3-1-3 Pyrenoid

Pyrenoid was found in all specimens. Therefore, the presence of the pyrenoid appeared to be one of the characteristics for the genus *Heterocapsa*. However, the number and position, and the presence or absence of the starch sheaths surrounding the pyrenoid and tubular invaginations in its matrix varied depending on culture strains, species and culture conditions (Fig. 3-1-2).

Pyrenoids were usually found solitary, and surrounded by several starch sheaths in almost all *Heterocapsa* species. The position of pyrenoid could be easily observed at high magnification under light microscope due to the presence of the starch sheaths. However, pyrenoid without starch sheaths was also found in *H. pygmaea* (CCMP 1322 and CCMP1490 strains), *H. rotundata* (TK12-D44 strain), *H. lanceolata* (TK6-D57 strain) and *H. orientalis* (D-87-B-3 strain). Under transmission electron microscope, *H. pygmaea* sometimes possessed two or more pyrenoids in their cytoplasm (Plate 17). Position of the pyrenoid was generally

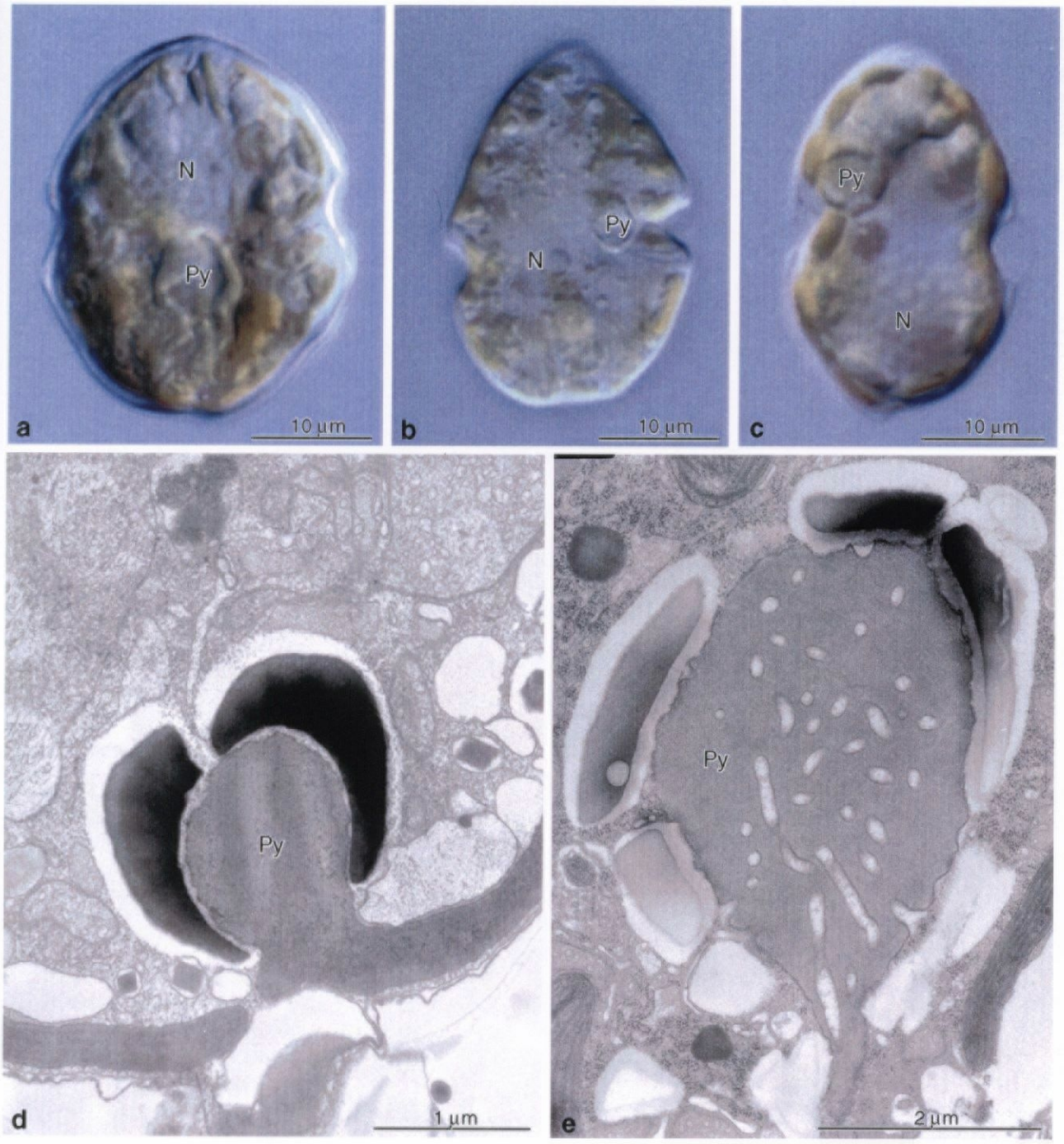


**Figure 3-1-2.** Positions and ultrastructures of pyrenoids.

**a**. A pyrenoid located in the posterior half of the cell that immediate beneath of a spherical nucleus (*H. orientalis*); **b**. a pyrenoid located in the middle of the cell with ellipsoidal nucleus (*H. illdefina*); **c**. a pyrenoid located above spherical nucleus in the anterior half of the cell (*H. niei*).
**d, e**. TEM images of pyrenoids, **d**, without tubular invaginations in the matrix (*H. niei*); **e**, many tubular invaginations are present in the matrix (*H. orientalis*).

stable in each species, for example, that of *H. triquetra* was located in the posterior of cells, whereas it was located in the anterior in *H. niei*. As shown in Figure 3-1-2, a-c, nuclei were located at the opposite side of the pyrenoids in the cytoplasm. These configurational relationships seemed to be stable characteristics. Since the pyrenoid and nucleus could be easily observed under light microscopy, it would be useful to distinguish to species of *Heterocapsa*.

Cytoplasmic tubular invaginations in the pyrenoid matrix were found in *H. arctica*, *H. illdefina*, *H. triquetra*, *H. ovata*, *H. orientalis* and *H. pseudotriquetra*. This character could be found in all cells of these species. In other species, pyrenoid matrices were free from any structures, such as tubular invaginations and thylakoids.

The pyrenoids of all species of *Heterocapsa* were associated with the chloroplasts by one isthmus or several isthmi.

### 3-1-4 Body scale

Organic body scales were recognized on the cell surface of all *Heterocapsa* species (Fig. 3-1-3). Scanning electron microscopy revealed that those of some species were sparsely distributed on the membrane (Fig. 3-1-3, e), but others were densely deposited and made a stratum (Fig. 3-1-3, a - c). Both of which were directly contacted with the cell body by its basal plate. In the cytoplasm, body scales were also found in the vesicle located nearby the Golgi bodies (Fig. 3-1-3, d). Many of these vesicles were situated beside basal body. These facts imply that the body scale are produced in the Golgi vesicles and released to outside of the cell from somewhere near the proximal part of the flagellum, consequently they surround the cell body.

The body scales of *Heterocapsa* species commonly consisted of the basal plate and the spine-like uprights by which the scale was made up three-dimensional structure. The

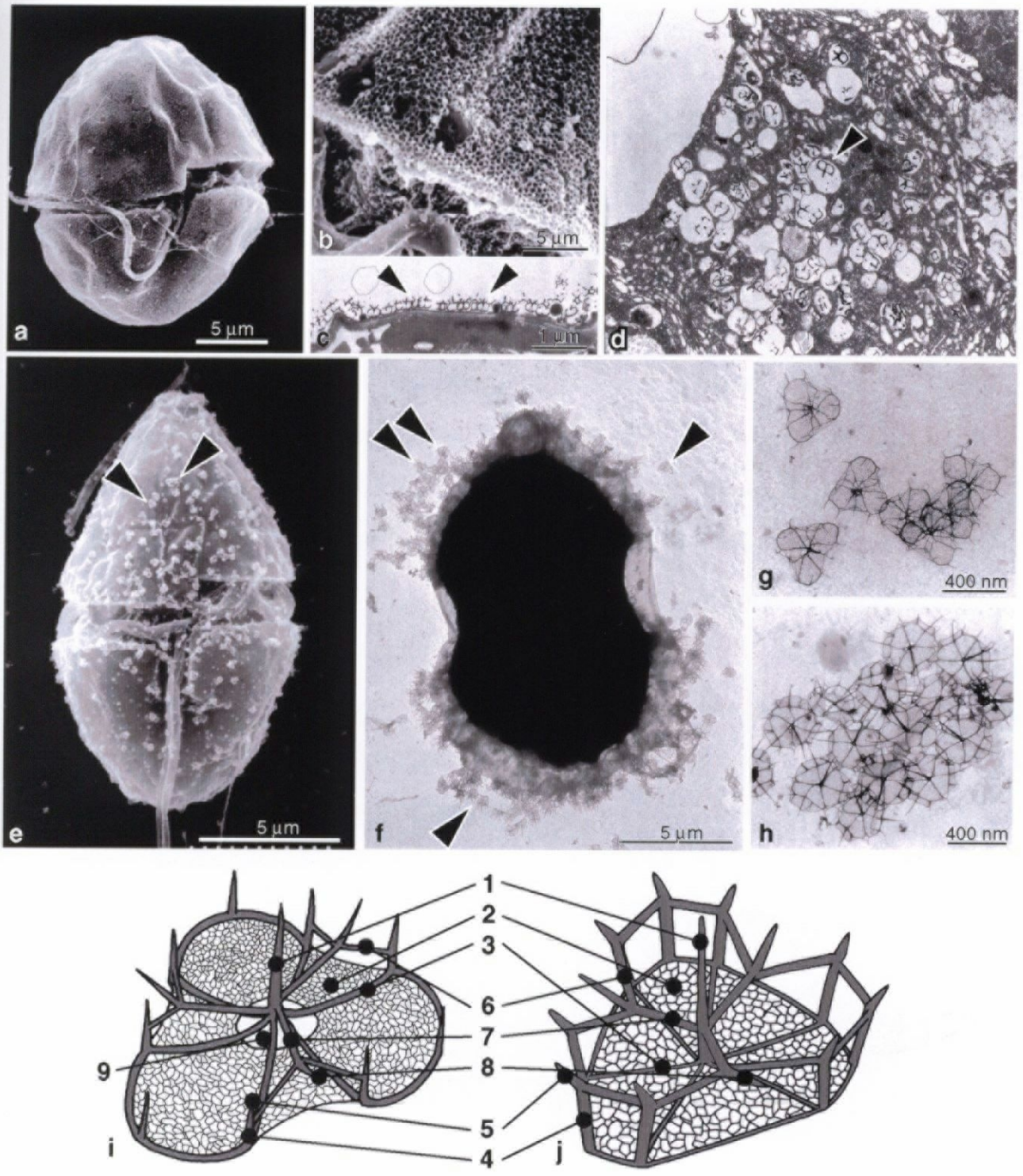


**Figure 3-1-3.** Body scales of *Heterocapsa* species.

**a**, **b**. Scanning electron microscopy of cell, note body scales surrounding the cell surface; **c**. Transmission electron microscopy of cell. Single layer of body scales is located on the plasma membrane; **d**. Body scales are seen within Golgi vesicles (a-d, *H. ovata*); **e**. SEM of cell; **f**. TEM of cell made by whole mount preparation (e, f, *H. circularisquama*); **g, h**. body scales under whole mounts (g, *H. rotundata*; f, *H. lanceolata*). Arrowheads show body scales. **i**, **j**. Diagrammatic illustrations of body scale ultrastructure. 1, central upright (or spine); 2, basal plate; 3, ridge; 4,peripheral upright; 5, peripheral spine; 6, peripheral bar; 7, radiate bar; 8, radiate spine; 9, central hole.

diagrammatic illustrations of the *Heterocapsa* body scale are given in Fig. 3-1-3, i and j. Scales were triradiate symmetry in the plain figure, namely it consisted of three equivalent parts. The basal plate was triangular, circular or hexagonal in outline, and the plate itself had fine reticular or fibrous texture. A long vertical upright rose at the center of the basal plate, and several uprights stood at each corner of the basal plate. These uprights are hereafter termed as the central upright and peripheral uprights, respectively. Numbers of the peripheral uprights varied in triploidy from each species, because all of the *Heterocapsa* scales were triradiate structure. From proximal end of the central upright, three or six ridges radiated along with the basal plate and connected with peripheral uprights. Distal parts of peripheral uprights were connected to each other by horizontal bars (peripheral bars). Moreover, three other bars radiated from proximal or middle part of central upright toward horizontal bars. Consequently, from each junction of bars and uprights, a short spine rose to somewhat radiate direction.

These ultrastructural features were commonly found in all *Heterocapsa* species. Possession of triradiate body scales composed of reticular basal plate and spine-like uprights or bars, should be a definitive characteristic of *Heterocapsa* species. Moreover, the number of these uprights and spines differed among species; the ultrastructure could be used as a specific characteristic of *Heterocapsa* species.

### 3-2 Descriptions of each species

In the present study, seven species in nine valid *Heterocapsa* species (see Section 1-3) could be re-examined and five new species introduced. In this section, these described species are firstly shown in alphabetical order of their species names, and undescribed species are subsequent to them. Descriptions of five new *Heterocapsa* species do not aimed to have nomenclatural validity in this thesis. The original descriptions with Latin diagnoses and

designation of holotype for these species are now in manuscript and will be published elsewhere as the first valid descriptions by myself and collaborators written in each section. Two *Heterocapsa* species that could not be investigated in the present study are also described additionally.

**3-2-1 *Heterocapsa arctica* Horiguchi** (Fig. 3-2-1, Plate 1, 13, 24)

Horiguchi, 1997, p. 491, Figs. 1-9.

Cells of *Heterocapsa arctica* are ellipsoidal and consist of a considerable large epitheca and a small hypotheca (Plate 1). The epitheca is 1.5 to 2.0 times as long as the hypotheca. Cell sizes are 22.5-37.5 μm (mean 29.6 μm) in length, and 10.0-15.0 μm (mean 11.6 μm) in width. The cingulum is displaced by almost a half of its width. The sulcus almost reaches the antapex of the cell and extends deeply into the epitheca. A chloroplast is located peripherally and occasionally perforated. The pyrenoid is spherical, surrounded by starch sheaths, and situated in the upper part of the hypotheca. The nucleus is ellipsoidal and located in the middle of the cell. Many lipid bodies of various sizes are scattered throughout the cytoplasm, largely concentrated in the apical part of the cell.

The cells of *H. arctica* resemble the gymnodinioid dinoflagellate under light microscope, but it possesses relatively thin thecal plates (Plate 1). The thecal plate arrangement are Po, cp, 5', 3a, 7", 6c, 5s (as, rs, las, lps, ps), 5"', 2"" (Fig. 3-2-1). The plate 2"" is slightly larger than the 1"" plate and covers the antapical part of the cell. All plates are relatively large and elongated to longitudinal direction due to its ellipsoidal cell body, however, the number and the arrangement are typical for the genus.

Ultrastructural features of a nucleus, chloroplasts, a pyrenoid and trichocysts were

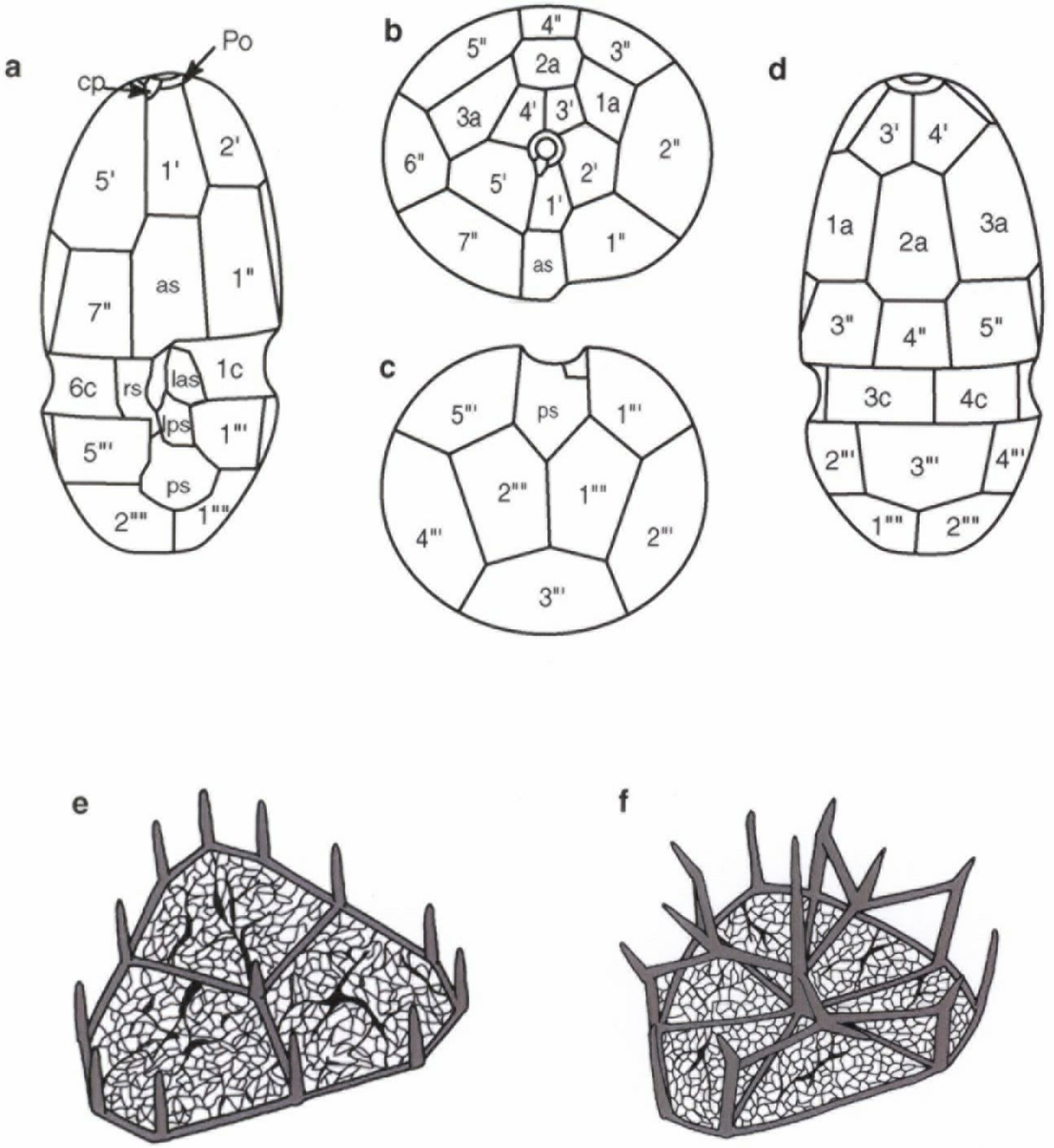


**Figure 3-2-1.** *Heterocapsa arctica* Horiguchi.

**a-d**. Diagmammatic illustrations of thecal plates; **a**, ventral view; **b**, apical view; **c**, antapical view; **d**, dorsal view.
**e, f**. Body scale; **e**, immature scale; **f**, mature scale.

observed (Plate 13). The dinokaryotic nucleus is ellipsoid, located in the middle of the cell. The chloroplast is situated peripherally in the cell. The pyrenoid is connected with the chloroplast with one stalk. It contains many tubular invaginations of chloroplast envelopes in the matrix, and surrounded by several starch sheaths. Amorphous electron opaque inclusions were present in each tubular invagination, which showed a stellate shape in the section (Plate 13, 2). Electron dense lipid globules are abundant in the apical and antapical part of the cytoplasm. Typical trichocysts of dinoflagellates are also present.

The body scales of *H. arctica* are triangular and similar to that of *H. triquetra* (Plate 24). Two types of body scales are present (Fig. 3-2-1, e, f). The one is structurally simpler scale without horizontal bars (Fig. 3-2-1, e), and the other is more complicated scale with horizontal bars (Fig. 3-2-1, f). These two types can be also seen in the original publication of *H. arctica* (Horiguchi 1997, Fig. 9). The basal plate of the scale is composed of reticulation, but the texture is relatively rough. The simpler scale possesses a central spine and twelve peripheral spines. All these spines are relatively short. From the proximal part of the central spine, three ridges radiate along the basal plate and reach the base of three peripheral spines, which stand on the central part of each limbus. The other complex type of the scale possesses horizontal bars between peripheral uprights except three uprights standing on the corner. Another bars elongated from almost proximal part of the central upright to the middle of the horizontal bars. Six ridges are present in the mature scale. Positions of ridges in the two types of scale differ from one another. Regarding the positions, three ridges of the simpler scale appear to have a structure homologous to three radiating bars of the developed scale. If this is true, the mature scale is made from simpler scale, by growing in an upward direction. Uprights of mature scales are actually thicker than the immature ones. Other structures of these two scales seem to be identical.

A cell of *H. arctica* is easy to be distinguished from all other *Heterocapsa* species from cell size and a remarkably large epitheca. The large epitheca is also found in *H. lanceolata* and

*H. rotundata*, but these two species are rather small in cell size. Body scales of *H. arctica* are superficially similar to those of *H. triquetra*, as indeed Horiguchi (1997) mentioned that body scales of the species were triangular type. However, number of peripheral bars is different from that of *H. triquetra*. Number of these ornamentations is identical to that of *H. rotundata*, although the scale of *H. rotundata* possesses a central hole. Therefore, *H. arctica* can be distinguished from others on the basis of the body scale ultrastructure.

The culture strain of *H. arctica* was originally collected from the arctic ice sample in 1986 by S. Apollonio and isolated by R. Selvin, and now it is maintained as CCMP 445 strain. The species *H. arctica* is described using this strain (Horighchi 1997), and the species is known only from this Arctic strain. It is usually maintained at 4 °C, and grows only under this extraordinarily low temperature condition.

**3-2-2 *Heterocapsa circularisquama* Horiguchi** (Fig. 3-2-2, Plate 2, 14, 25, 26)

Horiguchi, 1995, p. 130, Figs. 1-24.

Iwataki, Wong & Fukuyo in press,

Synonym

*Heterocapsa* sp.

Matsuyama *et al.*, 1995, p. 36, Fig. 2.

Cells of *H. circularisquama* are ellipsoid and consist of a conical epitheca and a hemispherical hypotheca (Plate 2). The epitheca is almost same size as the hypotheca. Cell sizes are 16.0–26.4 μm (mean 23.7 μm, n = 30) in length, and 11.2-19.2 μm (mean 15.5 μm, n = 30) in width (HCHS95 strain). The cingulum is relatively wide and is displaced by about 1/3 of its

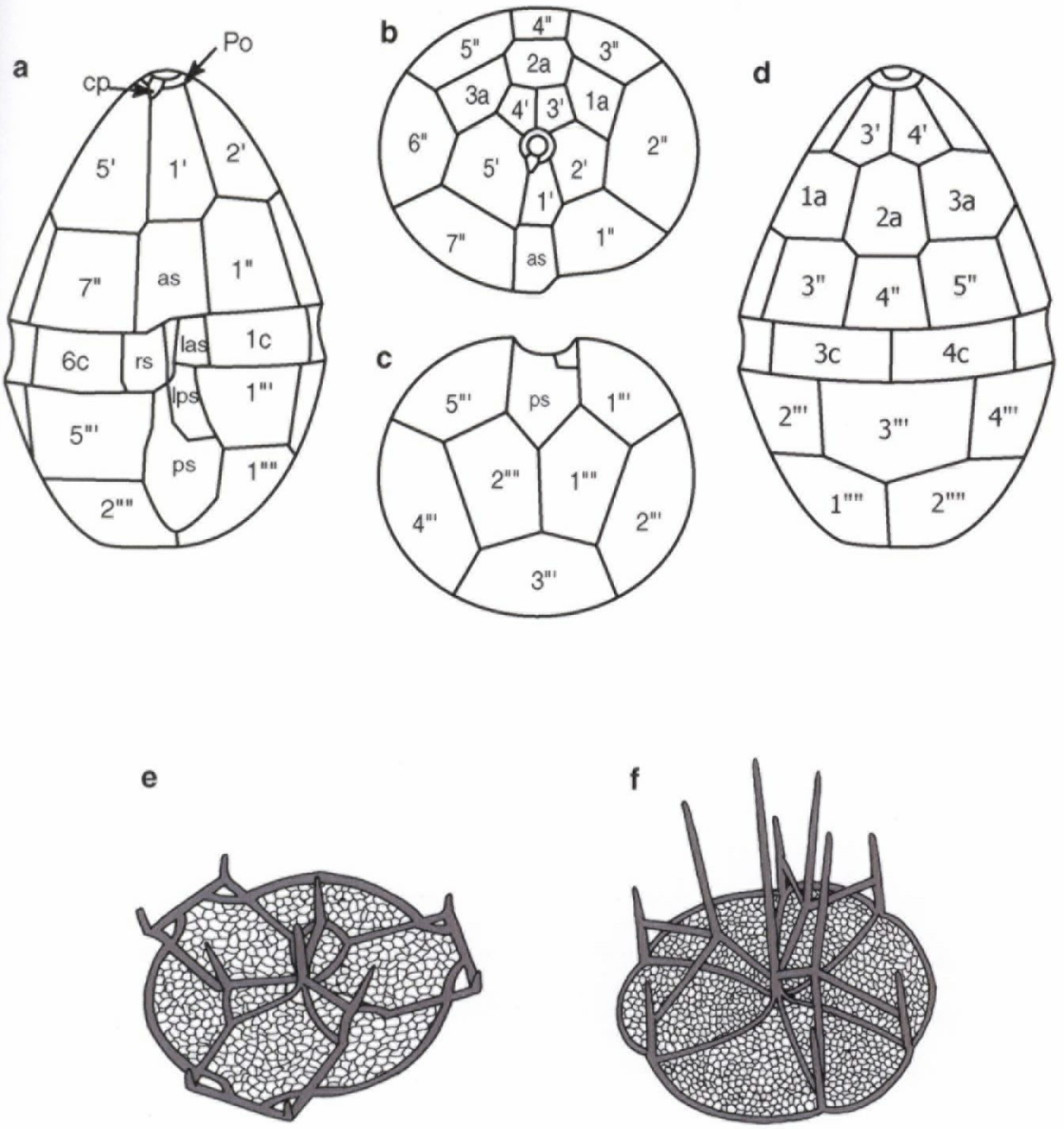


**Figure 3-2-2.** *Heterocapsa circularisquama* Horiguchi.

**a-d**. Diagmammatic illustrations of thecal plates; **a**, ventral view; **b**, apical view; **c**, antapical view; **d**, dorsal view.
**e, f**. Body scale; **e**, immature scale; **f**, mature scale.

own width. The sulcus almost reaches the antapex of the cell. The parietal chloroplast is single and occasionally perforated. The pyrenoid is spherical, surrounded by starch sheaths, and situated in the upper part of the hypotheca. The nucleus is elliptical and is located in the left side of the cell.

The thecal plates of *H. circularisquama* are quite thin (Plate 2). The plate formula is Po, cp, 5', 3a, 7", 6c, 5s (as, rs, las, lps, ps), 5"', 2"" (Fig. 3-2-2). The variation of the 2a plate is found.

Under transmission electron microscope, an ellipsoidal nucleus is elongated from the epitheca to hypotheca. The chloroplast is peripherally situated (Plate 14). A spherical pyrenoid is prominent from chloroplast with an isthmus. It does not possess tubular cytoplasmic invaginations, and is surrounded by starch sheaths. Electron dense lipid globules are found in the apical part of the cytoplasm. Typical trichocysts of dinoflagellates are also present.

The body scales of *H. circularisquama* are circular in outline, and it is the most distinctive feature of the species (Plate 25, 26). The basal plate of the scale is composed of fine texture of reticulation. A long central upright rises at the center of the basal plate, from which six ridges radiate towards the rim. Six peripheral uprights stand vertically from the junction between the ridges and the rim, where is slightly indented. At the distal part of each peripheral upright, one side of a peripheral bar is connected. Six peripheral bars connect each other and make a three pairs of peripheral upright. The junctions of each bar and almost proximal part of the central upright are connected by radiating bars. Moreover, spines are projected from all junctions between the uprights and the bars. Furthermore, quite thin threads sometimes connect with tips of these spines. However, this structure was not always observed. This structure of the body scale could be found as a major scale in all culture strains and preserved field samples, but another type of the body scale has also been found. Even though the latter scales are few in numbers, these have been found from all cultures of *H. circularisquama* (Plate 25, 26). It is

appropriate that the former type is a mature body scale and the other is an immature one as those of *H. arctica* scales. All spines of the mature scale are longer than the immature scale. The immature body scale also possesses six ridges, but these are not contacted with the rim.

Cell of *H. circularisquama* is much less diagnostic, hence it is difficult to discern from other species. Cells of *H. horiguchii* are slightly small and *H. illdefina* are somewhat dorsoventrally flattened, but appear quite similar with each other under light microscope. *H. circularisquama* is distinguishable based on the ultrastructure of body scales and possession of tubular invaginations in pyrenoid matrix.

The type culture strain of *H. circularisquama* was originally collected from Ago Bay in 1992 by Mizuguchi at the Fisheries Research Center of Mie, and the monoclonal culture was established by Horighchi at Hokkaido University. This species was also found from many coastal waters in the western Japan and Hong Kong. It is only one species that is has been known as a causative species for bivalves mass mortality.

### 3-2-3 *Heterocapsa illdefina* (Herman & Sweeny) Morrill & Loeblich III

(Fig. 3-2-3, Plate 3, 15, 27)

Morrill & Loeblich III, 1981, p. 63, Figs. 7, 8.

Sournia, 1982, p. 158.

Synonym

*Cachonina illdefina* Herman & Sweeney 1976

Herman & Sweeney, 1976, p. 204, Figs. 1-6.

*Cachonina niei sensu* Balech 1977

Balech, 1977, p. 60, Figs. 1-20.

Cells are elliptical and more or less dorsoventrally flattened, consisting of a conical epitheca and a hemispherical hypotheca (Plate 3). The epitheca is almost same size or slightly larger than the hypotheca. Cell sizes are 23.2-35.2 μm (mean 29.2 μm, n = 30) in length, and 18.6-24.0 μm (mean 19.8 μm, n = 30) in width (CCMP 446 strain). The cingulum is relatively wide and displaced by about 1/3 of its own width. The sulcus almost reaches the antapex of the cell. The light yellow green chloroplast is located in the cell peripherally and occasionally perforated. The pyrenoid is spherical, rarely surrounded by starch sheaths, and situated in the lower part of the epitheca or almost near the cingulum. The nucleus is elongate and located throughout the epitheca to hypotheca.

The thecal plates of *H. illdefina* are very thin (Plate 3). The thecal plate arrangement is Po, cp, 5', 3a, 7", 6c, 5s (as, rs, las, lps, ps), 5"', 2"" (Fig. 3-2-3). Variation of the plate 2a is present.

Under transmission electron microscope, an ellipsoidal nucleus is located in the hypotheca to the middle of the cell (Plate 15). The chloroplast is situated peripherally. The pyrenoid is usually single, but sometimes multiple. Electron transparent starch sheaths were rarely observed to surrounding the pyrenoid. It has only one connection with chloroplast. The tubular invaginations are present. Typical trichocysts of dinoflagellate are also present.

The body scales of *H. illdefina* are triangular in outline (Fig. 3-2-3, Plate 27). Basal plate of the scale is relatively rough. A long central upright rises at the center of the basal plate, from which six ridges radiate. Nine peripheral uprights stand vertically from nearby the corners of the triangle, forming three triplets. Each two outward upright of the triplets are connect with next uprights of another triplets by peripheral bars. They make junction which are somewhat uplifted. Three bars radiate from the proximal region of the central upright, and connected with three junctions. Six radiating ridges are in contact with the peripheral bars by small uprights. Moreover, spines are projected from all junctions between the uprights and the

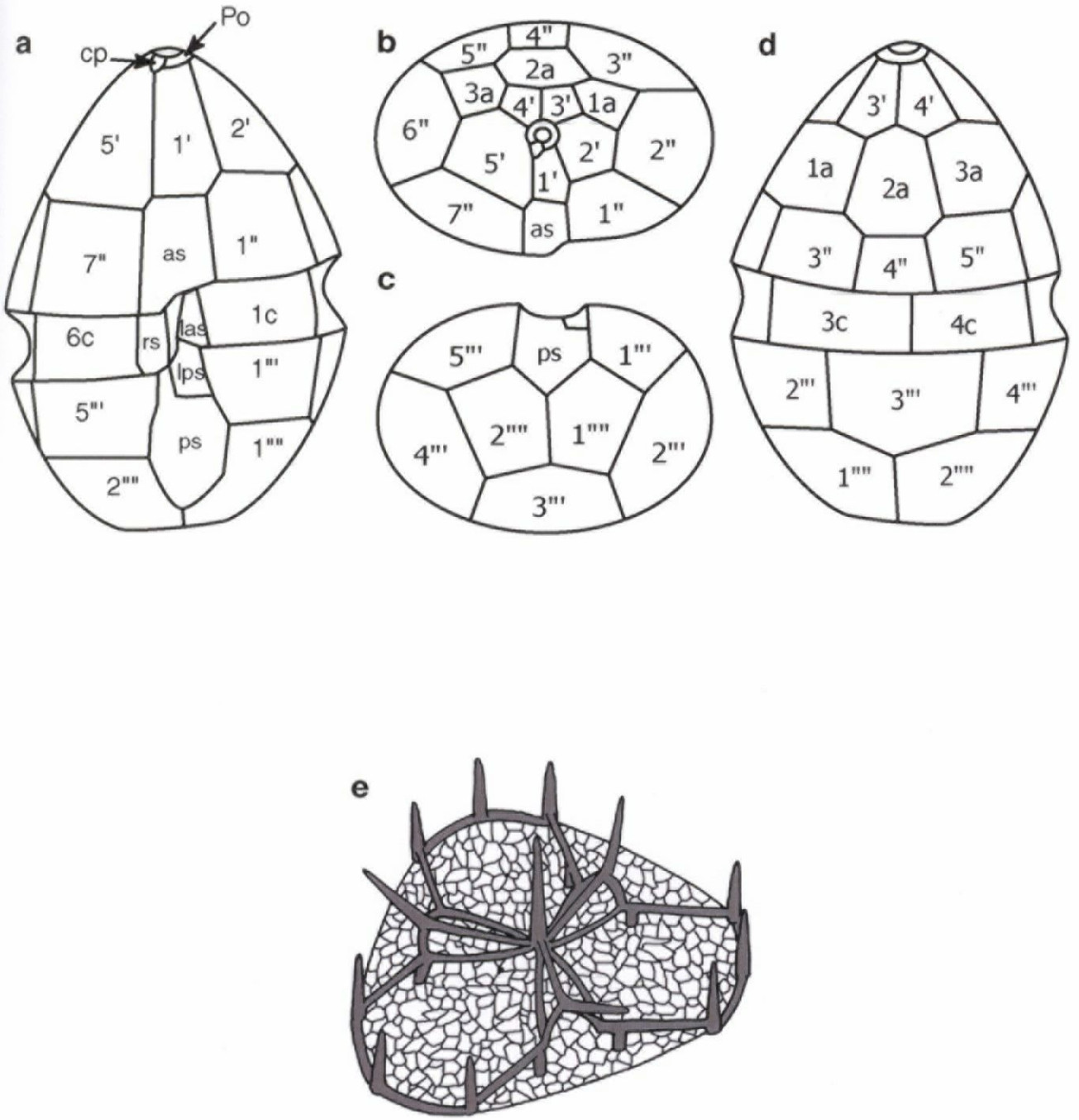


**Figure 3-2-3.** *Heterocapsa illdefina* (Herman & Sweeney) Morrill & Loeblich III.

**a-d**. Diagmammatic illustrations of thecal plates; **a**, ventral view; **b**, apical view; **c**, antapical view; **d**, dorsal view.
**e**. Body scale.

bars.

Cell of *H. illdefina* is quite nondescript considering the specific name "illdefina", it resembles some *Heterocapsa* species such as *H. circularisquama* and *H. horiguchii.* This species is discriminable from these species by tubular invaginations in pyrenoid and body scale ultrastructure.

The type culture of *H. illdefina* was originally collected from offshore of California in October 1973. Various morphological characters were re-observed by several authors (Balech 1977; Morrill & Loeblich III 1981; Horiguchi 1995), all these were same specimen, which is used in the original report of Herman & Sweeney (1976). Another occurrence of the species has not been known.

### 3-2-4 *Heterocapsa niei* (Loeblich III) Morrill & Loeblich III

(Fig. 3-2-4, Plate 4, 16, 28, 29)

Morrill & Loeblich III, 1981, p. 63, Figs. 1-3

Sournia, 1982, p. 158.

Steidinger & Tangen, 1996, p. 531, Pl. 3. 49.

Fukuyo, Inoue & Takayama, 1997, p. 69, pl. 22, Fig. 136.

Synonym

*Cachonina niei* Loeblich III 1968

Loeblich III, 1968, p. 92, Figs. 1-7.

Stosch, 1969, p. 559, abb. 1-4.

Cells are elliptical, consisting of a conical or hemispherical epitheca and a hemispherical

hypotheca (Plate 4). The epitheca is almost same size to the hypotheca. Cell sizes are 18.4-27.2 μm (mean 21.7 μm, n = 30) in length, and 12.0-19.2 μm (mean 15.9 μm, n = 30) in width (TG607-1 strain). The cingulum is displaced by about 1/3 of its own width. The sulcus is shallow and almost reaches the antapex of the cell. The brown chloroplast is located in the cell peripherally and is occasionally perforated. The pyrenoid is spherical, surrounded by starch sheaths. It is situated in the lower part of the epitheca. A spherical nucleus occupies a large part of the hypotheca.

The thecal plates of *H. niei* are quite thin, superficially resembling the gymnodinioid dinoflagellate under light microscope (Plate 4). The thecal plate arrangement is presumed by Po, cp, 5', 3a, 7", 6c, 5s (as, rs, las, lps, ps), 5"', 2"" (Fig. 3-2-4, a - d). Significant difference in the thecal plates could not be found.

A spherical dinokaryotic nucleus was observed in the hypotheca, under transmission electron microscope (Plate 16). The chloroplast is situated peripherally. The single pyrenoid surrounded by starch sheaths and is located in the epitheca. It connects with chloroplast by an isthmus. Tubular cytoplasmic invaginations in the pyrenoid matrix are absent. Electron dense lipid globules are located immediately beneath the chloroplast. Typical trichocysts of dinoflagellates are also present.

The body scales of *H. niei* are more or less triangular in outline, and the fine structure is rather complicated and is characteristic among the genus (Fig. 3-2-4, e, f, Plate 28, 29). The scale is about 300 nm in diameter. The basal plate of the scale is composed of fine reticulation. Central upright or spine is absent. Three ridges radiate from the center to the rim that divides the plate into three partitions. A spine or a hole is situated in the center of each partition. There is usually a small spine in the case of Caribbean strain (CCMP447 strain), while it is sometimes substituted by a hole in the Japanese strains (TG607-1, TG607-2 and NIES420 strains). These structures are observed only from the body scale of *H. niei*. Fifteen peripheral uprights stand on the rim of the basal plate. Each peripheral upright connects with the next

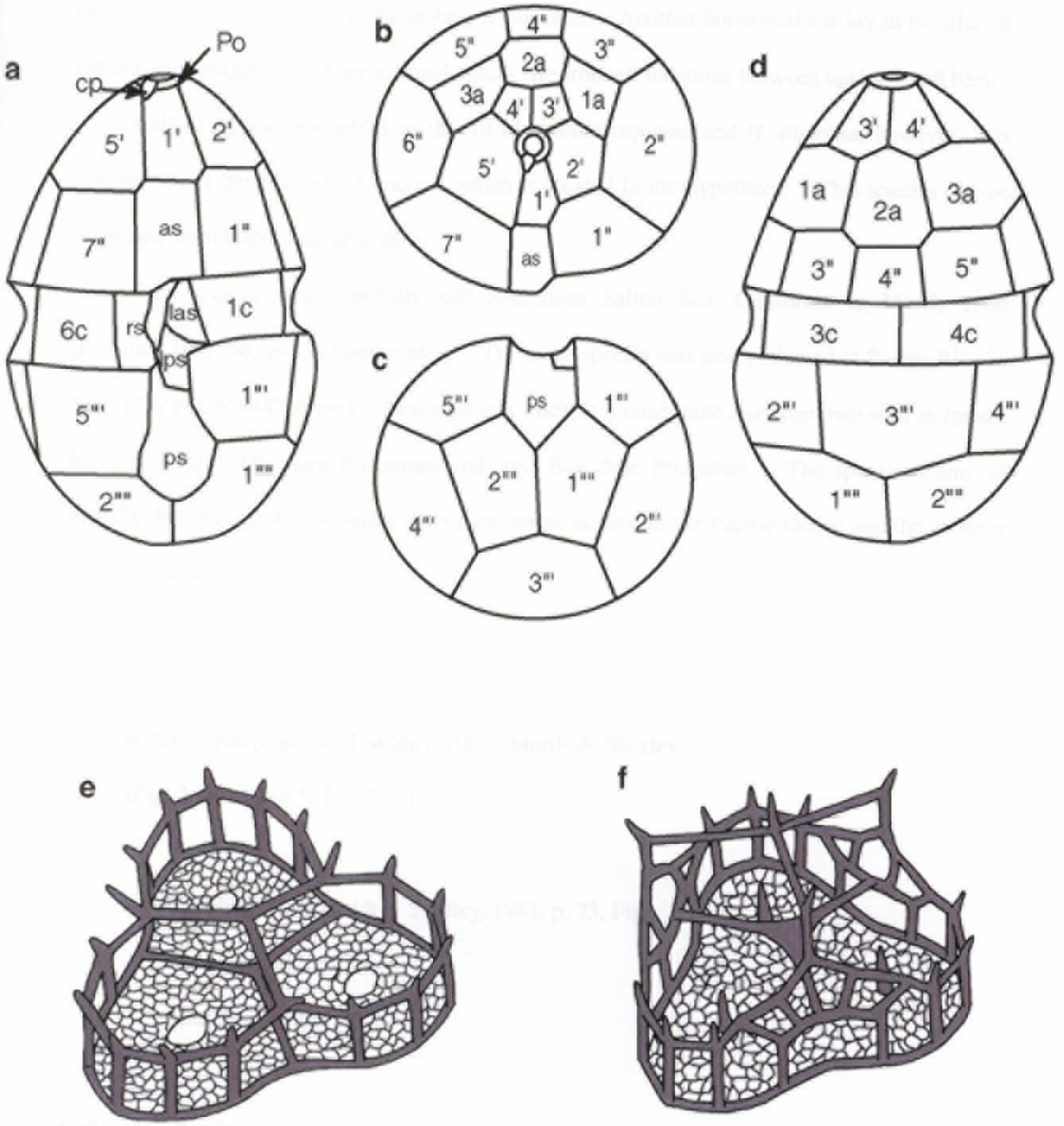





**Figure 3-2-4.** *Heterocapsa niei* (Loeblich III) Morrill & Loeblich III.

**a-d**. Diagmammatic illustrations of thecal plates; **a**, ventral view; **b**, apical view; **c**, antapical view; **d**, dorsal view.
**e, f**. Body scale; **e**, immature scale; **f**, mature scale.

uprights by horizontal bars at the distal part of them. Another horizontal bar lay in parallel on the radiating ridges. Moreover, small spines rise from all junctions between uprights and bars.

Cell of *H. niei* resembles to that of *H. circularisquama* and *H. illdefina*, however, it is different from the position of nucleus which is located in the hypotheca. This species can be identified from body scale structure.

This species was originally collected from Salton Sea, California in March 1966 (Loeblich III 1968, as *Cachonina niei*). The same species was also collected at Puerto Rico in June 1982 (CCMP447 strain). In the present study, it is confirmed also from two sites in Japan, Iriomote Island, Okinawa Prefecture and Ago Bay, Mie Prefecture. The species seems to broadly distribute from temperate to tropical areas, at least in the Pacific Ocean and the western Atlantic Ocean.

### 3-2-5 *Heterocapsa pygmaea* Loeblich III, Schmidt & Sherley

(Fig. 3-2-5, Plate 5, 17, 30, 31)

Loeblich III, Schmidt & Sherley, 1981, p. 73, Figs. 1-14.

Sournia, 1982, p. 158.

Sournia, 1990, p. 330.

Cells are elliptical, consisting of a conical or hemispherical epitheca and a hemispherical hypotheca (Plate 5). The epitheca is almost of the same size to the hypotheca. Cells are relatively small, 12.0-18.4 μm (mean 15.0 μm, n = 30) in length and 9.6-12.8 μm (mean 10.8 μm, n = 30) in width (CCMP1322 strain). The cingulum is relatively wide and displaced by about 1/2 - 1/3 of its own width. The sulcus almost reaches the antapex of the cell. The brown chloroplast is located in the cell peripherally. The pyrenoid is located in the epitheca.