Observations on the life cycle and microanatomy of *Thoracosphaera heimii* (Dinophyceae) with special reference to its systematic position

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Thoracosphaera heimii (Lohmann) Kamptner, a modern thoracosphaerid, and type species of the genus, was isolated in Natal, South Africa, and investigated using unialgal cultures and electron microscopy. These observations revealed:
(i) The formation of asexual *Gymnodinium*-like swarmers.
(ii) That a calcified coccoid cell, previously thought to be a coccolithophorid, develops directly from the *Gymnodinium*-like swarmer. No sexual fusion was observed during the formation of the calcified cell; thus it is not a resting cyst but entirely vegetative.
(iii) That the cells contain organelles distinctive of the dinoflagellates, viz. the dinokaryotic nucleus and trichocysts as well as starch which is accumulated in the cytoplasm.
(iv) That the cell covering is made up of an outer shell membrane, a calcified layer, an inner shell membrane, pellicle layer and a cytoplasmic membrane.
(v) That calcification may occur in Golgi-derived vesicles.

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Thoracosphaera heimii (Lohmann) Kamptner, 'n modêrne thorakosfeer en tiperende spesie van die genus, is in Natal, Suid-Afrika, geïsoleer en ondersoek deur middel van enkelalgkulture en elektronmikroskopie. Hierdie waarnemings het die volgende aangetoon:
(i) Die vorming van ongeslagtelike *Gymnodinium*-agtige swermers.
(ii) Dat 'n gekalsifiseerde coccoiede sel, voorheen as 'n coccolithofoor beskou, direk uit die *Gymnodinium*-agtige swermer ontwikkel. Geen geslagtelike versmelting is gedurende die vorming van die gekalsifiseerde sel waargeneem nie; dit is dus nie 'n rustende sist nie, maar geheel en al vegetatief.
(iii) Dat die selle, organelle kenmerkend van die dinoflagellate bevat, nl. die dinokariotiese kern en trigosiste sowel as stysel wat in die sitoplasma versamel word.
(iv) Dat die selomhulsel bestaan uit 'n buitenste membraandop, 'n gekalsifiseerde laag, 'n binneste membraandop, pellikellaag en 'n sitoplasmiese membraan.
(v) Dat kalsifisering moontlik in Golgi-afkomstige vessikels plaasvind.

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Introduction

Thoracosphaerids are members of the calcareous nanoplankton and are well known to micropalaeontologists, as microfossils from Cretaceous sediments, and to phycologists as a common constituent of the marine nanoplankton. Their systematic position has been tenuous ever since they were first described by Kamptner in 1927. Kamptner placed them within the coccolithophorids but did mention that possibly only by studying living material could we be sure that they were coccolithophorids.

Füttrer (1976, 1977) studied, in considerable detail, the dinoflagellate *Calciodinellum*, a genus originally described by Deflandre (1947) as a calcareous dinoflagellate, and several *Thoracosphaera* species with the aid of the scanning electron microscope. He concluded that *T. albatrosiana* and *T. tubelosa* were dinoflagellate cysts and were closely related to *Calciodinellum* and other described dinoflagellate cysts. Füttrer only used fossil material in his investigations. Tappan (1980) has placed *Thoracosphaera* in the Family Thoracosphaeraceae belonging to the Class Prymnesiophyceae (as Coccolithophyceae) because *T. heimii*, the type species of the genus, possesses no distinctive features to support the suggestion that it has dinoflagellate affinities. One can, however, also argue that no evidence has ever been provided to confirm that it is a member of the Prymnesiophyceae.

This paper attempts to provide some insight into the true systematic position of *Thoracosphaera heimii* based on detailed studies of unialgal cultures isolated from inshore waters from the south coast of Natal, South Africa. Culture studies have revealed some stages in the life cycle of this species and electron microscope observations have provided confirmation that it should be placed in the Class Dinophyceae.

Materials and Methods

Sea-water samples were collected weekly from Durban, Natal, South Africa and returned to the laboratory within 4 – 5 hours after collection. The phytoplankton was concentrated by passing the water through Nucleopore filters (10-μm and 5-μm pore size) and selected cells isolated and placed into unialgal culture.

The *Thoracosphaera heimii* isolates used in this investigation were isolated from a sample collected on the 6th Oc-

tober 1981. Single cells were isolated and inoculated in Provasoli Enriched Seawater medium (Provasoli 1968) and into G.P.M. medium (Loeblich III 1975). Cultures were then maintained in G.P.M. medium at 20 °C and subjected to an alternating cycle of 16 h light : 8 h dark.

For observing life-cycle stages cells were put into tissue-culture plates (MicroTest, Falcon) which have 60 flat-bottomed wells each of 0,1 cm^{-3} capacity.

For scanning electron microscopy, unfixed cells were rinsed in distilled water to remove the salt and placed into 0,1 mol dm^{-3} sodium hydroxide for 2 – 5 minutes to dissolve the membranes that surround the cells. After rewashing in distilled water the cells were placed on Nucleopore filters (0,8-μm or 10-μm pore size) and coated with gold. Specimens were viewed with a Jeol T-200 scanning electron microscope. Carbon platinum replicas were prepared as previously described by Pienaar (1976).

For ultrastructural observations, cells were fixed by adding 25% aqueous glutaraldehyde to G.P.M. medium in which the cells were growing, to give a 2,5% glutaraldehyde solution. Fixation was for 1 h at room temperature. Cells were then rinsed four times in G.P.M. medium each with a decrease in salt concentration; post fixed in 2% osmium tetroxide in 0,1 mol dm^{-3} sodium cacodylate buffer (pH 7,2) for two hours, dehydrated in a graded ethanol series and embedded in Spurr's low viscosity resin (Spurr 1969). Sections were cut using diamond knives and picked up on formvar coated grids, double stained with uranyl acetate and lead citrate (Reynolds 1963) and viewed with a Jeol 100CX transmission electron microscope.

Terminology

We are of the opinion that *T. heimii* is a member of the Class Dinophyceae and this has necessitated a change in terminology. Terms such as coccosphere, coccolith, or prismatolith, which are used in coccolithophorid terminology, should no longer be used. In this paper we have adopted terms that apply to dinoflagellates.

Füttrer (1976, 1977), in his papers, adopted dinoflagellate cyst terminology for *Thoracosphaera* species. However, we are also of the opinion that the calcified coccoid stage of *T. heimii* is not a cyst or even a resting stage but should be regarded as a vegetative stage. We therefore propose that terms used to describe cysts like 'test', 'archeopyle' or 'operculum' are not suitable in the description of a vegetative stage. As an alternative to these terms we suggest that 'shell', 'opening' and 'lid' be adopted. Whilst these terms have occasionally been used to describe cysts they are not generally regarded as accepted terms in cyst terminology.

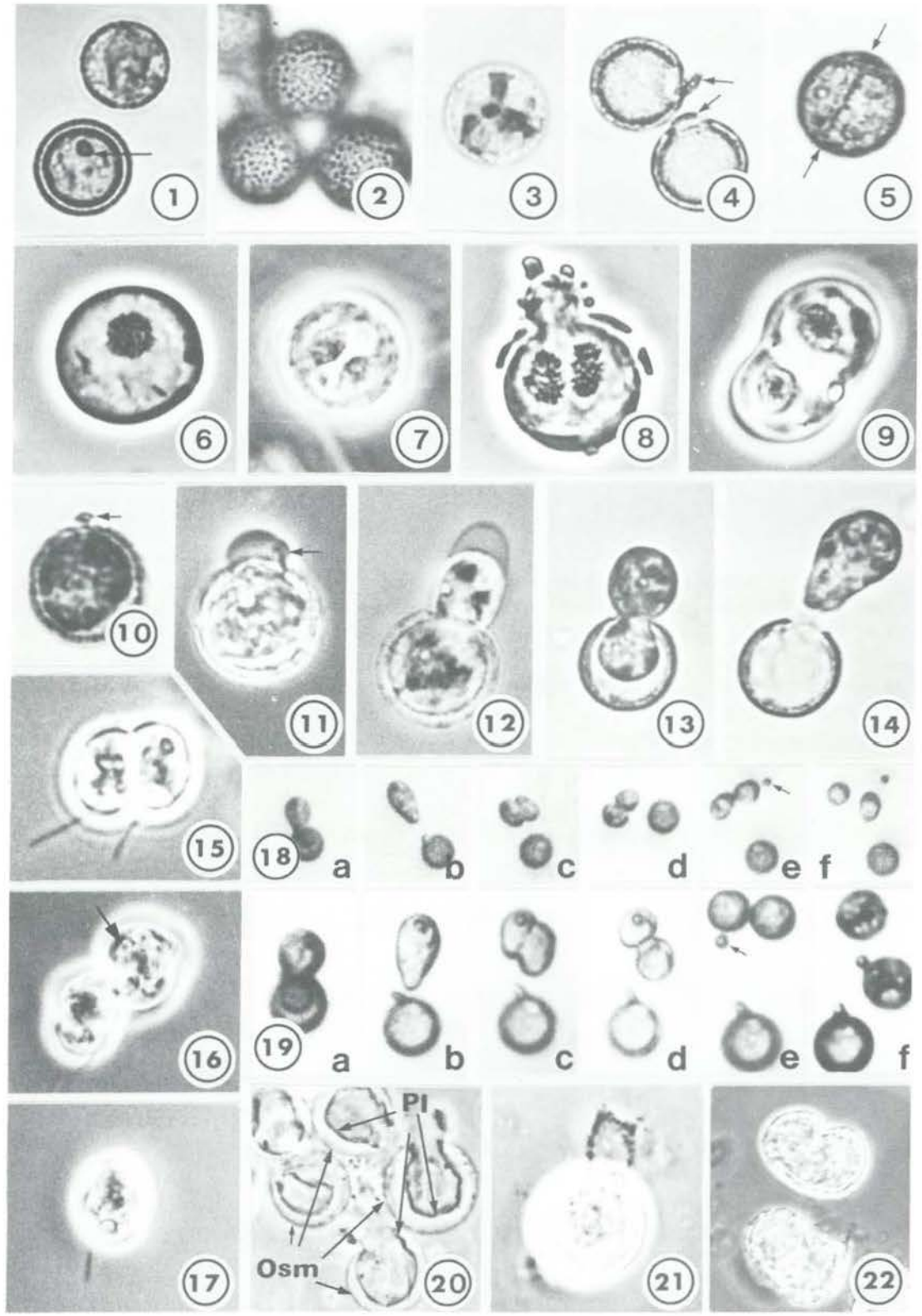
Observations

Light microscopy and cell cycle stages

Cells are spherical and are covered with a thick perforated shell of calcium carbonate (Figure 1 & 2). The thickness of the shell depends upon the age of the cell. In old cultures cells with varying thickness of the shell can be observed (Figure 1). Mature cells vary in total size from 9 – 20 μm (average 11,8 μm) with the thickness of the calcium carbonate shell being 1 μm. A single ribbon-shaped yellow-brown chloroplast can be seen in weakly calcified cells. In these young cells the chloroplast radiates radially from the centre of the cell (Figure 3) whilst in mature and old cells the chloroplast is parietal. A pyrenoid is not easily observed with the light microscope. A single parietal or centrally located nucleus is easily osberved in cells stained with acetocarmine (Figure 6). Acetocarmine dissolves the calcium carbonate of the shell enabling the dinokaryotic nucleus to be observed. Most cells observed possessed a peripherally situated red body (Figure 1). This is, however, not a stigma as it is often released from the cell during cell division (Figures 18e & 19e).

At maturity the cells are darker and a clear region with enlarged extremities traverses the cell close to the red body (Figure 5). This region resembles a vacuole and is very clear once nuclear division has taken place (Figure 7). This region is thought to be developed during cytokinesis. The release of motile cells takes place in much the same way as has previously been reported in the excystment of other dinoflagellate cysts. This begins with the opening of a lid region (Figure 10) which is located near the red body. The lid and resultant opening are circular in profile whereas the operculum and archeopyle in dinoflagellate cysts are usually angular or polygonal reflecting the plate tabulation of the parent cell (Evitt 1967). The region of the cell between the red body and the lid swells and the pressure caused by this phenomenon causes the lid to open (Figure 11) and the cytoplasm to be released in a vesicle (Figure 12). The lid at this stage is hinged at one side and remains attached to the shell (Figure 11). Eventually the binucleate cell emerges through the opening (Figures 8, 13 & 14). The released cell remains stationary just outside the opening for a few seconds (Figures 14 & 18b) as it is still enclosed and connected to the shell by a very thin envelope (Figure 12). The presence of this envelope can be confirmed by observation of empty shells when studied with phase contrast objectives (Figure 21). The opening through which the cell emerges is approximately 4 – 8 μm in diameter and is slightly larger than the lid (Figure 4). The newly released cell is elliptical, 9 × 12 μm in size, biflagellate and shortly after release begins swimming. The red body in motile cells is located towards the anterior of the cell. The two flagella are straight and extend towards the posterior pole and neither of them show the wavy appearance of the transverse flagellum. It is therefore difficult to confirm the presence or absence of the 'typical' transverse flagellum found in dinoflagellates.

Within a few minutes the cell changes its shape (Figures 15 & 18c) and commences division forming two *Gymnodinium*-like cells which remain attached to each other (Figure 16). At this stage two transverse flagella can be seen. One of the cells possesses the red body (Figure 16). The two daughter cells swim while still attached and after 2 – 25 min they separate to form *Gymnodinium*-like swarmers (Figure 17). The red body is frequently released from the cells during the final stages of cytokinesis (Figure 18e). The swimming cell is about 9 μm long and 7 μm wide and has a centrally situated girdle which is slightly spiral. The sulcus was difficult to see but both longitudinal and transverse flagella were seen. The colour of the swimming cells is a faint yellow-brown and they contain a single small chloroplast. These cells swim from 3 min to several hours. They then cease



Figures 1 – 22 *Thoracosphaera heimii* observed with the light microscope. (1) Young (top) and mature (bottom) cells. Note the presence of a red body (arrow), × 1300; (2) Perforated appearance of shells, × 1000; (3) A young cell showing a radially arranged chloroplast, × 1700; (4) Empty shells with the openings and lids (arrows), × 1300; (5) A dividing cell with a cleavage furrow (arrows), × 1700; (6 – 9) Cells stained with acetocarmine. (6) A cell showing interphase nucleus, × 2000. (7) A dividing cell with a cleavage furrow separating daughter nuclei, × 2000. (8) A cell prior to release showing daughter nuclei, × 1500. (9) Twin cell containing two nuclei, × 1500; (10 – 14) Serial stages of cell release. (10 & 11) Initial stages of release. Note the opening of lids (arrows), × 1700. (12) A cell with an envelope enclosing protoplast, × 1700. (13 & 14) Serial photographs of release of swimming cell, × 1700; (15 – 17) Different stages of the swimming cell. (15) Early stage of a divisional pair, × 1500. (16) *Gymnodinium*-like divisional pair. The red body (arrow) is visible in a cell of the pair, × 1300. (17) A *Gymnodinium*-like swimming cell, × 1300; (18 & 19). Time-lapse photographs of release and division of a swimming cell (18a – f, × 400) and a cell bearing no flagella (19a – f, × 700). The release of red body (arrows) is visible; (20). Cells treated with HCl. Calcified layer is dissolved and the outer shell membrane (Osm) and pellicle layer (Pl) are visible, × 1500; (21) An empty shell with a tubular envelope, × 1700; (22) Calcified twin cells observed in old culture, × 1000.

swimming, release their flagella and become spherical. These cells then produce a calcareous shell which is fully calcified within one to three days. Time-lapse photographs of cell release and division are shown in Figures 18a – f.

In addition to the cell cycle just described a second one is often observed. On maturation the spherical calcified cell releases an ellipsoidal cell but this cell is devoid of flagella. The cell remains in close proximity to the empty calcareous shell and changes into a spherical divisional pair bearing no flagella. This results in two spherical daughter cells after the release of a red body (Figure 19e & f). This process is complete within 10 min. A series of photographs (Figures 19a – f) illustrates this process. The daughter cells then produce a calcified shell.

These two types of cell cycles were frequently observed and we are of the opinion that they are the main cell cycle and are indicated by thick lines in Figure 23. Occasionally we were able to observe several subcycles (Figure 23, thin lines) which may or may not be artificial cycles induced through the culture conditions to which the cells were subjected.

As shown in cycle A (Figure 23), the divisional pair occasionally did not completely separate into two daughter cells and these twin cells began calcification resulting in the variously shaped twin calcified shells observed in some cultures (Figure 22). These cells have two nuclei (Figure 9).

Another unusual cycle (Figure 23, cycle B) was observed in culture where some of the daughter cells that were formed from separation of pairs and some twin divisional pairs without separation, develop a thin envelope instead of going through the process of calcification. These cells on maturation will also release swimming cells. This type of cycle is normally observed in older cultures.

On several occasions yet another cycle was observed (Figure 23, cycle C). Here the ellipsoidal cell released from a mature calcified cell changes its shape and forms only one *Gymnodinium*-like swimming cell. This cell possesses two longitudinal flagella, and does not form two *Gymnodinium*-like swimming cells. The number of nuclei in this cell is still uncertain. After swimming for a while it settles, becomes spherical and although not actually observed we feel that calcification commences without the separation into two daughter cells. A diagrammatic illustration of possible cell cycles of *T. heimii* described above is summarized in Figure 23.

Electron microscopy

Cell covering

The cell covering was studied using both scanning and transmission electron microscopy.

The calcium carbonate crystals could only be observed in unstained sectioned material (Figure 40). Sections which had been stained showed empty spaces where the calcium carbonate had either been dissolved or washed away (Figure 31). Various stages in the calcification of the shell were observed. In young cells, just commencing the deposition of calcium carbonate, the crystals are irregularly grouped over the surface of the cell (Figure 32). It is almost as if

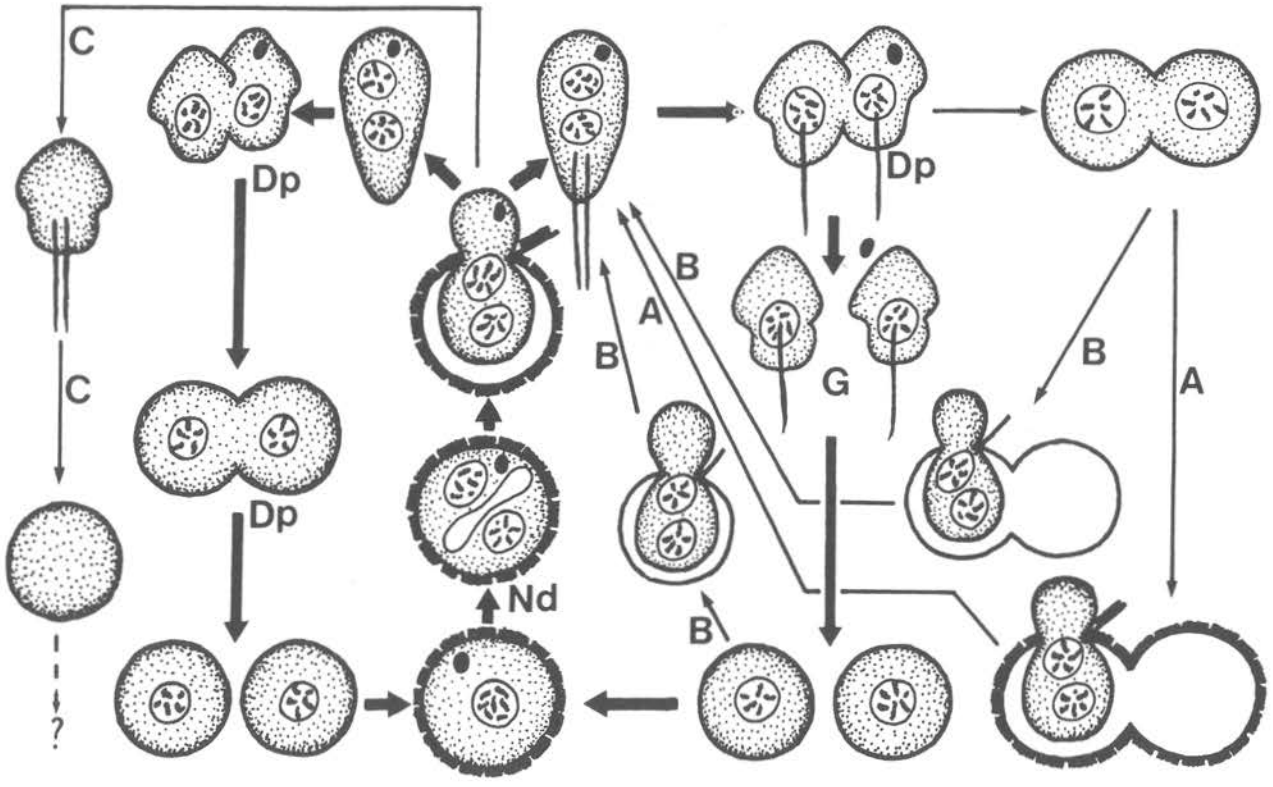


Figure 23 Diagrammatic illustration of possible cell cycles of *T. heimii*. Thick lines indicate main cycles and thinner lines indicate subcycles (A – C). Nuclei are illustrated if numbers of them are known. Calcified layer is indicated by thick wall surrounding cell. Nd: nuclear division stage, Dp: divisional pair, G: *Gymnodinium*-like swimming cells. See text for details.

numerous nucleation sites are initiated from which the calcium carbonate deposition proceeds, eventually resulting in a shell of uniform thickness (Figure 31). This process was also confirmed using the scanning electron microscope and carbon platinum replicas. Figure 24 shows cells at different stages in calcification. Initially calcification proceeds in such a manner as to produce skeletal elements possessing small square pores (Figure 25). At this stage these elements are still fragile. The calcification then proceeds by the addition of calcium carbonate crystals resulting in the shell having a more solid appearance (Figure 27). The pore-like areas are still clearly visible and are square in outline.

In Figure 25 it is apparent that the shell is composed of numerous crystals. These crystals are cubic or prismatic and are scattered around fragments of shell when the shell is prepared for replica preparation (Figure 28). The skeletal elements are composed of crystals of unknown number arranged in a more or less rectangular pattern eventually producing a square pore (Figure 26).

The proximal and distal surfaces of the shell are quite different in appearance. In the mature shell, the boundary of each element is obscure (Figure 29) but fragments of a young shell show the individuality of each element (Figure 30). The margin of the proximal surface of each skeletal element is dentate (Figure 30).

Sectioned material reveals that calcium carbonate deposition occurs in the space between the two membranes which are continuous and surround the entire cell. These two membranes are called the outer and inner shell membrane respectively. The outer shell membrane is not visible in mature cells that have been fixed for electron microscopy but can be clearly seen in young cells (Figure 32). This membrane can be seen with the light microscope even in fully calcified cells when the calcium carbonate is dissolved with 0,1 mol dm^{-3} HCl (Figure 20).

Immediately beneath the inner shell membrane is a non-membranous layer which is also continuous and surrounds the cell. This layer is composed of two bands, viz. the outer osmiophilic band and an inner more electron-lucent band (Figures 33 – 35). This layer may be homologous with the pellicle layer which has been found in many dinoflagellates (Loeblich III 1970; Morril & Loeblich III 1981).

In addition to the above, there is another membrane beneath the pellicle layer called the cytoplasmic membrane which encloses the cytoplasm (Figures 33 & 34). Flattened vesicles are often seen beneath the cytoplasmic membrane (Figure 33). Microtubules are also present immediately beneath the cytoplasmic membrane or the flattened vesicles although they are usually few in number (Figure 34).

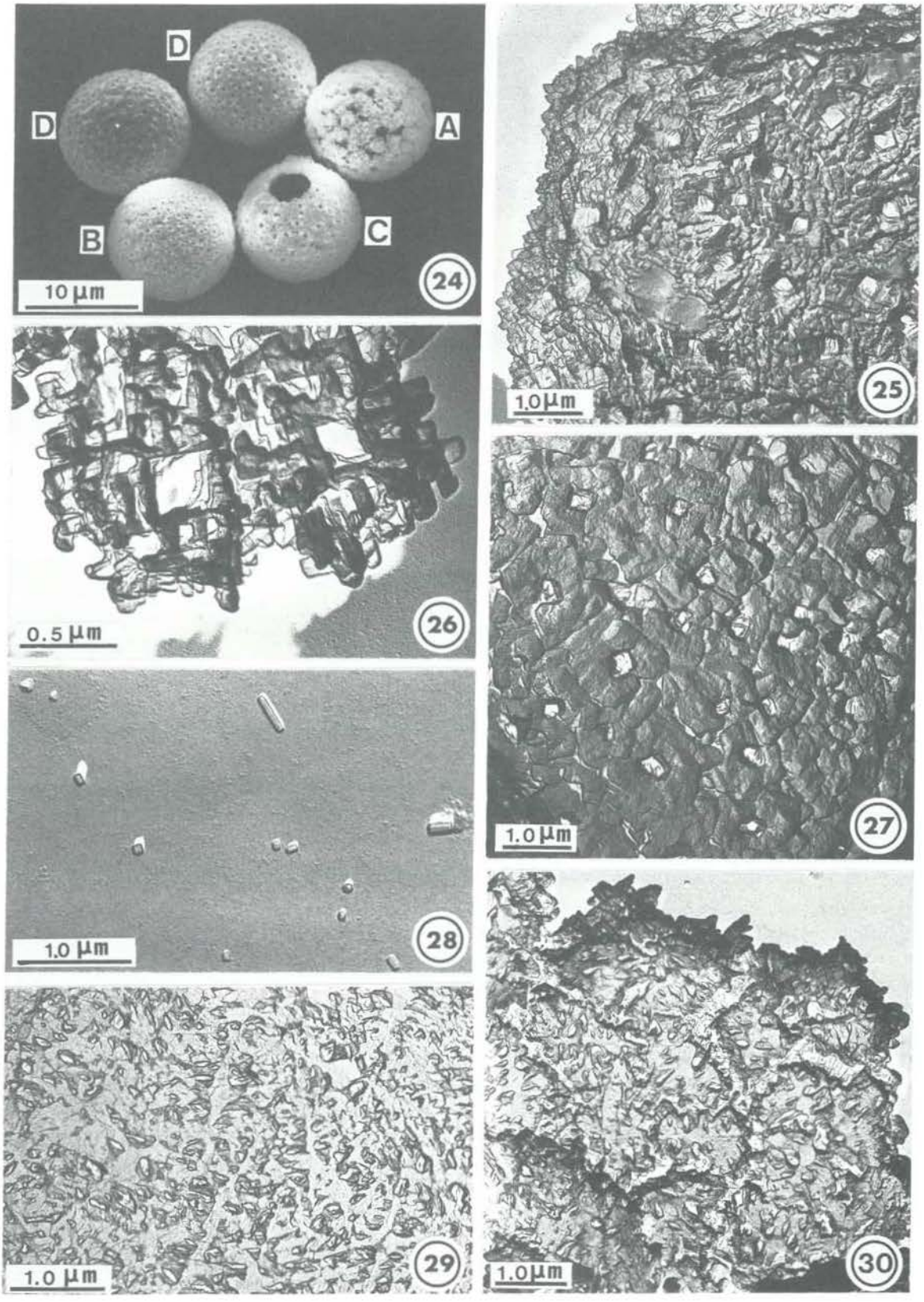
In young cells, the pellicle layer and the cytoplasmic membrane are situated close together and nothing could be seen between them (Figures 33 & 34). However, in mature cells the space between them is wide and occupied by additional membranes, vesicles, and dense material of unknown nature (Figure 35). No thecal plates or plate-like structures have been observed in either young or mature cells. A schematic illustration of the cell covering of *T. heimii* is given in Figure 37.

The cell covering of an uncalcified cell seems to be the same as that of a calcified cell except for the absence of a calcium carbonate layer. However, the outer and inner shell membranes have in many cases disappeared and the pellicle layer is thicker than in calcified cells. Only a few swimming cells were studied in ultrathin sections to examine the structure of the cell covering. It is therefore still obscure whether it is similar in construction to the vegetation cell. Figure 36 illustrates the cell covering of a swimming cell. In this figure another membrane can be seen beneath the inner shell membrane. This membrane is only found in swimming cells and corresponds to the pellicle layer in non-motile cells. This may suggest that during the cell's development into the vegetative stage the membrane is replaced by a pellicle. This suggestion will have to be verified by further investigation.

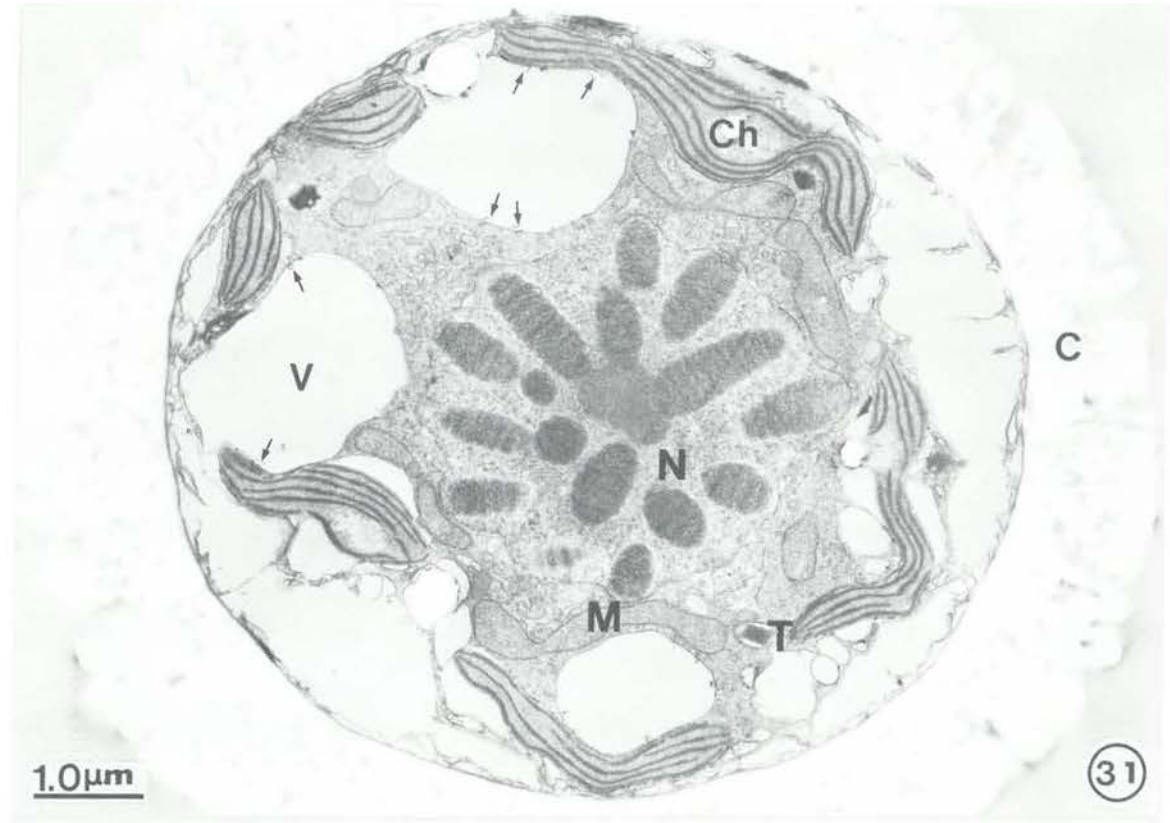
Intracellular structure

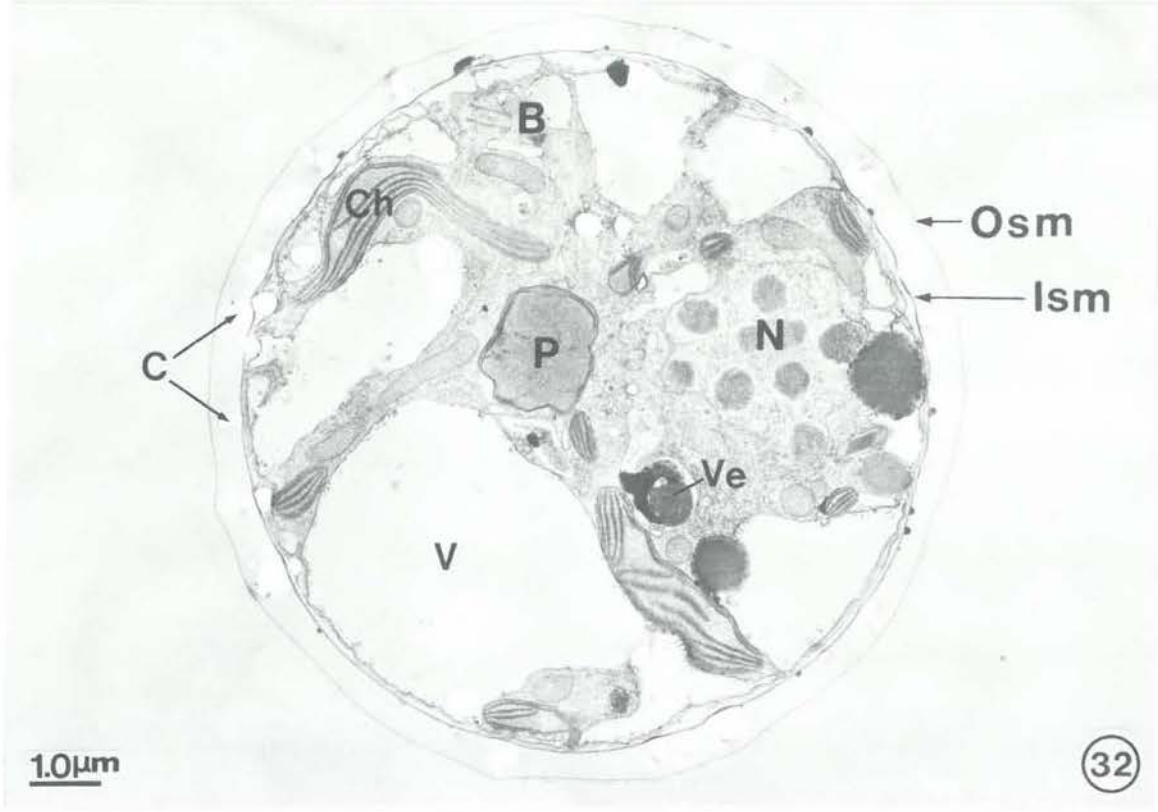
The nucleus is located in a central to peripheral position and is typically dinokaryotic (Figures 31 & 32). The chromosomes are condensed and even at the interphase stage have a fibrous appearance (Figure 31). The nuclear envelope and nucleolus (Figure 31) resemble previously described dinoflagellates. There is a single chloroplast which in young cells is radially situated whereas in mature cells it is located in a parietal position (Figures 31 & 32). The lamellae are composed of stacks of three thylakoids and there are no girdle lamellae (Figure 36). A single pyrenoid is centrally placed in young cells (Figure 32) but has been observed in a parietal position in mature cells. Vacuoles containing peculiar cylindrical crystals resembling a parallelogram, are evident in most cells studied. These crystals are electron lucent in stained material (Figure 39) whereas they are electron dense in unstained material (Figure 40). In young cells the vacuoles are large and extend from the periphery to the centre of the cell resulting in the organelles being situated in the narrow space between the vacuoles (Figure 32). This may be the reason why the chloroplast lobes radiate from the centre of the cell. Vacuoles are often flattened and situated peripherally between the chloroplasts and the cytoplasmic membrane (Figure 33). In addition to the cylindrical crystals, these vacuoles often contain different crystals similar to calcium carbonate and which once again are more easily observed in unstained material (Figure 40). This evidence suggests that the vacuole may play a role in calcification. The cylindrical crystals are always attached to the inner surface of the tonoplast (Figure 39) and appear to be produced in the dictyosomes which are located near the nucleus (Figure 42). The number of dictyosomes making up the Golgi system is not known. Located in close proximity to the Golgi apparatus are unusual organelles, bounded by a single unit membrane and which contain tubular elements and/or dense material (Figures 38 & 41). This organelle is probably related to the vacuole containing the cylindrical crystals as it contains similar crystals (Figure 41). The electron density of the material in this organelle is similar to the material found deposited between the cytoplasmic membrane and the inner shell membrane in the mature cell (Figure 35).

The mitochondria have tubular cristae (Figures 31 & 32) and are similar in structure to those reported in other dinoflagellates. Trichocysts characteristic of dinoflagellate cells (Bouck & Sweeney 1966) are present but are not as

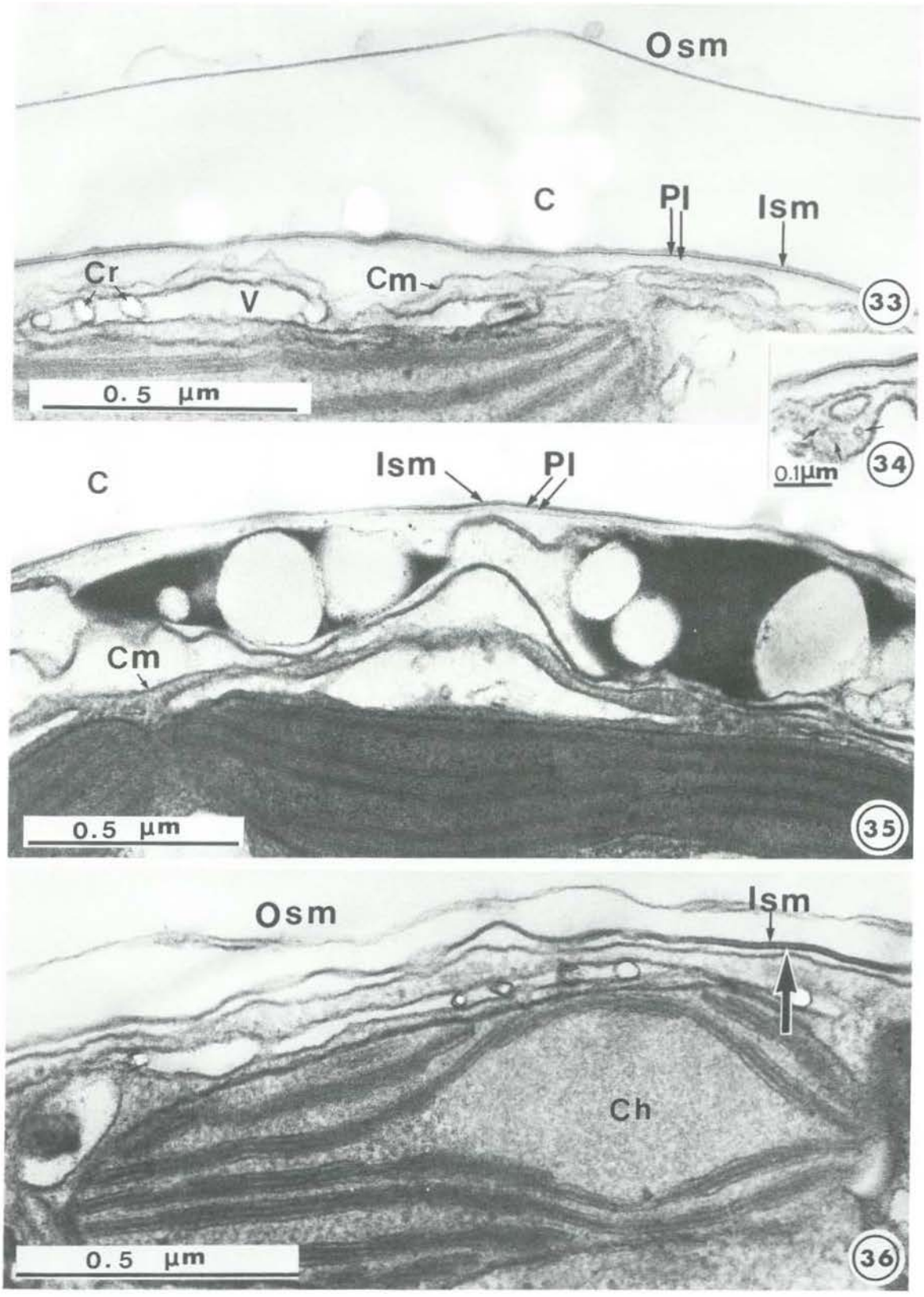


Figures 24 – 30 (24) Scanning electron micrograph of several cells showing various stages of calcification of the shell. A – D indicate the degree of calcification in order from young to mature stage. (25 – 30) Carbon platinum replicas of shell. (25) Distal surface structure of young shell showing individual crystals making up the shell. Central square pores are clear; (26) Two skeletal elements viewed from distal face. Crystals are arranged in a rectangular pattern; (27) Distal surface structure of mature shell showing more solid appearance. Individual crystals are no longer obvious but square pores are still clear; (28) Individual cubic and rectangular crystals separated during the preparation of replicas; (29) The proximal surface of the mature shell. Randomly arranged pores and ridges are visible but individuality of skeletal elements is obscure; (30) Collapsed shell viewed from proximal face. Dentate outline of skeletal elements is shown.





Figures 31 & 32 (31) Section of a mature cell showing organelles typical of the Dinophyceae. Nucleus (N), trichocysts (T), chloroplast (Ch) and mitochondria (M) are visible. Chromosomes are condensed and showing fibrous appearance. Vacuoles (V) containing cylindrical crystals (arrows) are also visible. Calcified layer (C) is seen as an empty space surrounding the cell; (32) Section of a young cell. In addition to organelles shown in Figure 31, a centrally located pyrenoid (P), one basal body (B) and vesicles containing dense material (Ve) are visible. Calcium carbonate crystals (C) are deposited in the space between the outer (Osm) and inner (Ism) shell membranes.



Figures 33 – 36 Section micrographs of cell covering. (33) Young cell just beginning to deposit the calcified layer (C). Outer (Osm) and inner (Ism) shell membranes, pellicle layer (Pl), cytoplasmic membrane (Cm) and vacuoles containing cylindrical crystals (Cr) are visible. Note that no material is seen between pellicle layer and cytoplasmic membrane; (34) Microtubules underlying cytoplasmic membrane (arrows); (35) Mature cell. In the space between pellicle layer (Pl) and cytoplasmic membrane (Cm), new membranes and dense material are deposited. Lower part of matured calcified layer (C) is seen above inner shell membrane (Ism); (36) Swimming cell. A unit membrane (big arrow) is visible beneath the inner shell membrane (Ism). A chloroplast (Ch) with three-band thylakoid lamellae and without girdle lamellae is also seen.

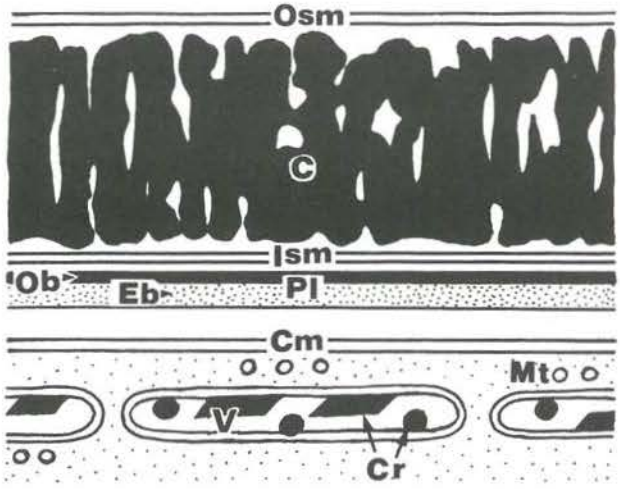


Figure 37 Diagrammatic illustration of common cell covering of *T. heimii* composed of outer shell membrane (Osm), calcified layer (C), inner shell membrane (Ism), osmiophilic (Ob) and electron-lucent (Eb) bands of pellicle layer (Pl), cytoplasmic membrane (Cm), microtubules (Mt) and vacuoles (V) containing cylindrical crystals (Cr).

abundant as in other dinoflagellates (Figure 43). A storage substance thought to be starch is located in the cytoplasm (Figure 38). Basal bodies are often seen in young cells (Figure 32).

Mitosis and the release of daughter cells

Several photographs were obtained indicating stages in mitosis. Figure 44 illustrates the anaphase nucleus which is typical of this stage of division in previously described dinoflagellates (e.g. Kubai & Ris 1969, Loeblich III & Hedberg 1976). The nuclear envelope invaginates into the nucleoplasm and forms cytoplasmic channels in which spindle microtubules can be observed. Two dictyosomes are often visible situated towards the polar region of the dividing cell. Figure 46 illustrates the telophase stage of mitosis. The daughter nuclei have already separated but a cytoplasmic channel is still visible. Vacuoles, once again containing cylindrical crystals are visible in this region and are likely to play a role in the separation of the daughter nuclei and associated cytoplasm. Large vacuoles situated on both sides of the equatorial region eventually become continuous with each other via the furrow which separates the nuclei. Crystals are always visible in this furrow (Figure 46). Flagella have at this stage already been produced (Figure 45). A cell at a stage just prior to release from the shell is shown in Figure 47. The opening from which the young cells escape begins as a distinct swelling of the space above the cytoplasmic membrane. The cytoplasmic membrane at this stage is still enveloping all the cell contents. After release the pellicle layer is always left behind inside the parent shell.

Swimming cell

The ultrastructure of the swimming cell is fundamentally the same as that of the calcified cell except that it possesses two flagella. The cell covering is made up of similar membranes as found in the young calcifying cells but, as mentioned before, there is another membrane instead of a pellicle layer and no crystals are evident (Figure 36).

Discussion

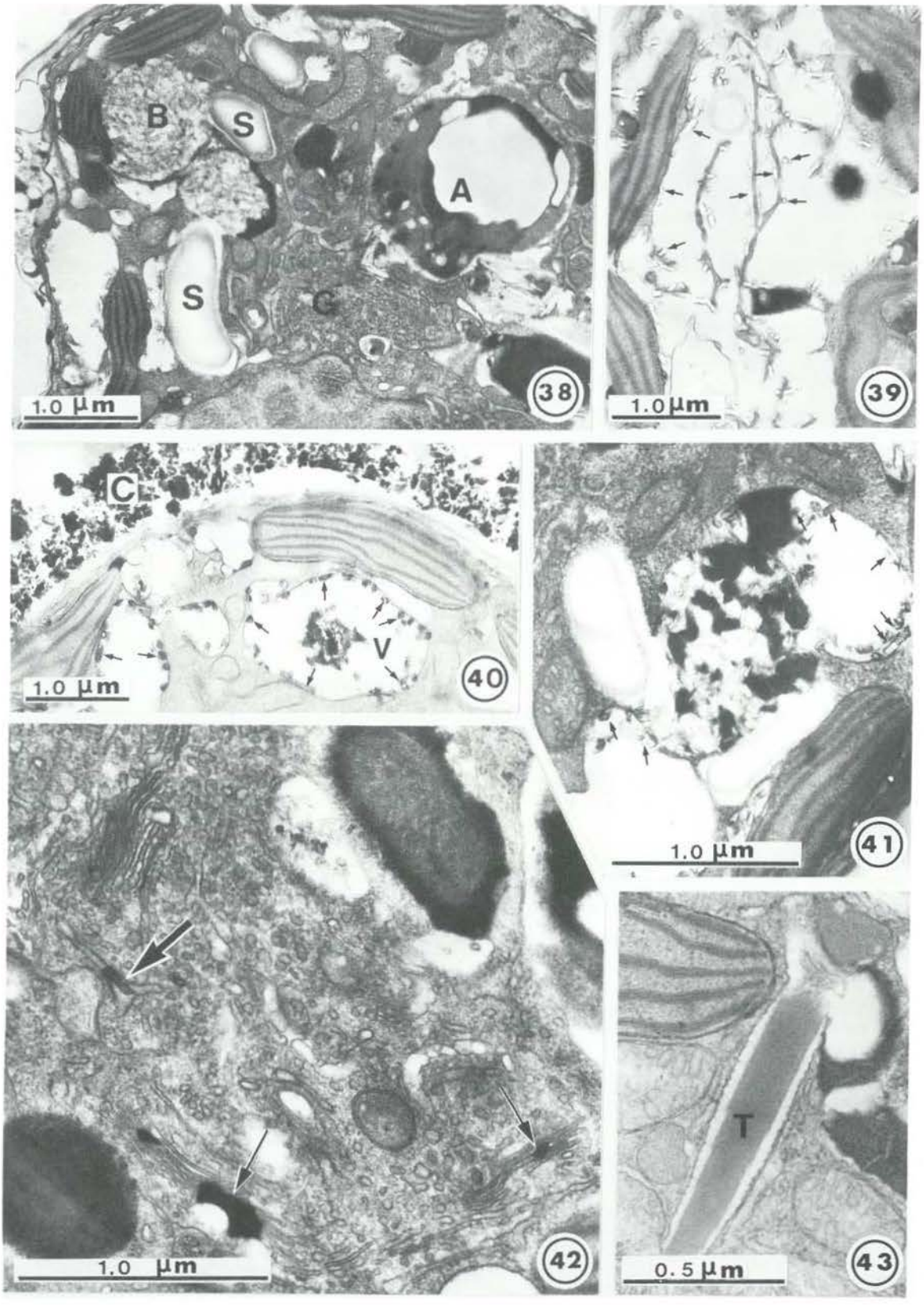
Since Lohmann (1920) described *Syracosphaera heimii* (which is now regarded as the type species of *Thoracosphaera*) the genus *Thoracosphaera* has been thought of as a member of the coccolithophorids (Prymnesiophyceae). This misinterpretation is not surprising when one considers that *Thoracosphaera* is surrounded by a calcium carbonate shell and that there existed, at the time, very little information on calcareous dinoflagellates. There were, however, some doubts as to the systematic position of the genus and further research was advocated (for bibliograpy, see Füttrer 1977). Füttrer (1976, 1977) eventually concluded, on the basis of studies of fossil material, that all thoracosphaerids were dinoflagellate cysts. He compared *T. albatrosiana* and *T. tuberosa* with the dinoflagellate cyst of *Calciodinellum operosum* which has angular openings similar to the archeopyles of dinoflagellate cysts.

Referring to Füttrer's results, Tappan (1980) treated *T. albatrosiana* and *T. tuberosa* as dinoflagellate cysts but she deferred placing Cretaceous and Palaeocene species of *Thoracosphaera*, viz. *T. heimii, T. saxea, T. tesserula, T. deflandrei* and *T. granifera* into the Dinophyceae until further research had been done on these species. Tappan placed these species in the Family Thoracosphaeraceae belonging to the order Isochrysidales in the Class Prymnesiophyceae (as Coccolithophyceae).

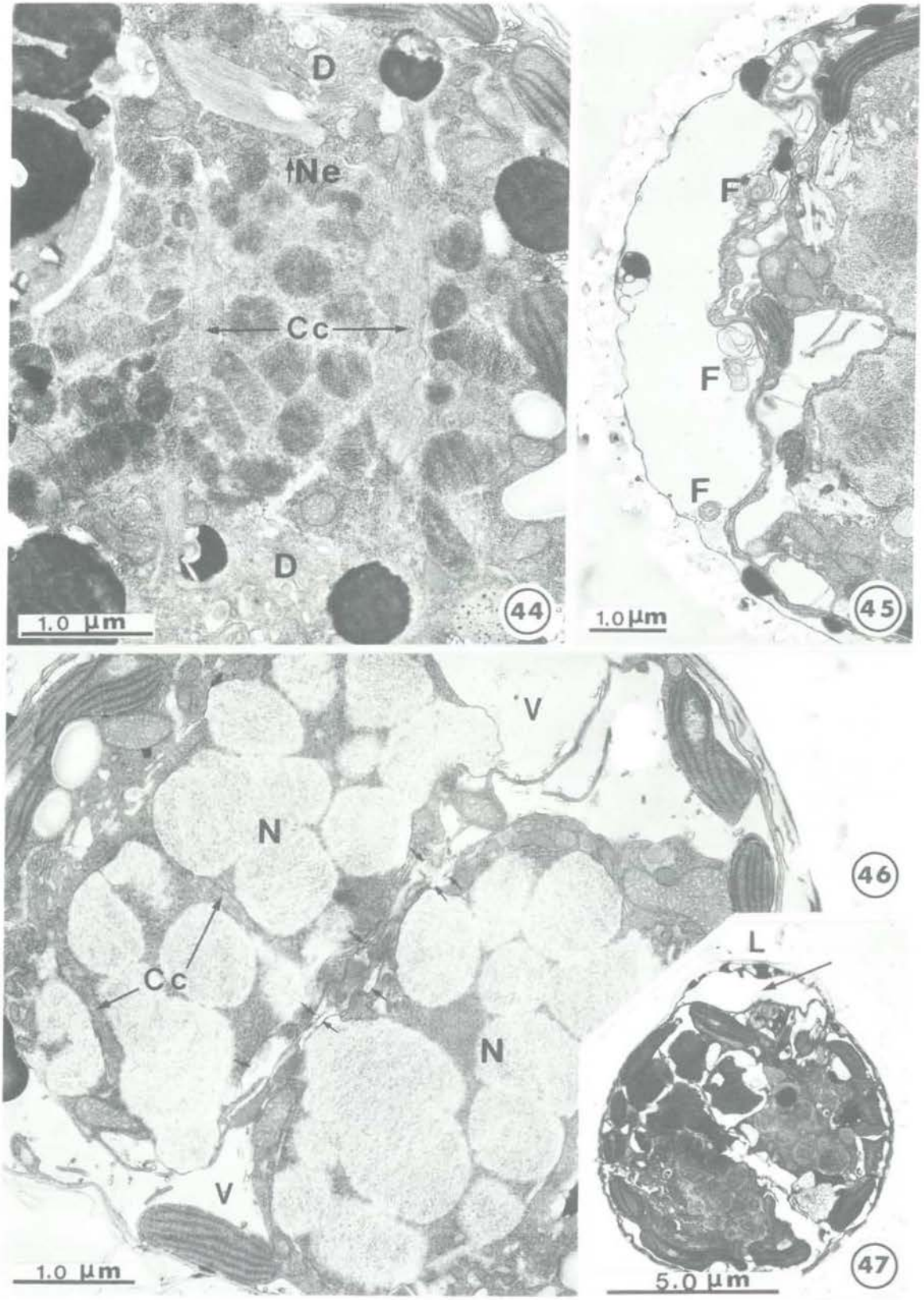
These doubts as to the correct systematic position of *Thoracosphaera* at either the division or class level is now clarified by the fact that we have observed this genus producing *Gymnodinium*-like swarmers, and by its ultrastructure. It is clearly a member of the Class Dinophyceae and we recommend that it be removed from the Prymnesiophyceae and transferred to the Class Dinophyceae together with other Cretaceous and Palaeocene species of *Thoracosphaera*.

Calcareous dinoflagellates were first described by Deflandre (1947) and later by Wall & Dale (1968) and Wall *et al.* (1970). These workers showed that some modern dinoflagellates produce calcareous cysts. It then became generally accepted that all calcareous dinoflagellates were in fact cysts. Füttrer (1976, 1977) suggested that all thoracosphaerids were actually calcareous cysts and probably closely related to the cyst genus *Calciodinellum*. Studies on the life cycles of dinoflagellates (Von Stosch 1964, 1965, 1973) have illustrated that dinoflagellate resting cysts are diploid zygospores which were produced as a result of the sexual fusion of gametes. In *T. heimii* we have not observed any fusion of gametes prior to the development of the calcified cell. We are therefore of the opinion that the calcified stage of *T. heimii* is not a resting cyst stage.

It is known that a 'division cyst' is formed in some dinoflagellates. In *Helgolandinium subglobosum* (Von Stosch 1969) and *Woloszynskia apiculata* (Von Stosch 1973) division cysts are produced from a swimming vegetative cell during the process of cell division. In the case of *T. heimii* the calcified stage is the dominant life-cycle stage and does not resemble a stage like a division cyst. In *T. heimii* this calcified stage is interpreted as a vegetative stage which is most unusual in the Class Dinophyceae.



Figures 38 – 43 Intracellular structure of *T. heimii*. (38) Part of the cell. Vacuoles containing dense material (A) and/or tubular structures (B) are visible situated near the Golgi region (G). Accumulated starch grains (S) in the cytoplasm are also visible; (39) Vacuoles containing cylindrical crystals (arrows) attaching to the membrane; (40) Unstained section. Calcium carbonate layer (C) and cylindrical crystals (arrows) in vacuoles (V) are preserved and are electron dense. Note that there is a crystal mass in the vacuole which is similar to that of the calcified layer. This crystal is surrounded by small, cylindrical crystals; (41) A vesicle containing cylindrical crystals (arrows) in addition to dense material and tubular structures; (42) Golgi apparatus. Dictyosomes (D) are hemispherically arranged. Note the presence of a cylindrical crystal (big arrow) and dense material (small arrows) in cisternae; (43) Typical dinoflagellate trichocyst (T).



Figures 44 – 47 Mitotic stages. (44) Anaphase stage. Nuclear envelope (Ne) is persisting and invaginated by cytoplasmic channels (Cc) through which spindle microtubules pass. Some chromosomes are attached to the membrane surrounding the channel of cytoplasm. Two dictyosomes (D) are visible situated at opposite poles; (45) Telophase stage. Flagella (F) are already present. Note that the cytoplasmic membrane is still continuous at the equatorial region; (46) Telophase stage. Note that the vacuole (V) containing cylindrical crystals (arrows) is separating daughter nuclei (N) and associated cytoplasm. Cytoplasmic channels (Cc) are still visible; (47) A cell prior to release. The opening and the lid (L) are visible. Distinct swelling of the cell is visible near the opening (arrow).

The taxonomic position of the genus *Thoracosphaera* within the Class Dinophyceae is still not clear. Füttrer (1976, 1977) suggested the affinity between *Thoracosphaera* and *Calciodinellum* and thought that they belonged to the order Peridiniales. The extant dinoflagellate genera, *Peridinium, Scripsiella* and *Ensiculifera*, that produce calcareous cysts belong to this order (Wall & Dale 1968). However, the Peridiniales are an assemblage of armoured dinoflagellates with characteristic thecal plate tabulation. *T. heimii* does not produce any type of cell, either motile or non-motile, which possesses thecal plates. We therefore believe that based on our present knowledge, *Thoracosphaera* should not be placed in the order Peridiniales. The order Phytodiniales might be a more suitable order in which to place *T. heimii* because of the production of *Gymnodinium*-like motile cells, and the coccoid non-motile vegetative cell. It should, however, be noted that some taxa in the order Phytodiniales are known to produce thecal plates during their motile or stalked vegetative stage (Baumeister 1943, 1957; Pfiester & Lynch 1980).

Another order to which *Thoracosphaera* could belong is the order Gymnodiniales because of the absence of thecal plates. However, there is also some disagreement that most genera placed in this order are characterized by a motile vegetative cell rather than a non-motile vegetative stage.

In our opinion *Thoracosphaera* is a very unusual dinoflagellate genus and our present knowledge has not yet reached a stage which enables us to accurately suggest its systematic affinity.

The 'typical' dinoflagellate cell covering termed a theca or amphiesma is well known as being homologous throughout dinoflagellate taxa, and is composed of three membranes, viz. the outer membrane which is interpreted as a plasmalemma and outer and inner plate membranes which form a thecal vesicle containing a plate or plate-like structure (Dodge & Crawford 1970). A pellicle membrane, pellicle layer (Loeblich III 1970) and cytoplasmic membrane (Kalley & Bisalputra 1971; Steidinger & Cox 1980; Morril & Loeblich III 1981) were also reported occurring beneath the inner plate membrane. It is still not certain which membrane is the functional plasmalemma. Dodge & Crawford (1970) and Dürr (1979) interpreted the outer membrane as the plasmalemma whilst Kalley & Bisalputra (1971) and Steidinger & Cox (1980) on the other hand interpreted the cytoplasmic membrane as the plasmalemma.

The cell covering of *Thoracosphaera* is very distinct and the homology with the typical amphiesma is not obvious. In *T. heimii* it is composed of an outer shell membrane, a calcium carbonate layer, an inner shell membrane, a pellicle layer and a cytoplasmic membrane all of which are continuous and surround the entire cell.

Further studies are needed before we are able to comment as to whether the cell covering of *T. heimii* is primitive or advanced or to comment further on its systematic position in the Dinophyceae.

To date there has been no detailed experimental work on the process of calcification in dinoflagellates. As yet we have not followed the process of calcification in *T. heimii* but hope to undertake such a study in the near future.

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