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SIMILARITY OF THE DINOFLAGELLATES *PERIDINIUM TROCHOIDEUM*, *P. FAEROËNSE* AND *SCRIPPSIELLA SWEENEYAE* AS DETERMINED BY CHROMOSOME NUMBERS, CELL DIVISION STUDIES AND SCANNING ELECTRON MICROSCOPY

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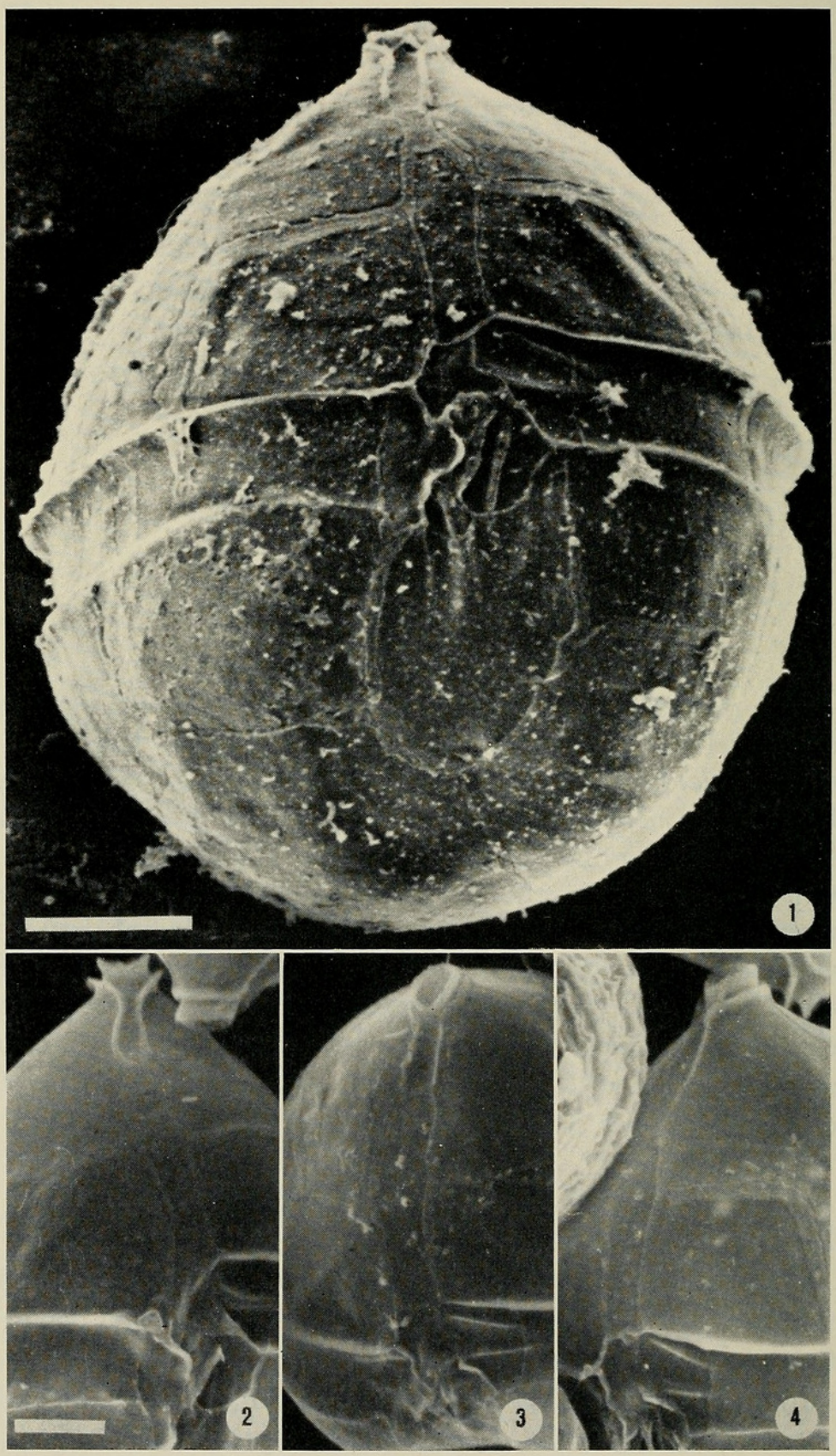
The proper systematic placement of the common, marine dinoflagellates (*Glenodinium trochoideum* Stein, 1883, *Peridinium faeroënse* Paulsen, 1905 and *Scrippsiella sweeneyae* Balech ex Loeblich, 1965) has been the subject of several studies with varying conclusions. Early systematic investigations focused exclusively on the cell wall features of cells from natural populations. However, more recent research has involved cultured strains. In order to clarify the systematics of these species we grew and compared material obtained from culture collections, reexamining cell morphology but also making use of cell life cycle information and numbers of chromosomes per cell.

History of Previous Investigations

Peridinium trochoideum (Stein) Lemmermann, 1910

Stein originally described *Glenodinium trochoideum* from the plankton of the Kiel harbor, West Germany. The magnifications for his plate illustrations varied from 450–690×, setting the length of his *G. trochoideum* cells at 45–69 μm and the width at 28–42 μm. Lemmermann (1910) transferred the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species to the genus *Peridinium*. Braarud (1958) recorded observations of cell division in a Norwegian strain of *P. trochoideum* and figured spiny cysts formed in culture media. Sousa e Silva (1962) also depicted (plate 10, fig. 3) a spiny cyst for *P. trochoideum*, described as having formed in her cultures of Portugese material. Dodge (1963) wrote that each *P. trochoideum* cell contains 44 chromosomes. Balech and Soares (1966) compared preserved material collected off the coast of Brazil with descriptions of *P. trochoideum* and *P. faeroënse* and concluded that these two species are synonymous. They reassigned both to the genus *Scrippsiella*, but in uniting the species, incorrectly chose the junior epithet *faeroënse*. Although similar in description to *S. sweeneyae*, *S. faeroënse* was considered unique due to details in the arrangement of the sucal plates and the narrowness of the first apical plate. Wall and Dale (1968) and Wall *et al.* (1970) collected spiny calcareous cysts off Bermuda and Woods Hole, Massachusetts. They identified the germinated cells as *P. trochoideum*. One cell arose from each cyst. Dickensheets and Cox (1971) examined isolate IUCC 1017, determining the plate pattern and illustrating the surface features of the cell wall. They mistakenly followed Balech and Soares in using the junior epithet. Kalley and Bisalputra (1970) examined the surface features of *P. trochoideum* (IUCC 1017) and confirmed Braarud's observations on the manner of cell division (Kalley and Bisalputra, 1975).

The source of the Woods Hole *P. trochoideum* (Strain "Peri") is recorded as "M. Parke (?)" presumably meaning the Plymouth strain 104 which is on deposit in the Indiana Culture Collection as No. 1017.

Peridinium faeroënse Paulsen, 1905

Paulsen described this species as *Peridinium faeroënse* from samples taken off the Faeroes. As mentioned above, Balech and Soares (1966) transferred this species to the genus *Scrippsiella*.

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FIGS. 1–4. Scanning electron micrographs of *Peridinium trochoideum*, Strain "Peri." 1. Ventral view. Scale line = 5 μm. 2–4. Enlargements of plate 1′ region. 2–4, Scale line = 3 μm.

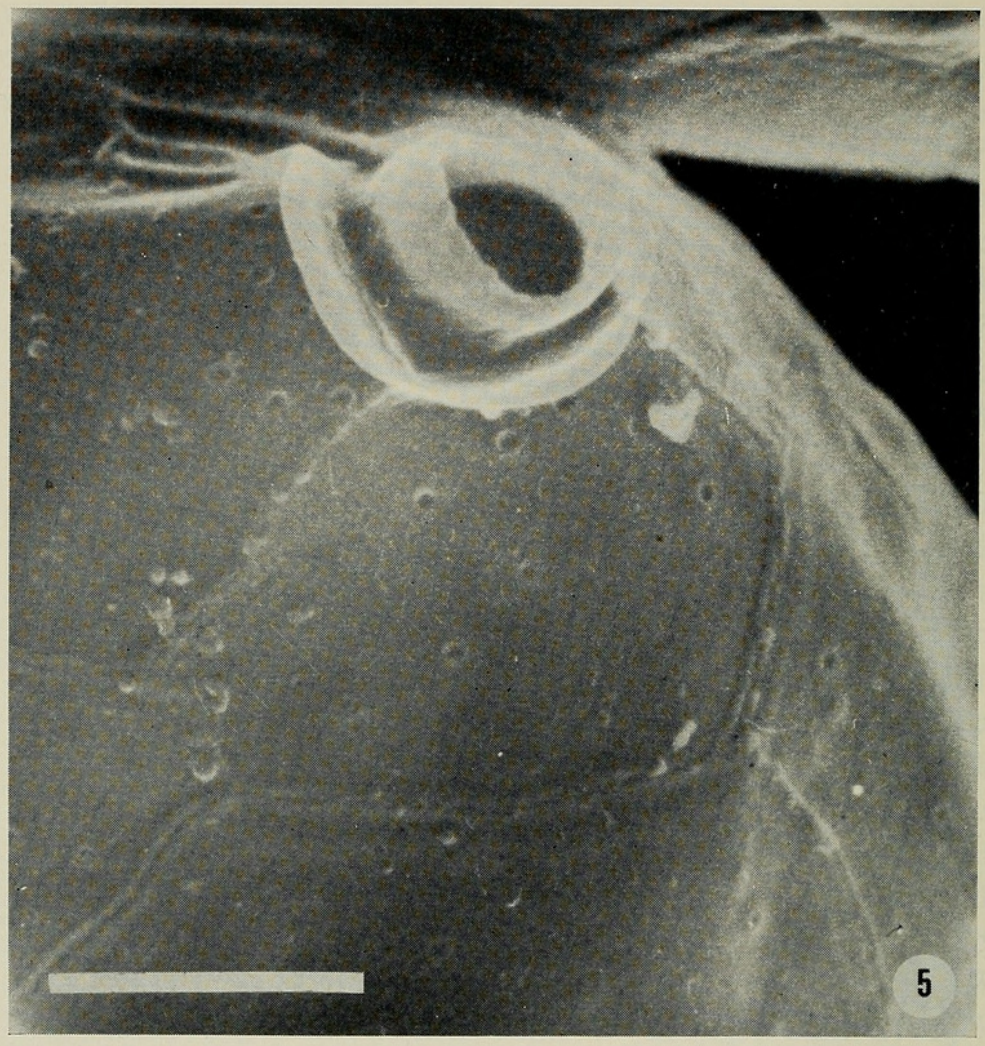

FIG. 5. Scanning electron micrograph of *Peridinium trochoideum*, Strain "Peri" illustrating dorsal side of apical pore and plate 3′. Scale line = 2 μm.

Plymouth isolate 180 listed as *P. faeroënse* is also on deposit in the Culture Centre of Algae and Protozoa, Cambridge, England as No. 1134/4.

Scrippsiella sweeneyae Balech ex Loeblich, 1965

Balech (1959) erected the genus *Scrippsiella* with S. *sweeneyae* as the type. His material was originally isolated by B. M. Sweeney from the San Diego region. Balech (1959) stated that there was a superficial resemblance between S. *sweeneyae* and *P. trochoideum*, however he concluded that the cingular and sulcar plate formation of S. *sweeneyae* warranted the establishment of a new genus. Wall and Dale (1968) collected smooth, lobed calcareous cysts from Woods Hole, Bermuda

Table 1. Cell dimensions in μm

Organisms	Length	Width	Reference
Peridinium trochoideum	45–69	28–42	Stein, 1883[a]
	18–40	—	Sousa e Silva, 1962
	34–47	25–36	Wall and Dale, 1968
	21–32.5 (26.8)	17.5–25 (20.8)	this paper[b]
Peridinium faeroënse	32–36	—	Paulsen, 1905
	30–38	18–22.5	Balech and Soares, 1966
	20–30.2 (26.6)	17.5–27.5 (21.4)	this paper[b]
Scrippsiella sweeneyae	24–32.5	19–24	Balech, 1959
	32–36	28–29	Wall and Dale, 1968
	21.2–30 (26.3)	17.5–27.5 (21.6)	this paper[b]

[a] Range of magnifications is due to variations of original plates. The actual sizes occur within this range.
[b] Twenty living cells (logarithmically growing) were measured at 400×. The mean is in parentheses.

and the Western Arabian Sea and these hatched yielding organisms determined to be S. *sweeneyae*.

The original strain of S. *sweeneyae* used by Balech remains on deposit at the Indiana University Culture Collection (IUCC No. 1656).

Materials and Methods

Peridinium trochoideum (strain "Peri") was obtained from R. R. L. Guillard, Woods Hole, Massachusetts. The isolate of *Peridinium faeroënse* came from the Culture Centre of Algae and Protozoa (No. 1134/4). *Scrippsiella sweeneyae* was procured from the Indiana University Culture Collection (No. 1656) and is a subculture of the type material used by Balech (1959). All cultures were grown in GPM medium (Loeblich, 1975) and were maintained at 21° C under a light intensity of about 250 foot-candles. Cultures were synchronized on a 12:12 hour, light-dark cycle for studies of cell division. Samples for chromosome counts were taken from logarithmically growing and stationary cultures. Nuclei were fixed, stained and squashed according to the acetocarmine technique of Cave and Pocock (1951). A through-focus series of photographs was taken of each cell on high contrast copy film and one composite tracing made of each set of finished photos. Chromosomes were counted from the tracings. For scanning electron microscopy, cells from logarith-

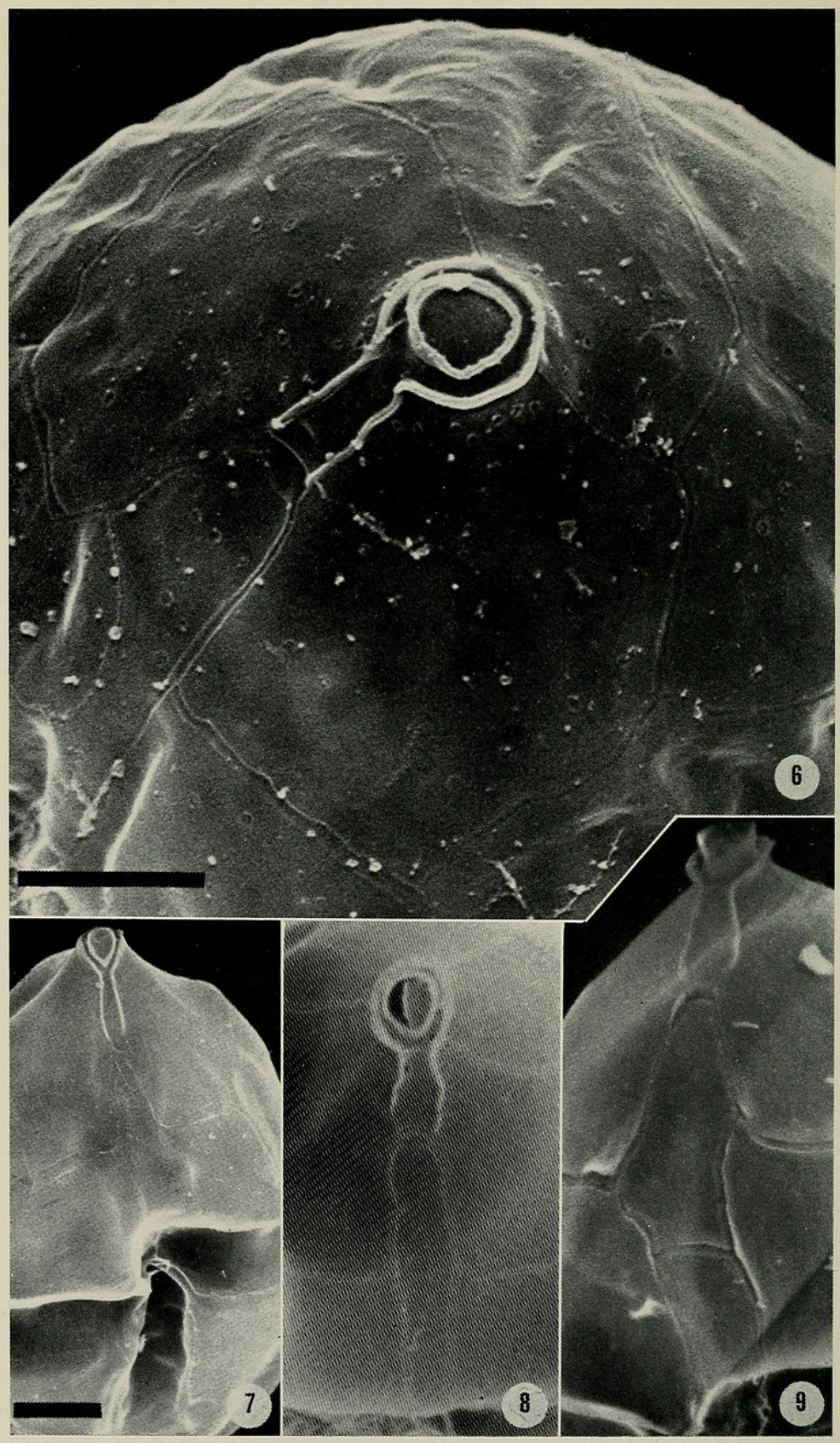
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Table 2. Chromosome counts

Organism	Culture phase	Chromosome number	Number of cells counted
Peridinium trochoideum	log	80–90	2
	log	94	1
	Stationary	80–90, 100	2
Peridinium faeroense	log	100	1
	Stationary	80–90	1
Scrippsiella sweeneyae	log	80–90	2

mically growing cultures were collected on 8 μm pore size (SCWP Millipore filter, 49 mm) by gravity filtration. When all but 2 or 3 ml of culture had passed through the filter, 20 ml of the fixative (4% glutaraldehyde in 0.05 M cacodylic acid (pH 7) and 0.15 M sucrose at 25° C) was added. Then, after most of this solution had filtered, the cells were rinsed in three cacodylate washes of decreasing concentration (0.1 M, 0.075 M, 0.04 M). Using a pasteur pipette and the remains of the final wash suspension the cells were washed off of the filter paper into centrifuge tubes. Dehydration was by means of an increasing ethanol series (25%, 50%, 75%, 95%, 100%, 100%). This was replaced by a Freon 113 series (25%, 50%, 80%, 100%, 100%, 100%, 100%) and critical point drying was performed using the Freon 13 procedure (Cohen, Marlow and Garner, 1968). Cells were coated with carbon and a gold-palladium mixture and examined with either the AMR 100 or JEOL JSM-35 scanning electron microscope.

Results

Cell Morphology: The results of the cell measurements appear in Table 1. The mean widths of cells of the three species are within less than 1% of one another while the mean lengths vary within about 2%. Fig. 1 provides a ventral view of *P. trochoideum* showing the cell shape, girdle position and apical pore characteristic of all three species. In particular it allows examination of plates in the sulcal region and the width of the first apical plate. The first apical plate appears long and narrow and extends from the bottom of the canal plate to the top of the anterior sulcal and transitional plates. The longitudinal flagellum emerges

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Figs. 6–9. Scanning electron micrographs of *Peridinium faeroënse* Plymouth isolate 180. 6. Apical view. Scale line = 2 μm. 7–8. Enlargements of plate 1′ region. 9. Individual with plate 1′ split transversely. 7–9, Scale line = 3 μm.

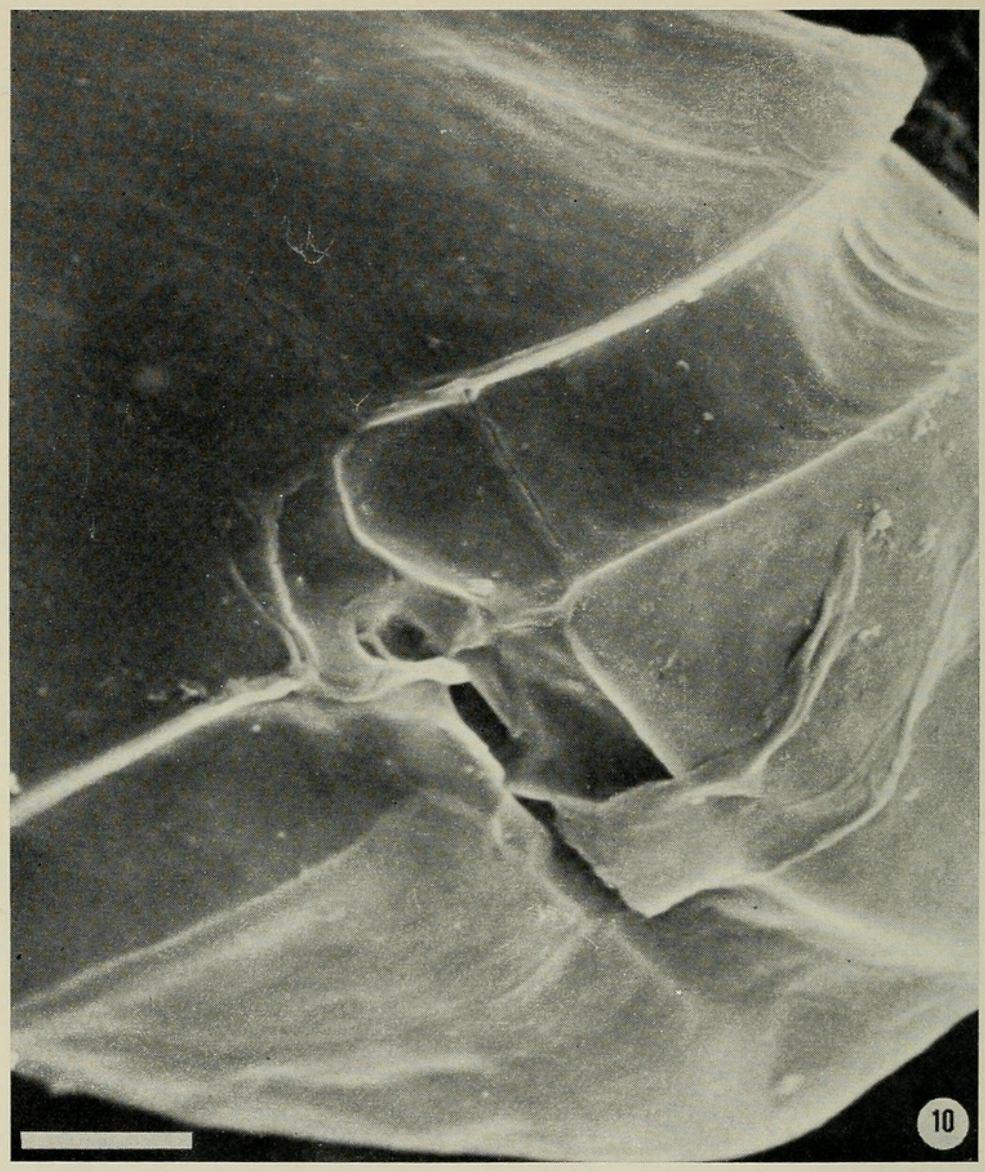


Fig. 10. Scanning electron micrograph of *Scrippsiella sweeneyae*, isolate IUCC No. 1656. Ventral region with frayed longitudinal flagellum. Scale line = 2 μm.

below these and is covered on the right side of the cell by a flange of the right sulcal plate. The first apical plates of three additional *P. trochoideum* cells are illustrated in Figs. 2–4. Fig. 5, a close-up of the second and third apical plates and apical pore of *P. trochoideum* reveals the apical pore structure with the inner flange protruding beyond the exterior flange and the random distribution of the trichocyst pores. The typical apical plate arrangement of *P. faeroënse*, similar to that found in *P. trochoideum* and *S. sweeneyae* is depicted in Figs. 6–8. Fig. 9, also of *P. faeroënse*, however, shows a wider first apical plate, split across the

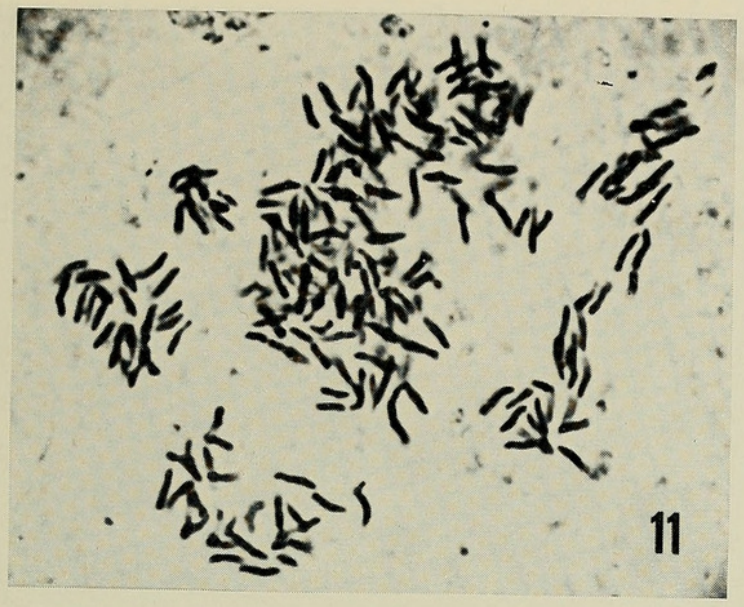


Fig. 11. Acetocarmine stained and squashed nucleus of *Peridinium trochoideum*, Strain "Peri" stationary phase cell at selected focal level. Note presence of V-shaped chromosomes.

center—an abnormality observed in more than one *P. faeroënse* specimen. Fig. 10 illustrates the ventral region of *S. sweeneyae.*

Chromosome Counts: Table 2 contains the results of the chromosome counts. There is no significant difference in chromosome number between exponentially growing cells and cells from stationary phase cultures. All counts for the three isolates ranged from 80 to 100. Fig. 11 shows the fixed, stained and squashed chromosomes of a cell taken from a stationary culture of *P. trochoideum.* Note the presence of both V-shaped and straight chromosomes.

Life Cycles: Cultures of the three species examined contained swimming pairs of what appeared to be fusing cells. However, despite continual observation we found no cysts of the type described by Wall and Dale.

The mode of cell division was determined with results in agreement with the earlier studies of Braarud (1958) and Kalley and Bisalputra (1975). Fig. 12, a scanning electron micrograph, shows a *P. trochoideum* cell shedding its theca. As the light microscopy study revealed, in each of the three strains the theca is shed prior to cytokinesis after splitting dorsally in the sutures between the epitheca and cingulum. Figs. 13–21 illustrate the final stages in the division cycle, identical in all three strains. A dividing cell throws off its theca permitting the emergence of a "peanut-shaped" cell presumably still covered by the pellicular layer and bearing its flagella. The flagella are soon discarded and the nonmotile cell settles to the bottom of the flask where cell division occurs. As is apparent here, the daughter cells are often of unequal size.

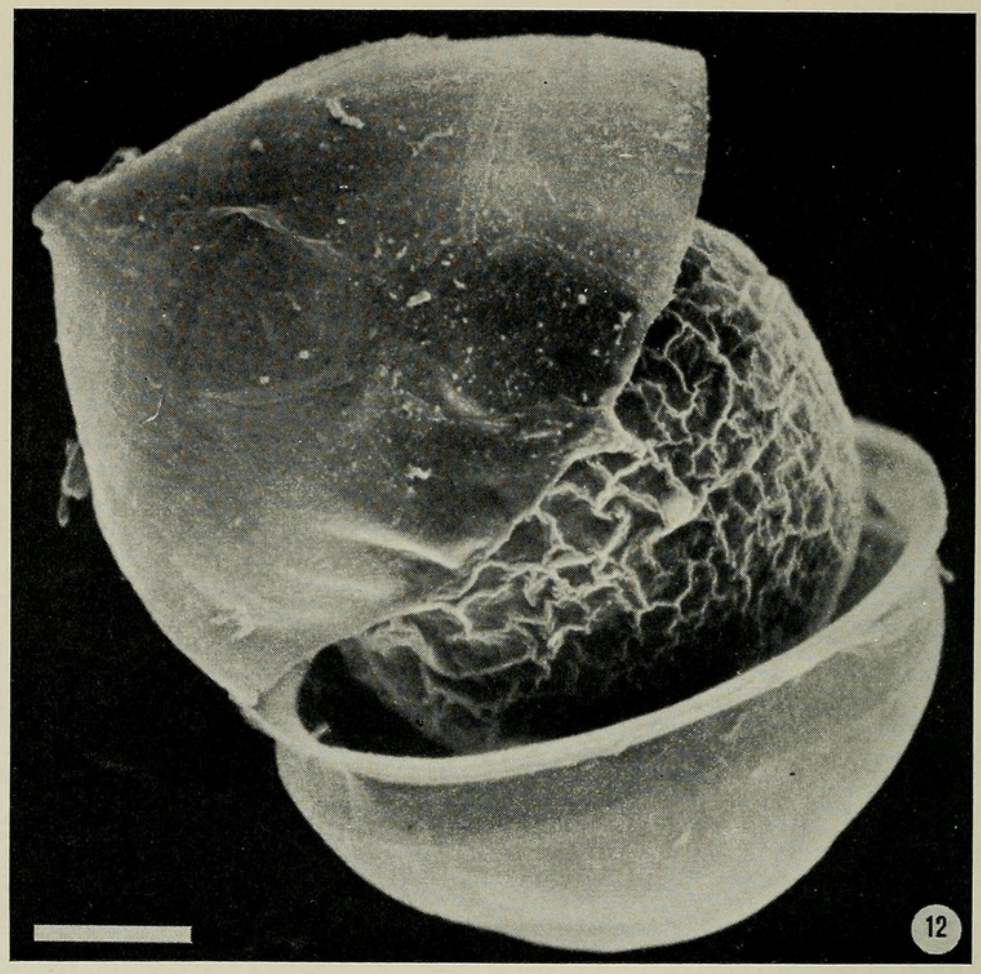


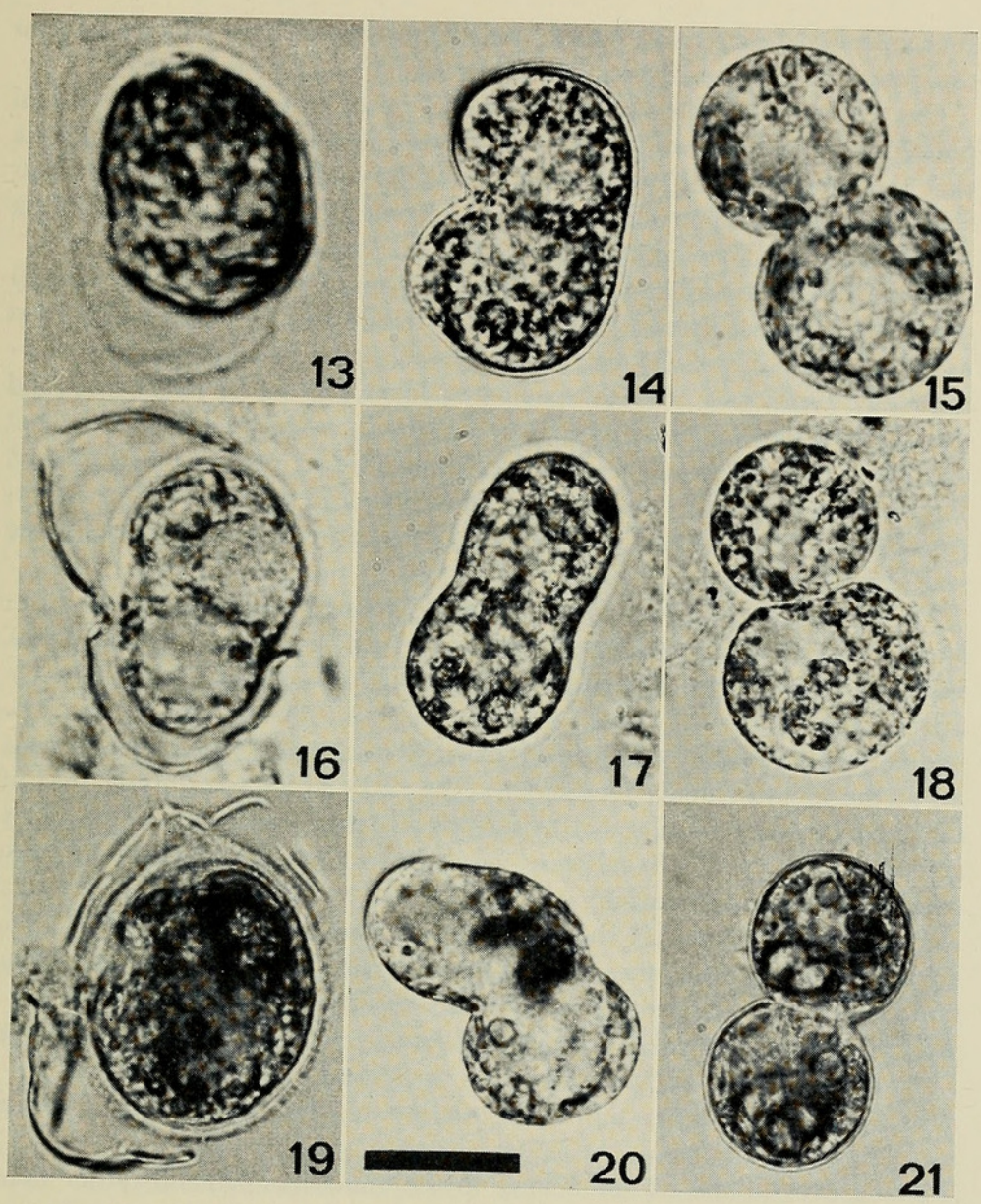
FIG. 12. Scanning electron micrograph of *Peridinium trochoideum*, Strain "Peri," cell undergoing ecdysis. Scale line = 5 μm.

DISCUSSION AND CONCLUSIONS

Our cultures of *P. trochoideum*, *P. faeroënse* and *S. sweeneyae* display no consistent differences while exhibiting striking similarities in morphology, size, chromosome number and life cycle.

The range in chromosome number for a given nucleus is due to procedural difficulties. We attribute the chromosome variation between species to the same cause. The overlap of chromosomes which results from squashing combined with the occurrence of V-shaped chromosomes makes accurate counting difficult. Dodge's count was done using unsquashed cells which may explain why our figure (80–100) differs with his (44) by a factor of two. A count made from an intact nucleus should be viewed as only an estimate. Differences in the chromosome numbers of the three species are not of sufficient magnitude to warrant the separation of these species.

With scanning electron microscopy, the greatest morphological varia-



FIGS. 13–21. Light micrograph of dividing cells. Scale line = 20 μm. 13–15. *Peridinium trochoideum*, Strain "Peri." 16–18. *Peridinium faeroënse*, Plymouth isolate 180. 19–21. *Scrippsiella sweeneyae*, isolate IUCC 1656.

tions were found not between the different strains but within a culture of *P. faeroënse* cells taken from a single flask. The presence of an extra suture in the first apical plate found in two *P. faeroënse* specimens illustrates the occurrence of abnormal thecal plate patterns in dinoflagellates, underscoring the danger in relying solely on thecal morphology when distinguishing species. An extra suture seems a more dramatic deviation from the norm than the imperceptible width difference of the

first apical plate cited by Balech and Soares as the basis for the species separation of S. *sweeneyae* and *P. faeroënse.*

On the basis of the similarities in cell size, cell morphology, thecal plate patterns, chromosome counts and type of cell division we conclude that the strains identified as *P. trochoideum* and *P. faeroënse* are conspecific with the type material of S. *sweeneyae.* The Wall and Dale (1968) findings, however, conflict with this conclusion.

Wall and Dale isolated from the ocean two morphologically distinguishable types of calcareous cysts which hatched organisms identified as S. *sweeneyae* and *P. trochoideum.* The cysts with numerous, low-rounded ridges gave rise to specimens identified as S. *sweeneyae* and the spiny cysts hatched organisms classified as *P. trochoideum.* That one species would produce such drastically distinct cysts seems unlikely. But there is a possible explanation. The organism attributed to S. *sweeneyae* by Wall and Dale may not be conspecific with any of the three strains we studied.

Wall and Dale's (1968) plate pattern diagrams of S. *sweeneyae* differ from the original description of the typical plate pattern (Balech, 1959, Figs. 1a–f) and atypical plate arrangements (Balech, 1959, Figs. 2a–c). Specifically, plate 1'''' never touches plate 3''' in the Wall and Dale material. In addition, the size of their S. *sweeneyae* specimens exceeds all of the measurements reported for the three isolates including that of the type material (Table 1). Although size represents a variable characteristic among dinoflagellates, we believe that the absence of any overlap may be meaningful.

We are assuming that the three strains studied are conspecific. We are also assuming that they are conspecific with the type material represented in Stein's description of *Glenodinium trochoideum* since—

1. Stein's drawings of *G. trochoideum* are so sketchy as to allow our isolates to be circumscribed by the original description.
2. for over 12 years the Plymouth isolate 104 has been considered *P. trochoideum.*

We believe that the strains belong to the genus *Scrippsiella* rather than to other genera of the Peridiniaceae, *e.g.*, *Peridinium* or *Protoperidinium* where they might have been placed. The isolates differ from the type of *Peridinium* in being marine, possessing an apical pore and in their production of calcareous cysts. They differ from the type of *Protoperidinium* in calcareous cyst production and number of cingular plates. Adopting the oldest species' name, the isolates should be called *Scrippsiella trochoidea* (Stein) Loeblich, 1976, p. 25.

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