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A description of the marine dinoflagellate, *Scrippsiella tinctoria* sp. nov.

Stephen R. INDELICATO and Alfred R. LOEBLICH, III

University of Houston, Marine Science Program, 4700 Avenue U, Galveston, Texas 77551, U.S.A.

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This paper describes a new marine dinoflagellate, *Scrippsiella tinctoria*, isolated from a Pacific Ocean neritic environment off San Diego, California. The thecal layer is tabulated by means of the chloral hydrate-hydriodic acid-iodine staining technique. Cell division and chromosome numbers of this species are similar to those described for other members of this genus. This species secretes copious amounts of at least one yellow water soluble compound into the culture medium.

Key Index Words: *Dinoflagellate*; *marine alga*; *Pyrrhophyta*; Scrippsiella tinctoria sp. nov.; *systematics*.

The Food Chain Research Group (FCRG) of Scripps Institute of Oceanography, La Jolla, CA, maintains in its culture collection an isolate identified as *Gymnodinium* sp., FCRG No. 47. This isolate was originally thought to be a member of the dinoflagellate order Gymnodiniales, an order whose members lack a thecal layer within the amphiesma. MORRILL and LOEBLICH (1981), however, found that this isolate possesses within its amphiesma, a thecal component that stains positively with a chloral hydrate-hydriodic acid-iodine mixture, indicating the presence of cellulosic compounds. In the same study it was also determined that this isolate contains an acetolysis resistant amphiesmal layer identified as the pellicle. The presence of a theca and a pellicle places FCRG 47 in the order Peridiniales. The use of KOFOID's (1909) system of designating thecal plates, to aid in the identification of the Peridiniales, revealed that this species is a new member of the genus *Scrippsiella* BALECH *ex* LOEBLICH 1965.

Isolate FCRG 47 actively secretes a yellow-colored, ultraviolet (UV) absorbing, water soluble compound into solution as the cultures grow. This compound (or compounds) may well contribute to the "Gelbstoff" (yellow substance) of sea water first noted by KALLE in 1937. The release of yellow-colored compounds by FCRG 47 and its contribution to Gelbstoff is currently under study.

Materials and Methods

Scrippsiella tinctoria sp. nov. (FCRG 47, LOEBLICH 173), was originally isolated as a clonal culture from marine waters off a sewage outflow near Pt. Loma, California on July 10, 1970 by Mr. J. B. JORDAN Both isolates have been cultured for a number of years in medium GPM (LOEBLICH 1975). Cultures for this study were maintained in 10 ml of GPM (pH 7.5; salinity 27 ppt) in Pyrex screw-capped test tubes at 21°C under a light: dark photoregime (12:12 hr) of 350 ft. candle illumination from cool white fluorescent lights.

Culture growth and behavior were observed by means of a Leitz Diavert inverted microscope. Live cells of *Scrippsiella tinctoria*

were taken from log-phase caltures and length and width were measured at 400× under a Leitz SM-Lux microscope. This process was facilitated by gently passing the microscope slide containing a drop of culture through a bunsen burner flame. The sudden rise in temperature causes *S. tinctoria* to discard its flagella, ceasing cell locomotion without changing cell morphology or size.

The staining of thecal plates of *S. tinctoria* for tabulation was performed using the chloral hydrate-hydriodic acid-iodine method described by von STOSCH (1969) and redescribed by SCHMIDT *et al.* (1978). This stain acts upon the cellulosic component of thecal plates. Cells harvested from log-phase and stationary-phase cultures were fixed in methanol-formic acid for 10 min. and resuspended in tertiary butanol containing 6% dioxane. A drop of this suspension was mixed with a drop of chloral hydrate-hydriodic acid on a microscope side and covered with a coverslip. A few crystals of iodine placed beneath the coverslip helped to intensify the stain. Considerable pressure applied to the coverslip was required to flatten the relatively thin thecal plates of this species. This process also provided an easy method for making chromosome counts. Although the chromosomes themselves did not stain, the process of squashing the cell and extracting out the photosynthetic pigments with methanol-formic acid allowed the condensed chromosomes to become highly visible (see Fig. 12).

All photomicrographs were taken through a green interference filter on Kodak Technical Pan film 2415 using a Leitz Orthoplan microscope equipped with Leitz Wetzlar lenses and an Orthomat camera.

Description of Scrippsiella tinctoria

Division Pyrrhophyta PASCHER, 1914

Order Peridiniales HAECKEL, 1894

Family Calciodinellaceae DEFLANDRE, 1947

Scrippsiella BALECH *ex* LOEBLICH, 1965

Scrippsiella tinctoria sp. nov.

Scrippsiella tinctoria is a small, armored, ortho peridinioid dinoflagellate (Figs 1, 2, 8), which superficially resembles *Scrippsiella sweeneyae* BALECH *ex* LOEBLICH 1965, the type species of the genus *Scrippsiella* (BALECH 1959). This organism has a typical dinoflagellate dinospore morphology with a mean length of 23.2 μm (range 19.2-28.8 μm, std. dev. 2.14 μm, n=30) and a mean width of 20.4 μm (range 17.3-24.3 μm, std. dev. 1.84 μm, n=30). The ratio of mean cell length : mean cell width=1.13. In anterior view, the cell is nearly circular in outline except for a notch where the sulcus is incised. There is no dorso-ventral compression of the cell and the longitudinal axis is perpendicular to the dorso-ventral axis. The epitheca is equal in size or slightly larger than the hypotheca. In ventral view, the epitheca is conical to broadly oval in outline with an apical pore at the apex. The apical pore complex in some individuals may be somewhat flattened and slightly depressed, never extended as in some other species of *Scrippsiella* (BALECH 1959). The hypotheca is rounded and sometimes moderately flattened at the cell's posterior. The plate tabulation is pp, pr, 4′, 3a, 7″, 6c, 5s, 5‴, 2⁗ (Figs 1,2). The pore plate (pp) is small, circular, and apically located (Fig. 3). It is slightly overlapped by the 2′, 3′ and 4′ plates which are symmetrically located on the lateral and dorsal sides. The preapical (pr or "canal plate") is small, rectangular, and located ventrally, just anterior to the 1′ plate. Plate 1′ is large and relatively wide with an ortho arrangement. Plates 2′ and 4′ are large, similar in size, and hexagonal in shape. Plate 2′ is located to the left and ventral to the pp. Its longest border is with the pp-pr complex and its other borders are approximately equal in length and adjoin the 1′, 1″, 2″, 1a, and 3′ plates. Plate 4′ is located to the right of the pp. It shares borders with the pp-pr complex, and the 3′, 3a, 6″, 7″ and 1′ plates. The three intercalary plates form a contiguous series : *i.e.*,

the 2a plate borders both the 1a and the 3a plates. Together they are offset slightly to the left side of the organism. The 2a plate is the largest of the three, being irregularly hexagonal ("hexa") and sharing its longest border with the 4'' plate and its smallest with the the 5'' plate. The smallest intercalary is the 1a plate, which is hexagonal in shape. The 3a plate is pentagonal, sharing a long border with the 6'' plate and a short border with the 5'' plate. The seven precingular plates are somewhat variable in shape and size. Most commonly the 1'', 2'', 6'' and 7'' plates are the largest with the 3'', 4'' and 5'' being much smaller. Variants were noted that lacked a precingular plate; these were either lacking a suture between the 4'' and 5'' or the 5'' and 6'' plates.

The hypothecal plates have the typical peridinioid arrangement of 5''' and 2'''' (Fig. 4). After examining plate squashes from approximately fiifty individuals, no detectable variations in the hypothecal tabulation were discovered. The five postcingular plates are of approximately equal size and are arranged symmetrically around the anterior border of the hypotheca. The two antapical plates are slightly larger than any of the postcingular plates. The 1'''' plate is the larger of the two. Both are pentagonal in shape, sharing common borders with the posterior sulcal plate and the 3''' plate. The 1'''' plate also borders the 1''' and 2'''; the 2'''' plate borders the 3''' and 4'''.

The cingulum is moderately incised and descends sinistrally to one-half its width at the sulcus. It consists of six plates, five of approximately equal length (2c-6c) and a smaller 1c (transitional) plate which extends into the sulcal region (Fig. 5). From the dorsal side, two sutures can be seen, one on either side of the 4c plate.

The sulcus is ventral, depressed, and extends into the hypotheca. It consists of 5 plates : an anterior sulcal (as), a left sulcal (ls), a right sulcal (rs), an internal or medial sulcal (ms), and a posterior sulcal (ps) (Figs 6, 7). Generally, the posterior sulcal plate is the largest. Its posterior end is rounded and deeply extended into the hypotheca. The anterior end is deeply notched, forming two lobes. The left lobe is larger and butts against the posterior border of the 1c plate. The lobe on the right side of the posterior sulcal plate is much smaller and butts against the right sulcal plate. Situated partially in the notch of the posterior sulcal is the left sulcal plate (which is actually more to the center of the sulcus). This plate is smaller than the posterior sulcal plate. The right sulcal plate is small and somewhat trapezoidal. It sometimes appears to be an extension of the distal end of the cingulum. The right border of the rs plate is contiguous with the 6c plate while the left border lies adjacent to the left sulcal plate. Its anterior end extends to the anterior sulcal plate and shares a small border with the 7'' plate. The anterior sulcal plate forms a bridge which extends from the right anterior border of the 1c plate, to the left anterior border of the right sulcal plate. This plate also has a small border with the 7'' plate and sits immediately posterior to the 1' plate. The internal sulcal plate is small and ovoid and is usually hidden in part by the left sulcal plate (Fig. 7).

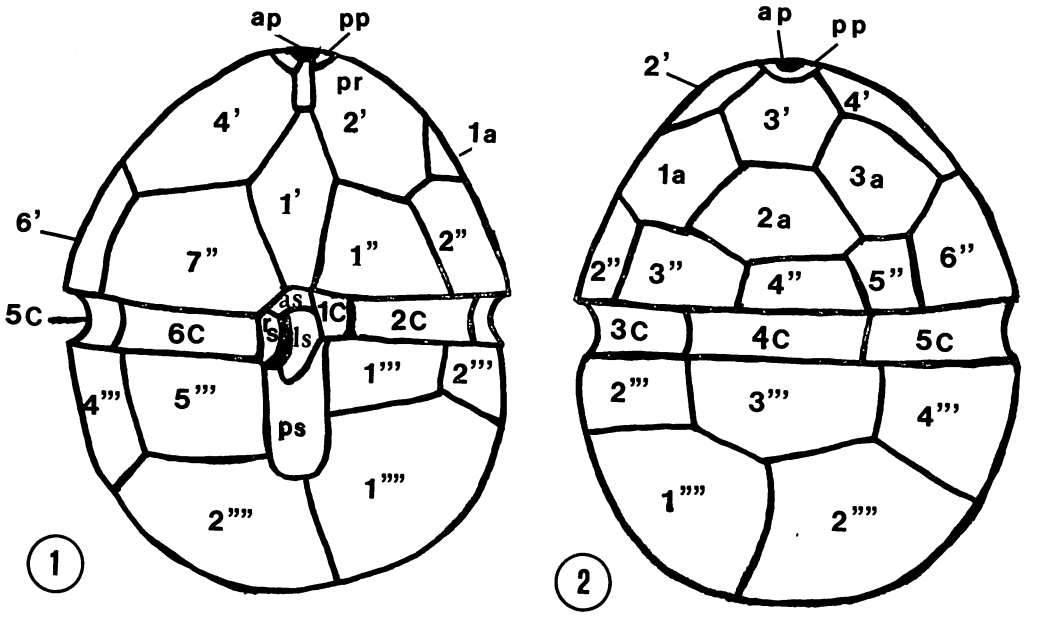
The nucleus is large and spherical with a mean diameter of 10.7 μm (range 7.4-12.8 μm, std. dev. 1.18 μm, n=29). It is usually located centrally or slightly posterior to the cingulum. The chromosomes are long, intertwined, approximately 10 μm long, and easily seen without staining (Fig. 12). Chromosome counts from seven individuals led to an estimate of 80-100 chromosomes per cell. Due to the long and intertwined nature of the chromosomes, obtaining precise counts was difficult.

Several large, round, yellow, refractile "accumulation bodies" or "physodes" (INDELICATO 1984) can also be seen in each cell. The cells in culture tend to secrete a yellow-colored water soluble pigment into the culture medium. The relationship between "physodes" and the cellular secretions of *S. tinctoria* is currently under study.

Scrippsiella tinctoria divides asexually in a

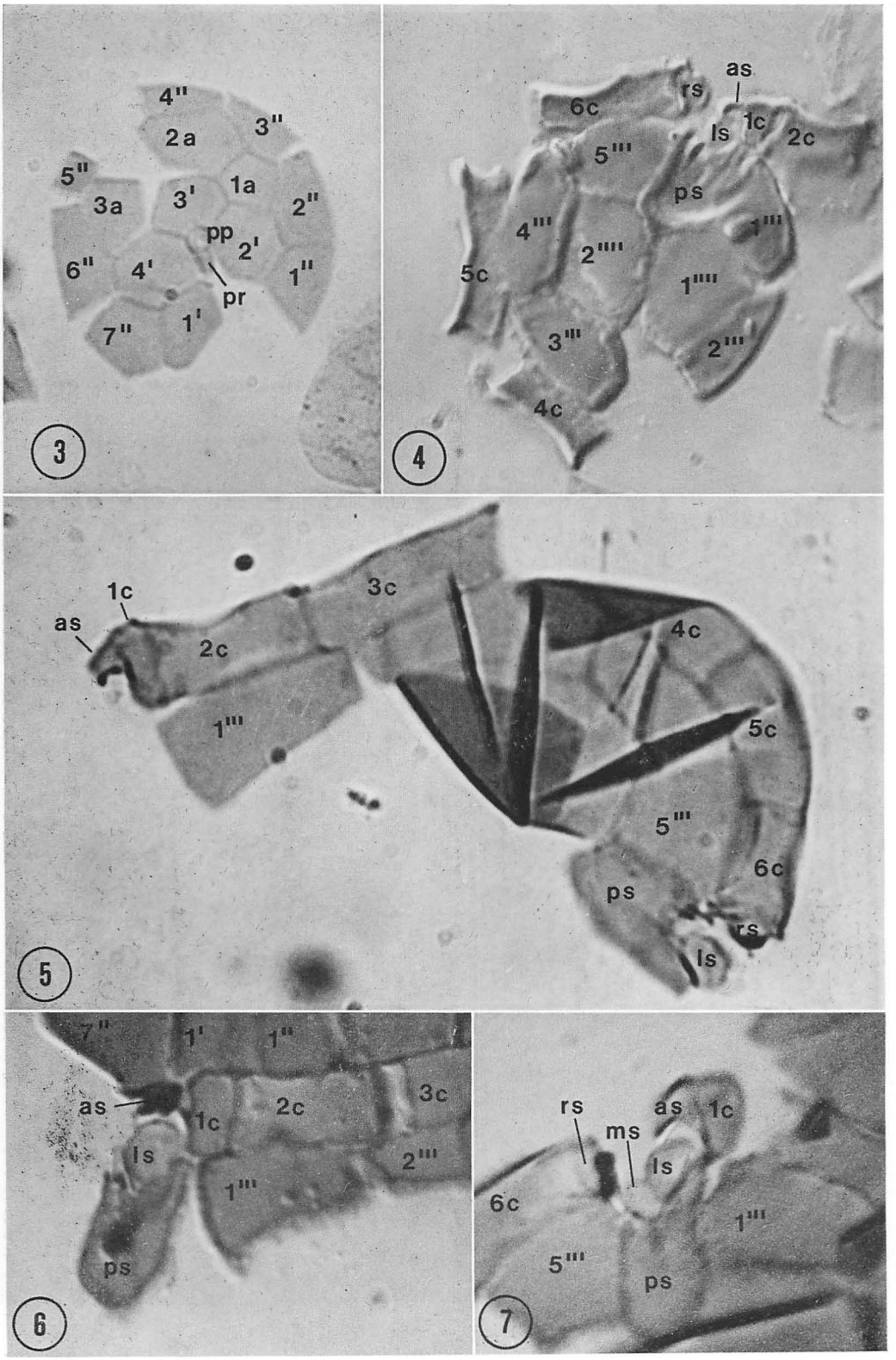
manner similar to *Scrippsiella trochoidea* (STEIN) LOEBLICH, *Scrippsiella faeroense* (PAULSEN) BALECH and SOARES and *Scrippsiella sweeneyae* (BRAARUD 1957, KALLEY and BISALPUTRA 1975, FINE and LOEBLICH 1976). Swimming vegetative cells were observed settling to the bottom of the culture container and subsequently undergoing ecdysis (Figs 9, 10). This occurs by the cytoplasm and pellicle first pulling away from the old cell wall, followed by the separation of the plates along the epithecal-cingular suture. The theca is thus spilit transversely with the cingulum remaining attached to the hypotheca (Fig. 10). The "naked" protoplast then sheds the old theca as well as the parental pellicular layer (Fig. 11), and undergoes mitosis. During mitosis a "peanut"-shaped cell is formed as the two daughter cells initiate cytokinesis. Each daughter cell is nearly spherical toward the end of cytokinesis (Fig. 13), however the two daughter cells are not always equal in diameter. New cell wall material forms concurrently with, or prior to the shedding of the old theca (MORRILL and LOEBLICH 1981). No sexual cycle has as yet been observed in this organism.

Latin diagnosis: Epitheca conica ad late ovalem, poro apicali non elevato. Hypotheca rotundata, magnitudine epithecae quasi aequa. Cingulum centrale, decendens, per 1/2 partis latitudinis dispositum, sine laciniis. Sulcus magnus in hypothecam extendens.



Figs 1, 2. *Scrippsiella tinctoria*: Composite sketch of the thecal plate arrangement (pore plate (pp), preapical plate (pr), apical plates (1′-4′), intercalary plates (1a-3a), precingular plates (1″-7″), cingular plates (1c-6c), anterior sulcal plate (as), left sulcal plate (ls), right sulcal plate (rs), posterior sulcal plate (ps), postcingular plates (1‴-5‴), and antapical plates (1⁗-2⁗)). 1) Ventral view. 2) Dorsal view.

Figs 3-7. *Scrippsiella tinctoria*: Theca stained with chloral hydrate-hydriodic acid-iodine. 3) Epithecal plates. Note tabulation (pp, pr, 4′, 3a, 7″). ×4500. 4) Hypothecal and sulcal plates with some attached cingular plates. Note tabulation (6c, 5s, 5‴, 2⁗). ×4500. 5) Complete cingular plate series with attached hypothecal and sulcal plates. Note tabulation (6c). ×6000. 6, 7) Views of sulcus with surrounding plates. Note the 5 sulcal plates (in Fig. 7 the left sulcal plate is moved aside to expose the underlying medial sulcal plate (ms)). ×6500.


4''
2a
3''
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pp
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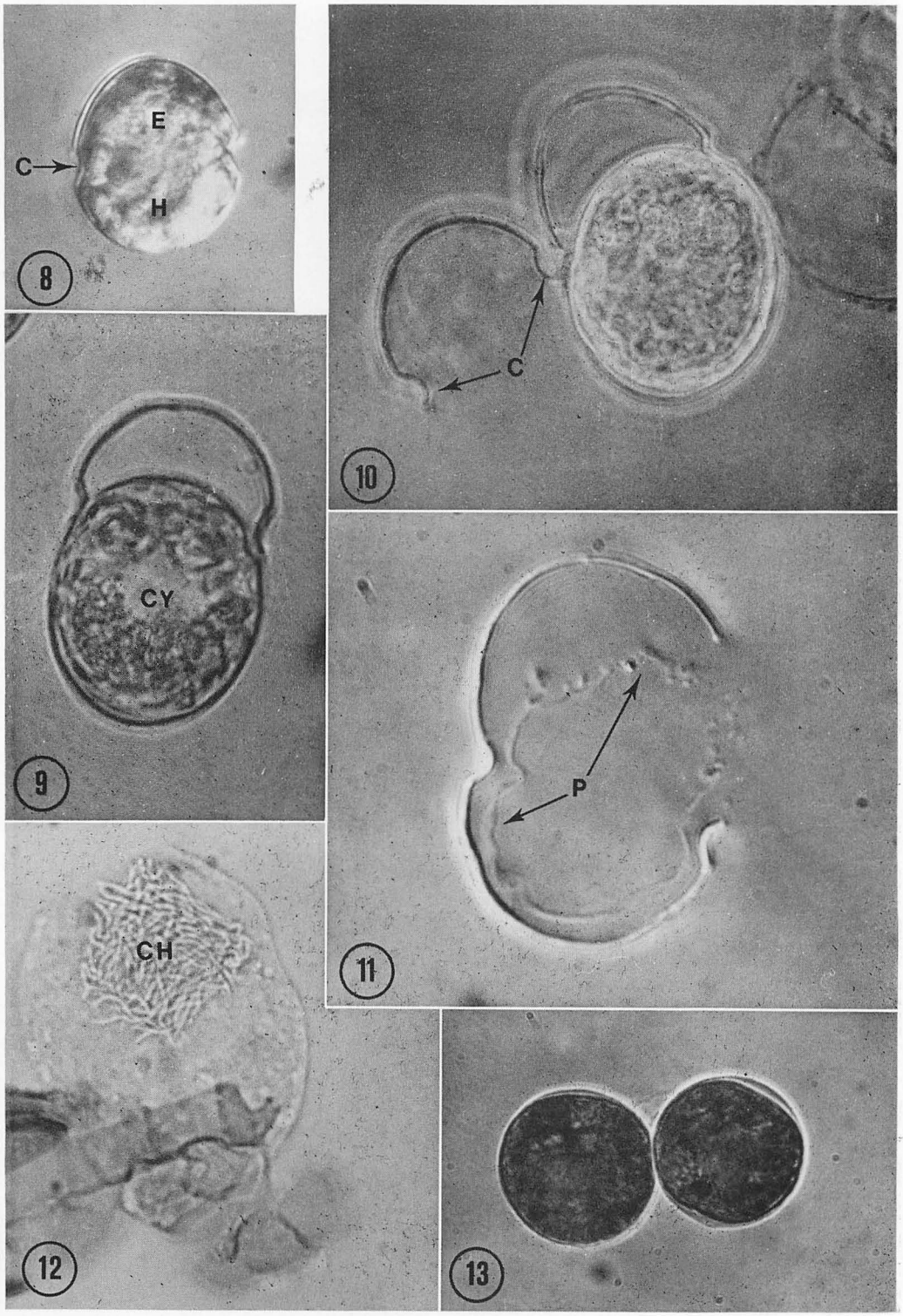
E
C
H
8
CY
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P
11
CH
12
13

Formula laminarum: pp, pr, 4′, 3a, 7″, 6c, 5s, 5‴, 2⁗ dispositione orthoperidinioidea. Cellula 19.2-28.8 μm long., 17.3-24.3 μm transdiametro. Nucleus magnus (magnitudine mediana 10.7 μm, 7.4-12.8 varians), sphericus, positu in centro ad aliquantulum posteriorem. Chromosomata longa, implicata et visibilia sine tinctione. Cellulae pigmentum brunneum, in aqua solubile secernunt.

Habitus: in aqua marina, in loco Point Loma, California dicto. Holotypus: figure inter verba 1.

Etymology of *S. tinctoria*: Latin, tinctorius, "of dyeing", referring to its ability to discolor the surrounding medium yellow.

Discussion

When *S. tinctoria* is compared to the type species of the genus *Scrippsiella*, *S. sweeneyae* [see BALECH 1959], there can be no doubt that it should be placed in the genus *Scrippsiella*. Morphological similarities include identical major thecal plate tabulations, the presence of an apical pore, and uninterrupted intercalary plates. It is important to note here that the 6 cingular plates of *S. tinctoria*, the small 1c or "transitional" plate and the 5 larger plates, are homologous to the 6 cingular plates of *S. sweeneyae*. That is to say the sutures of the cingular plates arise at the same position on the hypothecal plates in both species. These two species also share similar cell dimensions, chromosome numbers, and a marine habitat (FINE and LOEBLICH 1976). *Scrippsiella tinctoria* differs morphologically from *S. sweeneyae* in the absence of a pronounced apical horn and in the relative size and arrangement of sulcal plates. *Scrippsiella tinctoria* also has an internal sulcal plate, a feature which has not yet been found in *S. sweeneyae*. The ability of *S. tinctoria* to secrete a water soluble yellow pigment into solution makes this organism unique in comparison to the other species of *Scrippsiella* (INDELICATO 1984). The absence of a pronounced apical horn also separates this organism from most of the other scrippsielloid species except possibly *S. subsalsa* (Ostenfeld) Steidinger and Balech. The 2a and 3a plates in *S. subsalsa*, however, are separated by the 2′ plate which differs from the contiguous series of intercalary plates of *S. tinctoria*. A similar species, *Ensiculifera loeblichii* COX and ARNOTT [here considered to be a member of the genus *Scrippsiella*] contains only five cingular plates in comparison to the six cingular plates of *S. tinctoria*.

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Figs 8-13. *Scrippsiella tinctoria*: Light micrographs. 8) Entire cell. Note epitheca (E), hypotheca (H), and cingulum (C). ×1500. 9) Cell beginning ecdysis. Cytoplasm (CY) balls up and swells to break out of theca. The fission line occurs at the epitheca-cingulum border. ×2300. 10) Continuation of ecdysis. The cingular plates (C) remain attached to the hypothecal plates. ×1300. 11) Discarded theca. Note the old pellicle (P) which remains after ecdysis. ×1500. 12) A squashed cell with photosynthetic pigments extracted out with methanol-formic acid to show the chromosomes (CH). ×4800. 13) A dividing cell with equal sized daughter cells. ×2300.

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インデリカト, S.R., ・レーブリッヒ, A.R. III: 海産渦鞭毛藻 *Scrippsiella tinctoria* sp nov.

カリフォルニア州サンディエゴの太平洋沿岸から単離した海産渦鞭毛藻の新種 *Scrippsiella tinctoria* を記載した。鎧板の配列は抱水クロラール・ヨウ化水素酸・ヨウ素染色法により調べた。本種の細胞分裂の様式と染色体数は本属の他の種で記載されたものと類似している。本種は少なくとも一種の黄色水溶性物質を多量に培養液中に分泌する。