Hisayoshi NOZAKI*: **Morphology and taxonomy of two species of *Astrephomene* (Chlorophyta, Volvocales) in Japan**

野崎久義*: 日本産 *Astrephomene* (緑藻, オオヒゲマワリ目) 2種の形態と分類

(Plates XI-XIII)

The genus *Astrephomene*, a colonial green flagellate, was described by Pocock in 1953 with a single species *A. gubernaculifera*. Although this alga resembles members of the family Volvocaceae in colonial organization, it does not undergo inversion during colony formation, a characteristic of the volvocacean algae. Therefore, Pocock (1953) erected a new family Astrephomenaceae. Since her description, no other species has been established in this family. No detailed studies on *Astrephomene* from Japan have been reported.

Recently, I isolated two morphologically distinct colonies of *Astrephomene* by re-wetting soil samples from Kanagawa Prefecture. The vegetative colonies and reproduction of the two algae identified one with *A. gubernaculifera* Pocock, however the other is believed to be a new species. The morphology, reproduction and taxonomy of the two species of *Astrephomene*, observed by light microscopy under controlled laboratory conditions are described in this report.

Materials and methods Soil samples from which *Astrephomene gubernaculifera* was isolated were collected in a paddy field at Yoshidashima, Kaiseimachi, Kanagawa Prefecture in April 1981. Other soil samples were collected from a paddy field at Nagae, Hayama-cho, Kanagawa Prefecture, in December 1980. The methods of isolation and culture, as well as mating, zygote germination and staining are those described in the previous study on *Volvulina steinii* Playfair (Nozaki 1982).

Observations and discussion

Astrephomene gubernaculifera Pocock, Trans. Roy. Soc. S. Afr. **34**: 111. f. 4-30. pl. 2, C-Q. 1953. (Figs. 1, 2; Pl. XI A-R).

Motile colony ellipsoidal to spherical, containing 64 or 32 (rarely 128 or 16)

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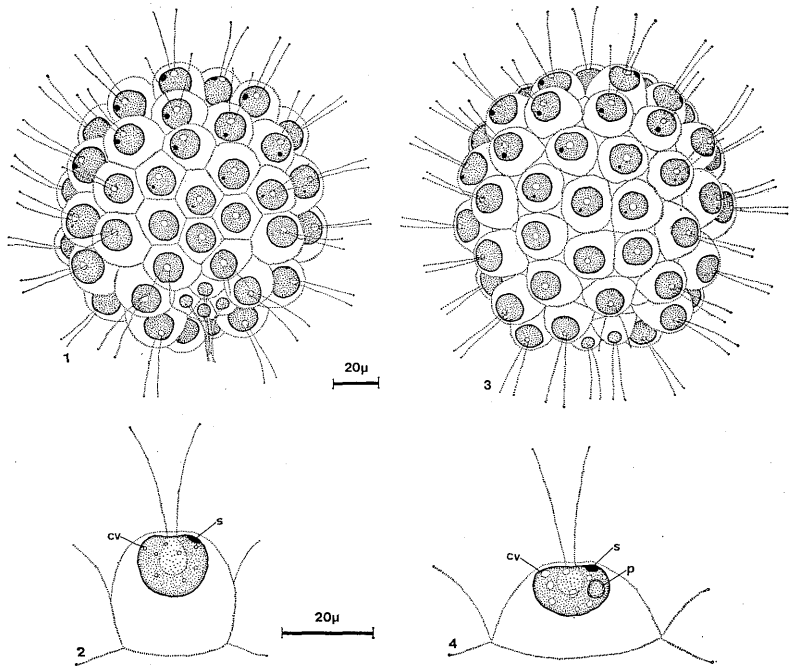
cells embedded in gelatinous cellular sheaths in the periphery of the colony. The 64- or 32-celled colony containing four or two, respectively, small somatic cells in the posterior pole. Cellular sheaths pentagonal or hexagonal in front view, compactly adhering to one another to form a hollow colonial structure. Cells nearly spherical, with two equal flagella, a stigma and many contractile vacuoles on the surface. Chloroplast single, massive, cup-shaped, showing no pyrenoids in any age of the culture. Reproductive cells up to 18 μm in diam.; somatic cells up to 10 μm in diam.; mature 64-celled colonies 90–150 μm long. Asexual reproduction by autocolony formation, each cell except the posterior somatic cells dividing into a daughter colony without inversion. Just after the divisions, each reproductive cell in the newly formed colony emitting one flagellum, while the posterior two or four somatic cells emitting two. The repro-



Figs. 1, 2. *Astrephomene gubernaculifera* Pocock. 1: 64-celled colony. 2: Reproductive cell. cv, contractile vacuole; s, stigma.
Figs. 3, 4. *Astrephomene perforata* Nozaki. 3: 64-celled colony. 4: Reproductive cell. p, pyrenoid.

ductive cells of the colony liberated from the mother colony becoming biflagellate. Sexual reproduction heterothallic and isogamous. During colony clumping or after the dissociation of colonies into the individual cells, each reproductive cell escaping from its cellular sheath to be a spherical gamete. Plasmogamy initiated by the union of anterior mating papillae of the two gametes during gamete clumping, proceeding to form a planozygote. Planozygotes developing two, or sometimes more, pyrenoids and settling down into smooth-walled aplanozygotes. Mature aplanozygotes spherical, 12–20 μm in diam., containing reddish-brown granules. Upon germination, a single biflagellate gone cell escaping from the zygote wall. Gone cell spherical, containing the reddish-brown granules of the former zygote, secreting a gelatinous envelope. The two flagella projecting outside the envelope. 2–4 hours after the escape, the gone cell settling down and dividing into a gone colony within the envelope as in the asexual reproduction.

Type locality. The Cape Flats, South Africa.

Distribution. South Africa (Pocock 1953). Australia (Playfair 1915, Pocock 1953). The United States (Pocock 1953, Stein 1958, Brooks 1966). Mexico (Brooks 1966). Italy (Alfinito 1980). Japan.

The vegetative morphology of this alga agreed well with that described by Pocock (1953). However, there were slight differences with regard to reproduction (Pocock 1953, Stein 1958, Brooks 1966). Asexual reproduction was observed to be essentially the same as those reported by Pocock (1953) and Stein (1958) but with the additional feature of a flagellar emission from the cells of the newly formed daughter colony (Pl. XI J). Although the details of gone colony formation was studied by Brooks (1966), the gelatinous envelope within which the gone colony is formed (Pl. XI Q, R) was not mentioned.

Astrephomene perforata Nozaki, sp. nov. (Figs. 3, 4; Pl. XII A–J, Pl. XIII A–L).

Colonia ellipsoida aut spherica, ex 64 vel 32 cellulis composita, in vaginis cellulosis gelatinosis inclusis. Colonia 64 vel 32 cellularis 2 cellulas somaticas parvas continens. Vaginae cellulosae e fronte coloniae visu quasi circulares, vaginis propinquis interconnexae. Fenestrationes triangulares vel quadratae parvae inter quamque vaginam cellulosam formatae. Cellulae lenticulares auto sphericae. Chloroplastus poculiformis, in culturis junioribus nullas pyrenoides continens, in culturis vetustioribus in eius margine singularem pyrenoidem formans

Reproductio non-sexualis coloniis filialibus effecta, ex omnibus cellulis, cellulis somaticis exceptis, sine inversione formatis. Reproductio sexualis isogametis effecta, ex omnibus cellulis reproductivis sine divisione liberatis formatis. Cum germinatione zygota zoosporam biflagellatam singularem generans.

Motile colony ellipsoidal to spherical, containing 64 or 32 (rarely 128 or 16) cells embedded in gelatinous cellular sheaths in the periphery of the colony. Irrespective of the number of the constitutive cells, 64 or 32, the colony containing two small somatic cells in the posterior pole. Cellular sheaths roughly circular in front view, interconnected with the neighbors to form a hollow colonial structure. The interconnecting sheaths forming small triangular, sometimes square, fenestrations between each of them. The whole colony embedded in watery gelatinous matrix. Cells lenticular to spherical, with two equal flagella and many contractile vacuoles on the surface. Chloroplast single, massive, cup-shaped, possessing no pyrenoids in younger cultures, but showing a single one, sometimes two or three, in the brim or top of older cultures. Stigma single, large in the anterior cells, gradually diminishing towards the posterior pole. Reproductive cells up to 19 μm in surface diam.; somatic cells up to 10 μm in surface diam.; mature 64-celled colonies 110–200 μm long. Asexual reproduction by autocolony formation, each cell except the posterior somatic cells dividing into a daughter colony without inversion. 2nd and 3rd divisions parallel, perpendicular to the first division, forming a group of eight cells arranged in two rows of four each; the group gradually becoming more convex towards the outside of the parental colony to form a spheroidal colony within the parental cellular sheath with further divisions. Just after the divisions, each reproductive cell in the newly formed colony emitting one flagellum, while the posterior two somatic cells emitting two. The reproductive cells of the colony liberated from the mother colony becoming biflagellate. Sexual reproduction heterothallic and isogamous. During colony clumping or after the dissociation of colonies into the individual cells, each reproductive cell escaping from its cellular sheath to be a gamete. Gametes biflagellate, spherical, with a single pyrenoid, bearing an anterior mating papilla. Plasmogamy initiated by the union of the papillae of the two gametes during gamete clumping, proceeding to form a planozygote. Planozygotes quadriflagellate, spherical, with two, or sometimes more, pyrenoids. After swimming, several planozygotes settling down into a group of clumping aplanozygotes. Mature aplanozygotes smooth-walled, 12–20 μm in diam., containing reddish-brown

granules. Upon germination, a single biflagellate gone cell escaping from the zygote wall. Gone cell spherical, containing reddish-brown granules of the former zygote, secreting a gelatinous envelope. The two flagella projecting outside the envelope. 2-4 hours after the escape, the gone cell dividing into a gone colony within the envelope as in the asexual reproduction. The two flagella usually detached from the envelope just before the cell divisions, but sometimes remaining attached to it and functional until 4- to 16-celled stage.

Type locality. Nagae, Hayama-cho, Kanagawa Prefecture, Japan. Soil samples were collected by the author in December 1980.

Holotype. Figs. 3, 4.

Although there was considerable morphological difference in the vegetative colony between the two species, these showed essentially the same colony organization and reproduction.

Astrephomene perforata differs from *A. gubernaculifera* in the shape of cellular sheaths, having pyrenoids in the vegetative cells and the number of posterior somatic cells. The cellular sheaths of *A. gubernaculifera* adhered compactly to one another and seemed to be pentagonal or hexagonal in front view because of their mutual compression (Fig. 1; Pl. XI E, F). In contrast, the sheaths of *A. perforata* seemed to be roughly circular in front view and interconnected with the neighboring sheaths (Fig. 3; Pl. XII E, F). The interconnecting sheaths showed small triangular or square fenestrations between each of them. This difference could be clearly recognized in the specimens stained with haematoxylin (Pl. XI E, F; Pl. XII E, F), irrespective of the age of the colony or culture conditions. The specific name *perforata* comes from these interconnecting sheaths. In addition, colonies of *A. perforata* showed a watery gelatinous matrix surrounding the interconnecting sheaths (Pl. XII G). It could only be recognized by ink preparation. However, as was mentioned by Starr (1980), colonies of *A. gubernaculifera* did not show such a matrix even by ink preparation (Pl. XI G).

The chloroplasts of the vegetative cells of *A. gubernaculifera* did not show pyrenoids in any age of the culture (Fig. 2; Pl. XI A-D), as has been reported by Pocock (1953) and Stein (1958). In *A. perforata*, one to three pyrenoids appeared in the brim or top of the cup-shaped chloroplast of each vegetative cell in the older cultures (Fig. 4; Pl. XII C). The chloroplasts usually showed pyrenoids three or four days after inoculation growing in the synthetic medium.

When stained (Rosowski & Hoshaw 1970), the pyrenoid could be clearly recognized two days after the inoculation, but could not in one-day-old cultured cells.

The 64- or 32-celled colony of *A. gubernaculifera* contained four or two posterior somatic cells, respectively (Fig. 1; Pl. XI C, D), while that of *A. perforata* did two ones irrespectively of the colony cell number, 64 or 32 (Fig. 3; Pl. XII D).

I wish express my deep gratitude to Prof. H. Kasaki of Toho University and Dr. S. Kato of Kanagawa Prefecture, for their kind guidance and encouragement. My thanks are also due to Prof. T. Yamagishi of Nihon University for his useful discussion and reading and correction of the manuscript, and to Mr. T. Yamauchi of Keio Senior High School for his reading the latin description.

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Explanation of plates XI–XIII

Pl. XI. *Astrephomene gubernaculifera* Pocock. E,F, I, J: Stained with haematoxylin. G, Q, R: Ink preparation. I–L, N–P: Phase contrast. A: Surface view of mature 64-celled colony. B: Optical section of the colony in Fig. A. C: 64-celled colony showing four somatic cells. D: 32-celled colony showing two somatic cells. E: Mature colony. Arrow indicates cellular sheath. F: Young colony. Arrow indicates cellular sheath. G: Colony. H: Asexual reproduction. Arrow indicates the 8-celled stage. I: Four somatic cells and daughter colonies in parental cellular sheaths. J: Daughter colony in parental cellular sheath (arrow heads) showing biflagellate posterior cells (arrow) and uniflagellate other cells. K–R: Sexual reproduction. K: Gamete bearing a mating papilla (arrow) near the base of the flagella (arrow heads). L: Planozygote. M: Mature aplanozygotes. N–O: Zygote germination. P: Gone cell. Q: 8-celled stage in gone colony formation. R: Gone colony in gelatinous envelope. Scale in Fig. A is 50 μm for Figs. A–I. Scales in Figs. J, K are 20 μm. Scale in Fig. L is 20 μm for Figs. L–R.

Pl. XII. *Astrephomene perforata* Nozaki. E, F, J: Stained with haematoxylin. G: Ink preparation. J: Phase contrast. A: Surface view of mature 64-celled colony in two-day-old culture. B: Optical section of the colony in Fig. A. C: Colony showing pyrenoids (arrow) in five-day-old culture. D: 64-celled colony showing two somatic cells. E: Mature colony. Arrow indicates fenestration. F: Young colony. Arrow indicates fenestration. G: Colony. H: Asexual reproduction. Arrow indicates the 8-celled stage. I: Two somatic cells and daughter colonies in 64-celled parental colony. J: Newly formed colony showing biflagellate posterior cells (arrows) and uniflagellate other cells. Scale in Fig. A is 50 μm for Figs. A–I. Scale in Fig. J is 20 μm.

Pl. XIII. Sexual reproduction in *Astrephomene perforata* Nozaki. B–D, G–J: Phase contrast. K, L: Ink preparation. A: Colony clumping. B: Gamete release. C: Gamete clumping. Note the gametes aggregating with their flagellar tips sticking together in the center. D: Gamete bearing mating papilla (arrow) near the base of flagella (arrow heads). E: One-day-old aplanozygotes. F: Mature aplanozygotes. G–I: Zygote germination. Arrow

indicates hyaline body. J: Gone cell. K: 8-celled stage in gone colony formation. L: Gone colony in gelatinous envelope. Scale in Fig. A is 600 μm. Each scale in Figs. B-L is 20 μm.

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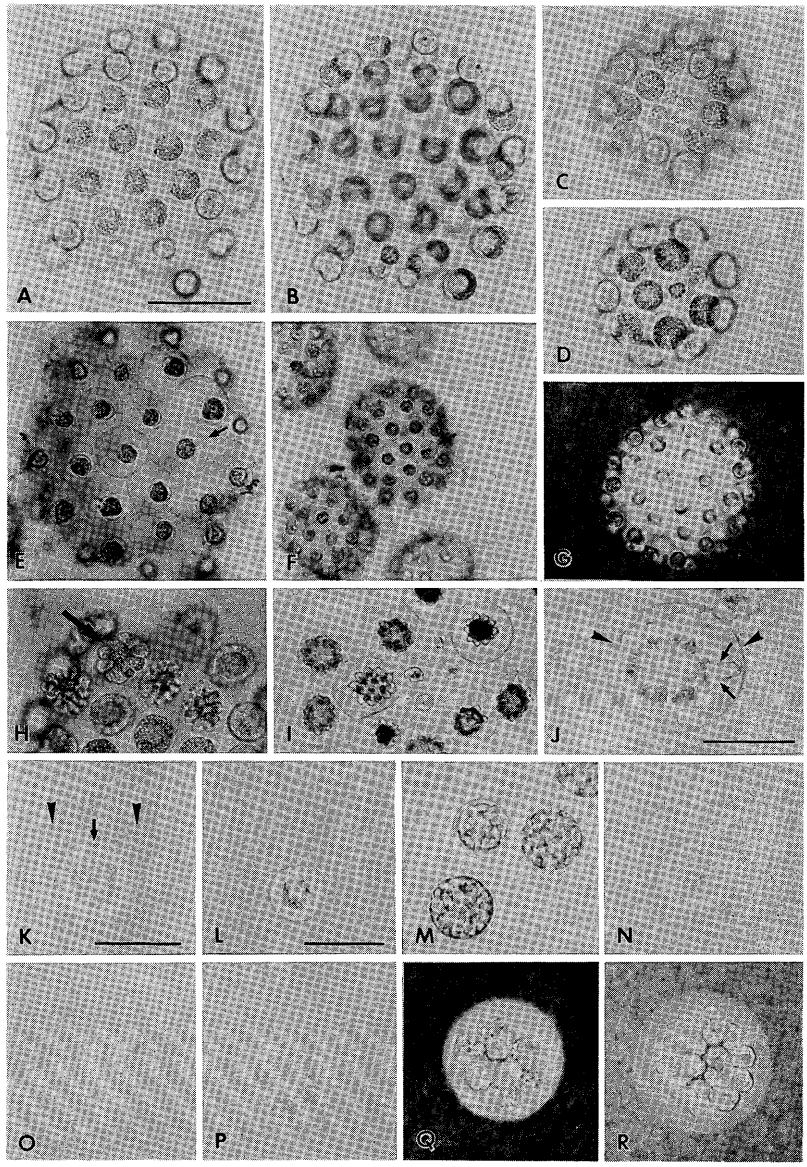
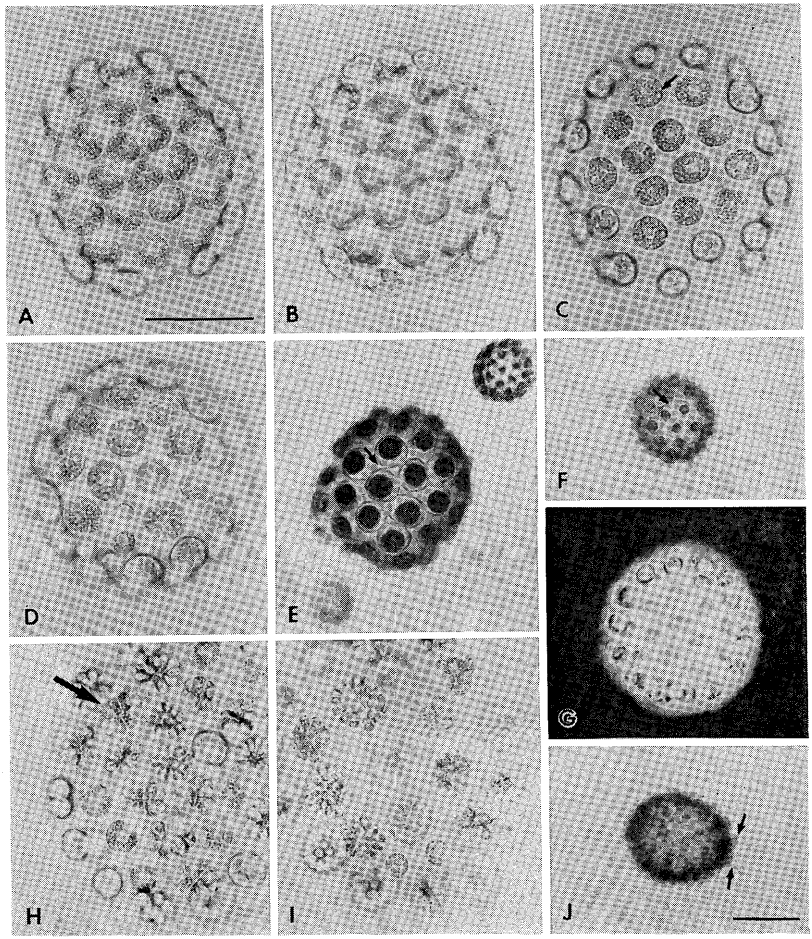
Astrephomene（緑藻，オオヒゲマワリ目）は Volvox 科に類似した群体をもっているが，群体形成時に逆転をしないことから Astrephomene 科に分類されている出現頻度の低い淡水藻である。現在までに一種 *A. gubernaculifera* Pocock が報告されているだけであり，日本産の本属に関する詳細な報告はいまだない。

筆者は神奈川県開成町吉田島と葉山町長柄にある水田の表土から形態の異なる 2 種類の *Astrephomene* を単離・培養し，それらの形態と生殖を詳細に観察した。その結果，開成町から得た藻は *A. gubernaculifera* と同定し，葉山町産のものは新種 *A. perforata* Nozaki として記載した。*A. perforata* は *A. gubernaculifera* と以下の 3 点で異なる。1）細胞の被鞘が連結されて群体を構成し，被鞘間にすき間がある。2）栄養細胞において培養齢とともにピレノイドが出現する。3）64細胞性の群体でも32細胞のものでも非生殖細胞を 2 個もつ。

□Grolle, R.: **Nomina Generica Hepaticarum; references, types and synonymies** 62 pp. 1983. Acta Bot. Fenn. 121. Helsinki.　東ドイツの有名な苔類研究家である Dr. Grolle は命名規約委員もしていて，豊富な文献学的な知識をもとに，苔類の学名の有効名につき多数の見解を明らかにしている。今回のカタログは E. R. Far et al. (1979) Index Nominum Genericorum を基本におき "invalid" な属名も併せて示し，これがどんな理由で invalid であるか，どの valid name を用いるべきかを示し，属名の author name を統一ある方法で示し (Sayer et al., 1964 に従って)，分類学的に異名として取り扱われるべきもの，及びその属名が所属する科や亜科を記号で示してある。巻末に現在確認されている（化石も含めて）苔類及びツノゴケ類の全部の科，亜科及びそれに分類される属（及びその異名）が示されている。この分類系にはいろいろ目新しい所もあり，苔類，ツノゴケ類を72科に分類している。このような仕事は文献学的な研究と共に，苔類そのものについて相当深く突っこんだ見解をもっていないと出来るものではないが，著者は見事にこれを果たし，ほぼ完ぺきと言える形までこの仕事を成しとげている。苔類の分類系を見るときに一つの重要な文献となる。　（井上　浩）



H. NOZAKI: Two species of *Astrephomene*



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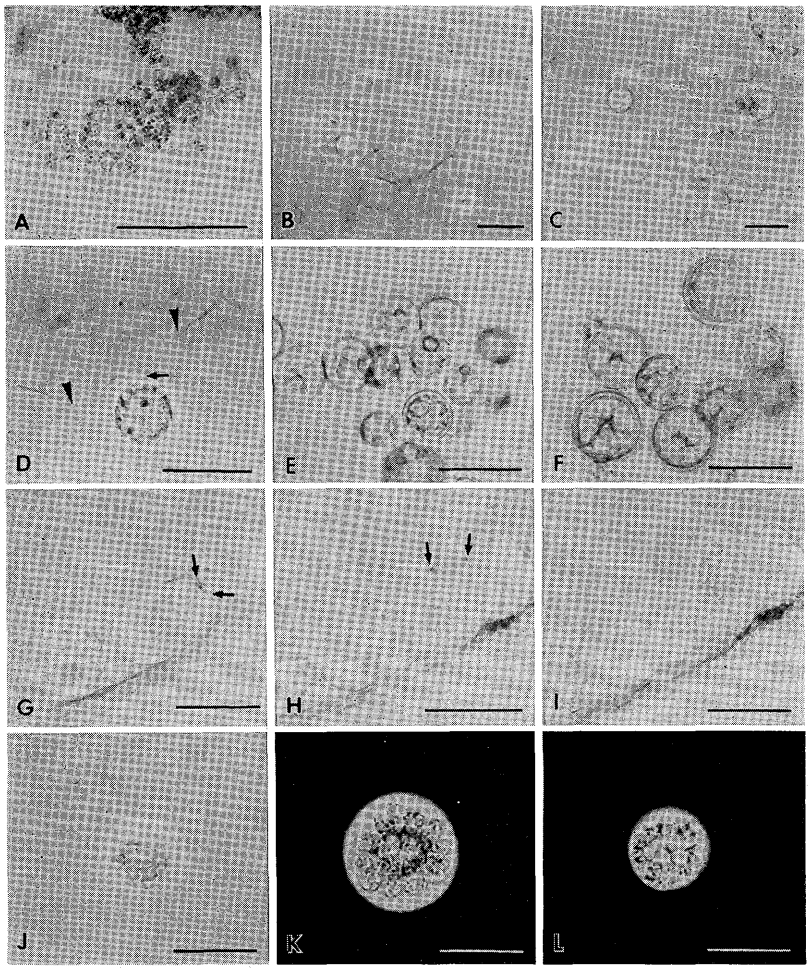

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