Sueo KATO*: Laboratory culture and taxonomy of two species of *Vacuolaria* (Class Raphidophyceae)

加藤季夫*: ラフィド藻 *Vacuolaria* 属2種の培養と分類

(Plates VIII-IX)

The genus *Vacuolaria* was established by Cienkowsky (1870) on the basis of specimens collected from a mountain stream in Switzerland, with *V. virescens* as the type species. *V. viridis*, the second species of this genus, was originally described as *Anisonema viridis* by Dangeard (1889) but Senn (1900) transferred it to *Vacuolaria*. Most authors recognized *V. viridis* (Senn 1900; Pascher 1913; Fott 1935, 1968, 1970; Pringsheim 1963). However, Spencer (1971) was of the opinion that the two species were conspecific.

I have studied the morphology of unialgal cultures of *V. virescens* Cienk. and *V. viridis* (Dang.) Senn at the light microscope level and in this report consider a possible resolution of the taxonomic controversy.

Materials and methods Algal samples were collected from ponds in Ibaraki Prefecture and Tokyo in the summers of 1977 and 1978 (Tab. 1). A single cell of *Vacuolaria* was picked up from the sample by a micropipette and transferred into a small hole bored into agar plates containing AF-6 medium. The hole was also filled with liquid AF-6 medium. About a week after the onset of incuba-

Tab. 1. Unialgal cultures of *Vacuolaria* investigated.

Species & Strain No.	Localities	Dates
V. virescens		
R-9	Shishizuka-Ooike pond, Ibaraki Pref.	Aug. 16, 1977
R-12	Shishizuka-Ooike pond, Ibaraki Pref.	Aug. 16, 1977
V. viridis		
R-335	a small pond, Tokyo Kyoiku University campus, Tokyo	July 23, 1978
R-352	a small pond, Horai Park, Tokyo	Aug. 31, 1978

* [redacted] Yokohama, Kanagawa 222. 神奈川県横浜市[redacted]

tion, four to eight cells were obtained from the original cell. The cloned progeny were transferred into a test tube containing AF-6 medium. AF-6 medium contained the hydrogen ion buffer, piperazine-N, N′-bis [2-ethanesulfonic acid] (PIPES), in addition to its defined components (Kato 1982). The pH of the medium was adjusted to 6.7 with 0.1 N NaOH.

The cultures were maintained at 20°C under fluorescent lights of 1500 lux intensity with a 12: 12 LD cycle. Gelatinous sheaths were stained with a 0.1% aqueous neutral red solution.

Results and discussion

Vacuolaria virescens Cienkowsky, Arch. Mikr. Anat. 6: 426. pl. 23, f. 19. 1870.

Motile cells were usually ovoid to pyriform (Pl. VIII A-B, F), but sometimes fusiform (Pl. VIII C), measuring 42-74 μm long and 26-42 μm wide. Small spherical cells, less than 30 μm in diameter, were also observed (Pl. VIII D). Two heterodynamic flagella arose from a notch which was subapical in position (Pl. VIII C). One flagellum projected anteriorly (swimming flagellum) and beat rapidly, while the other extended posteriorly along the cell surface (trailing flagellum) and moved slowly. The swimming flagellum was generally one cell long, while the trailing one was the same length or shorter. The bright-green chloroplasts were disc-shaped, 4-6 μm long and 3-4 μm wide (Pl. VIII E). The cell usually appeared green because the chloroplasts were several layers thick. The nucleus was spherical to oval, 12-18 μm long and 12-16 μm wide, and situated slightly anterior of the middle of the cell (Pl. VIII F). At the anterior surface of the nucleus, a supranuclear cap, consisting of a large Golgi apparatus and its products (Schnepf & Koch 1966), was observed (Pl. VIII F). The contractile vacuole was conspicuous (Pl. VIII F). The periplast was thin and flexible, and numerous mucocysts (small refractile bodies) were located just beneath the cell membrane (Pl. VIII E). Cell division usually took place in the encysted condition (Pl. VIII G) and the daughter cells divided repeatedly resulting in a palmelloid colony with thick gelatinous sheaths (Pl. VIII G, H).

My results agreed with the description of *Vacuolaria virescens* given by Cienkowsky (1870). However, there are two differences. According to Cienkowsky (1870), the motile cells of *V. virescens* were 138 μm long and possessed two flagella both projected anteriorly, whereas those of my cultures were 42-74

μm long and possessed two flagella, one projected anteriorly and the other extended posteriorly. However, I consider the present alga to be *V. virescens*. My results also agreed very well with those for *V. virescens* studied by Tschermak-Woess (1954), Skuja (1964), Heywood (1968) and Ioriya (1970).

Vacuolaria viridis (Dang.) Senn, in Engler & Prantl (ed.), Die Natürlichen Pflanzenfamilien 1 (1a) : 170. 1900.

Motile cells were obovoid (Pl. IX A-C) and flattened (Pl. IX D), measuring 32-56 μm long and 24-36 μm wide. In old cultures spherical cells were sometimes observed (Pl. IX E). Two heterodynamic flagella arose from a conspicious notch which was subapical in position (Pl. IX C). The swimming flagellum beat rapidly, while the trailing one oscillated slowly. Both flagella were generally longer than the cell. The bright-green chloroplasts were disc-shaped, 3-5 μm long and 3-4 μm wide (Pl. IX F) and formed a single layer at the periphery. The nucleus was spherical, 12-15 μm in diameter, and situated in the middle of the cell (Pl. IX G). The supranuclear cap, the contractile vacuole (Pl. IX G, H) and the mucocysts were present. Cell division took place while the cell was motile (Pl. IX I). Palmelloid colonies have not been observed either in the field or in culture.

My results agreed well with the descriptions of *V. viridis* given by Dangeard (1889), Pascher (1913) and Fott (1935).

According to Pascher (1913) and Fott (1935, 1968, 1970), cell shape and flagella length distinguish *V. viridis* from *V. virescens*. On the other hand, Spencer (1971) examined *V. virescens* from North Carolina, U.S.A., and found all of the variations characteristic of both species. She therefore believed that differences both in cell shape and in flagella length were not valid taxonomic criteria and treated *V. viridis* as a synonym of *V. virescens*. However, the specimen she considered to exhibit the form attributed to *V. viridis* (Spencer 1971, p. 276, f. 3) is too elongated and not flattened. Therefore, I believe that her specimen shouldn't be regarded as *V. viridis*. My observations on clone cultures suggest that the two species can be distinguished by cell shape. The cells of *V. viridis* were obovoid and flatted, while those of *V. virescens* were ovoid to pyriform and not flattened. I also observed that the flagella of *V. viridis* were longer than those of *V. virescens*. But, flagella length varied considerably within the same strain. Therefore, it shouldn't be used as a feature distingushing the two species, as avered by Spencer (1971).

My observations also show that *V. viridis* clearly differs from *V. virescens* in chloroplast arrangement. In healthy cultures the cells of *V. viridis* appeared bright-green, whereas those of *V. virescens* appeared green. This difference was due to a difference in chloroplast arrangement. The chloroplasts of *V. viridis* occurred as a single layer, while in *V. virescens* they were several layers thick, as already reported by others (Fott, 1935; Heywood 1977). Accordingly, *V. viridis* should be recognized as a distinct species.

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Explanation of plates VIII-IX

Pl. VIII. *Vacuolaria virescens* Cienk. Strain R-9. A-B: Lateral view of cells. ×500. C: A fusiform cell showing a notch (no). ×500. D: A small spherical cell. ×500. E: Part of a cell showing chloroplasts (ch) and mucocysts (m). ×1250. F: Nomarski micrograph of a pyriform cell showing a nucleus (n), a supranuclear cap (s.c) and a contractile vacuole (c.v). ×500. G: Phase contrast micrograph of daughter cells (d) just after cell division. ×500. H: A palmelloid colony stained with a 0.1% aqueous neutral red solution. ×125.

Pl. IX. *Vacuolaria viridis* (Dang.) Senn. Strain R-352. A-B: Lateral view of cells. ×500. C: A cell showing a conspicuous notch (n). ×500. D: Anterior view of a cell. ×500. E: Part of a cell showing chloroplasts (ch). ×1250. G: Nomarski micrograph of a cell showing a nucleus (n) and a contractile vacuole (c.v). ×500. H: Nomarski micrograph of a cell showing a supranuclear cap (s.c). ×500. I: Nomarski micrograph showing cell division. ×500.

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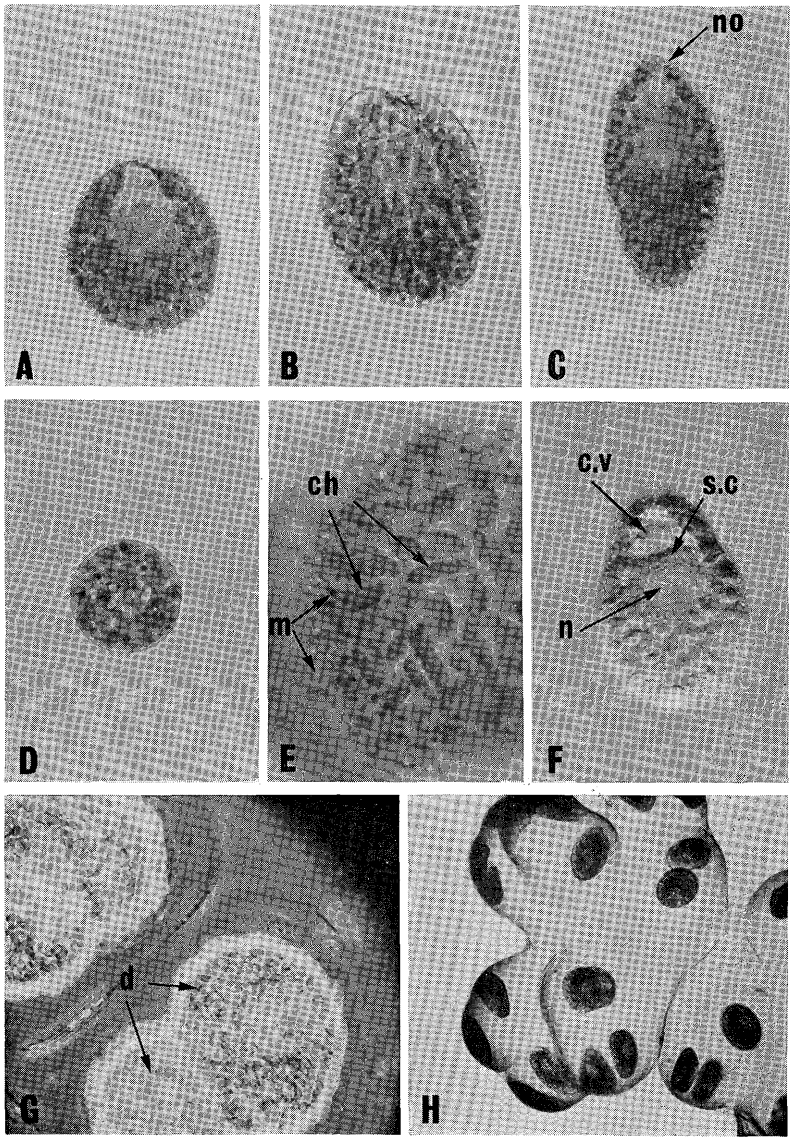
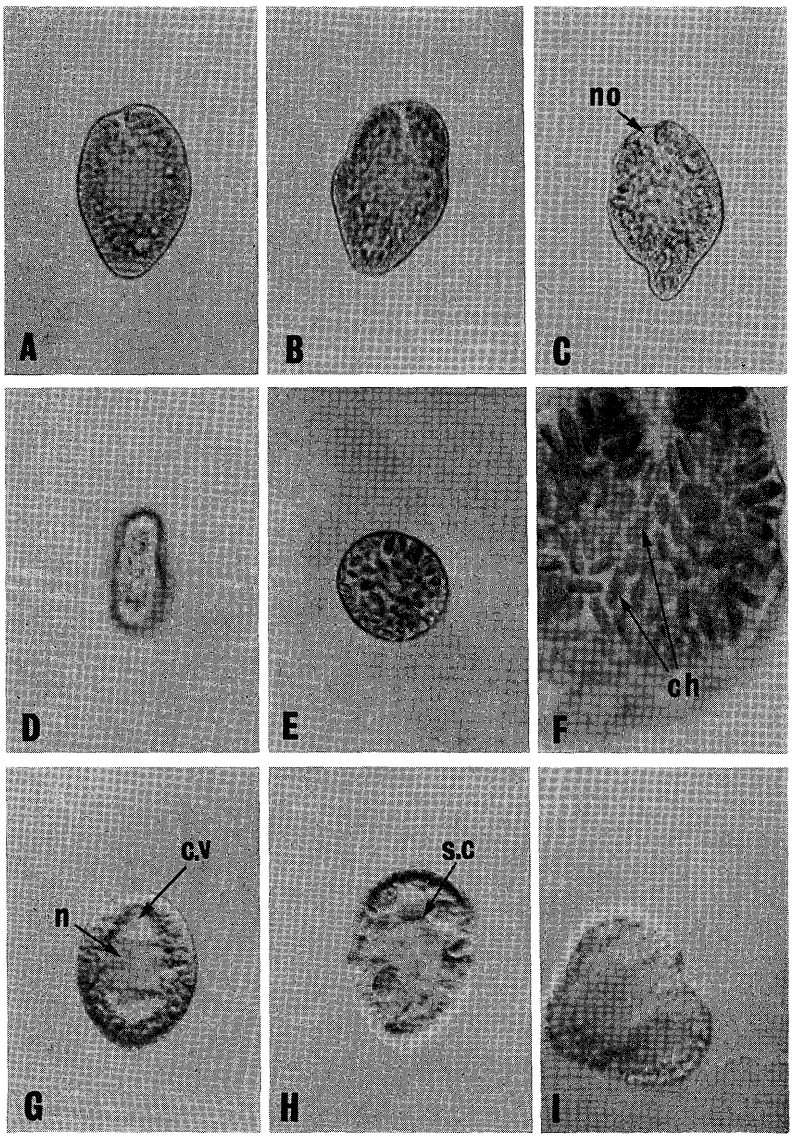
ラフィド藻の単離・培養方法について工夫，改良を行ない，幾つかの培養株を得た。これら培養株のうち，同種か別種かで論議のある *Vacuolaria virescens* Cienk. と *V. viridis* (Dang.) Senn のそれぞれ2株について形態を調べたところ，両者は細胞の形および葉緑体の配列で明かに異なることが判明した。このことから，両者は別種として扱われるべきであると結論した。



S. KATO: Two species of *Vacuolaria*



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