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Observations on *Trentepohlia lagenifera* (HILD.) WILLE (Chlorophyceae, Trentepohliaceae)[1)]

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Trentepohlia lagenifera (HILD.) WILLE, an alga of the Chlorophyceae, was investigated in wild and cultured specimens. Specimens used were collected and isolated from tree trunks in Miyajima Island and adjacent areas, Hiroshima Prefecture. In wild specimens, morphological observation was made in detail and its results were discussed. In cultured specimens, two morphologically different forms were observed. The form A formed small spot colonies on agar plate and its cell shape and size were similar to those of the wild specimen. On the other hand, the form B formed coarse, broadly expanding, fluffy colonies on agar plate. Its cells were much longer than those of the wild specimen. These two forms were described as cultured forms.

Key Index Words: *aerial algae*; *Chlorophyceae*; *morphological forms*; Trentepohlia lagenifera.

Trentepohlia lagenifera (HILD.) WILLE, known as an alga of the aerial Chlorophyceae, is widely distributed in the world, occurring mainly in tropical and subtropical regions (HILDEBRAND 1861, HARIOT 1889, DE WILDEMAN 1891, PRINTZ 1939, CRIBB 1968, etc.). It is in Japan, commonly found in south and southwestern parts (HIROSE *et al.* 1977). This alga is growing on barks and leaves of trees as well as on surface of stones and ground. The algal colony is usually yellow to reddish-orange or olive-green to yellow-green.

During our study of epiphytic algae on tree trunks in Miyajima Island and its adjacent areas, Hiroshima Prefecture, we frequently found reddish-orange to orange-colored colonies of the alga. They agreed well with the descriptions of *T. lagenifera* by HILDEBRAND (1861) and PRINTZ (1939). Moreover, there were two morphological forms observed in the culture of this alga.

The purpose of this paper is to describe in detail the morphology of both the wild and cultured specimens, and to compare the two forms observed in culture.

Materials and Methods

Colonies of *T. lagenifera* were collected with barks from tree trunks by striping it with knife in Miyajima Island and adjacent areas, Hiroshima Prefecture. Specimens used in this study are listed in Table 1 with their sample numbers, collection dates, localities and names of host trees.

In the laboratory, a portion of the colony carefuly scraped off from bark surface was used for observation with light microscope. Another portion of the colony scraped was

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Table 1. Summary of data on the specimens studied.

Specimen No. (HIRO)	Collection Date	Locality	Host tree
sh-164	Jan. '81	Miyajima-cho, Miyajima Isl.	*Eurya japonica*
sh-165	Jan. '81	Miyajima-cho, Miyajima Isl.	*Ilex pedunculosa*
sh-166	Jan. '81	Miyajima-cho, Miyajima Isl.	*Symplocos theophrastaefolia*
sh-184	Apr. '81	Ogauchi-cho, Hiroshima City	*Cryptomeria japonica*
sh-212	June '81	Miyajima-cho, Miyajima Isl.	*Juniperus rigida*
sh-219	June '81	Miyajima-cho, Miyajima Isl.	*Eurya japonica*
sh-221	June '81	Miyajima-cho, Miyajima Isl.	*Myrica rubra*
tn-223	Oct. '81	Okimi-cho, Nomishima Isl.	*Symplocos lucida*

sonicated for about one minute to obtain a more uniform suspension of small pieces of algal filaments. The suspension was aspirated onto a sterile agar plate which had been prepared by adding 1.5% agar to Bold's Basal Medium (1N BBM) as modified by BISCHOFF and BOLD (1963). Aspiration was acomplished in the manner described by WIEDEMAN *et al.* (1964). Petri dishes were placed under standard conditions (about 3000 lux light intensity on a diurnal light cycle of 12 hr light and 12 hr darkness at 22±1°C). After about one month, distinguishable colonies selected under stereoscopic binocular microscope were removed from the agar plate and inoculated on 1N BBM agar slants as unialgal cultures.

Some of these unialgal cultures were purified to the axenic state by the following method before being studied in detail. Short portions near the apex of algal filaments projecting into air on agar slant were picked off and transferred onto proteose agar medium (STARR 1964). After two to three weeks, algal filament which were still axenic were transferred to agar slant of 3N BBM (BROWN and BOLD 1964) and maintained as stock cultures.

Unialgal and axenic cultures are deposited in the Botanical Institute, Hiroshima University (CCHU).

Results

1. Observation on wild specimens

1) Colony

The colonies of *Trentepohlia lagenifera* collected formed fine cushion-like expansions of filaments closely placed on tree trunks. They were orange to orange-yellow by the presence of orange-red haematochrome pigments in their cells. On the shaded side of tree trunks, however, colonies were usually green to yellow-green, which was due to a little amount of haematochrome pigments.

2) Morphology of filaments and cells

Filaments were irregularly branched, mostly short and torulose, and erect and

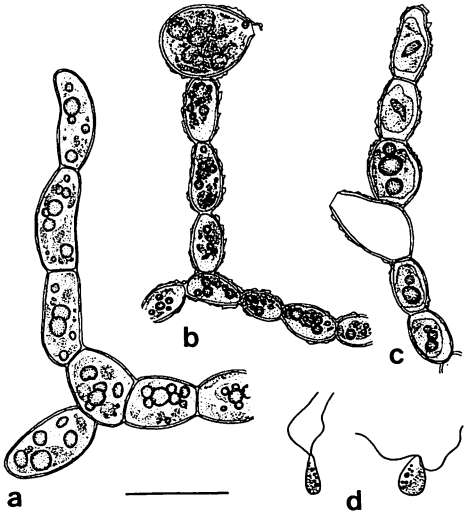

Fig. 1. *Trentepohlia lagenifera.* Wild specimens. a. Filament without granular secretions b. Sessile, terminal sporangium on filament with granular secretions; c. Sessile, intercalary empty sporangium; d. Biflagellate swarmers. Scale bar: 20 μm.

prostrate filaments were not sharply distinct from one another. Cells were ellipsoidal to barrel-shaped, and most of those at the surface portion of a colony had granular secretions on the outer cell wall (Fig. 1b, 2a). However, cells at the under portion of the colony or those of the filaments growing on the shaded side of tree trunks did not have such secretions usually (Fig. la).

Cell size was measured on filaments selected randomly from some small portions of a colony. The size of cells was variable in each

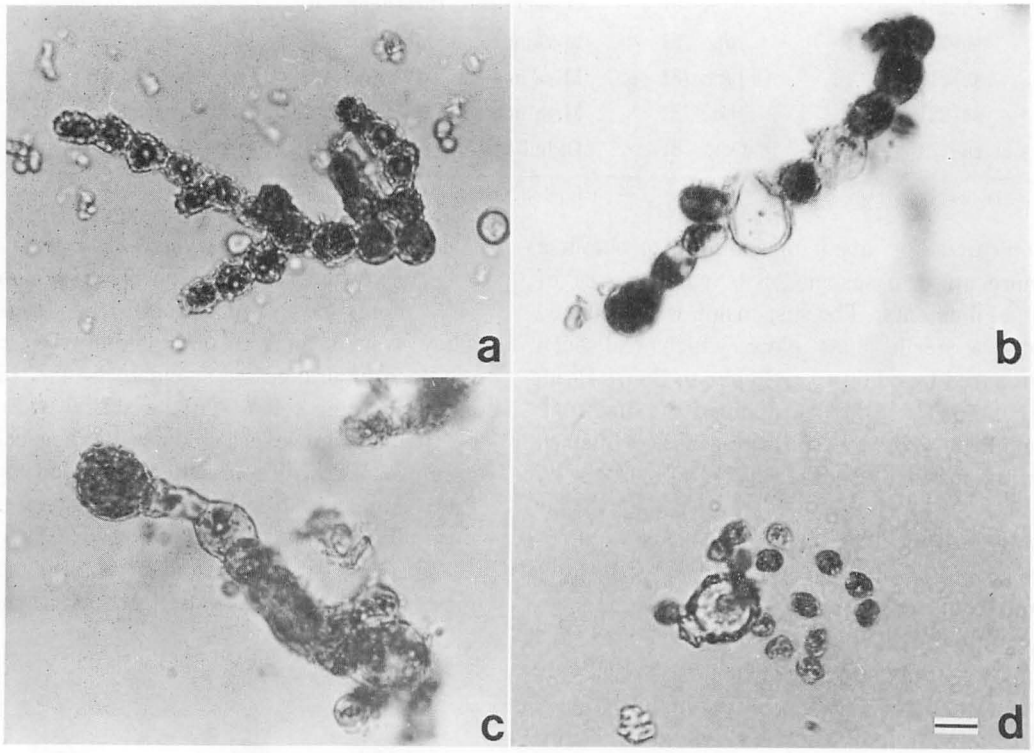

Fig. 2. *Trentepohlia lagenifera*. Wild specimens. a. Filament; b. Sessile, intercalary empty sporangium; c. Stalked sporangium; d. Liberation of four-flagellate swarmers from sporangium detached from stalk cell. Scale bar (10 μm) in d applies also to a-c.

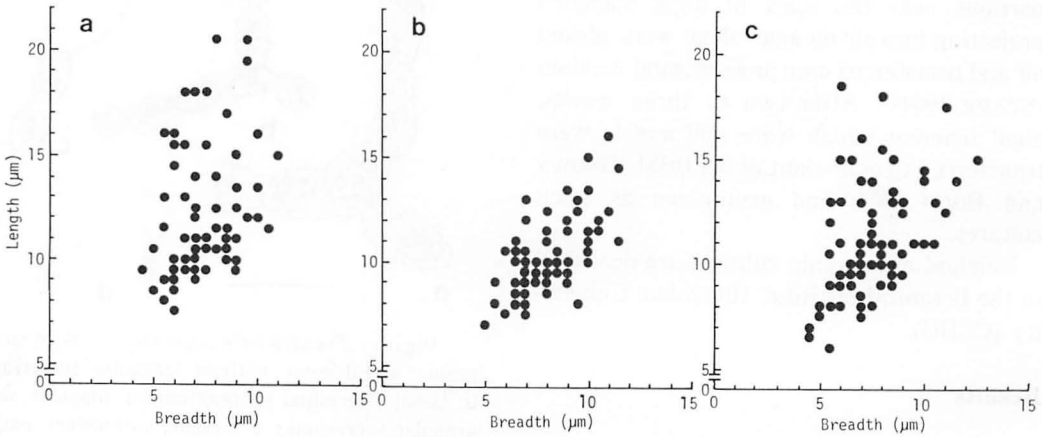

Fig. 3. Scatter diagram showing the cell size of *T. lagenifera* from wild specimens. a. Specimen no. sh-164; b. sh-219; c. tn-223.

sample, as can be seen in the scatter diagrams presented in Fig. 3, but it usually showed a small variation within a given filament. Cell size was 6.0–20.5 μm (average 12.3 μm) in length and 4.5–12.5 μm (average 7.7 μm) in breadth.

Chloroplasts were usually difficult to observe due to the presence of many haematochrome pigments. However, in cells of the filament at the under portion of a colony and on the shaded side of tree trunks, chloroplasts were visible because haematochrome pigments were scarce or absent. In these cells, parietal chloroplasts were ribbon-like or broken up into small discs (Fig. 1a). Pyrenoids and starches could not be observed in our specimens.

3) Reproductive cells

Both sessile and stalked sporangia were found in our specimens. Sessile sporangia occupied very diverse positions and were formed singly in terminal or intercalary on filaments (Fig. 1b, c, 2b). Sessile sporangia were usually flask-shaped with a short ostiole, 10.0–15.0 μm in diameter and contain 4, 8 or usually 16 swarmers. Swarmers escaped one by one from the ostiole of sporangium within about one minute after adding the water. They were flattened ovoid or pear-shaped, with two anterior flagella of equal length which were longer than the body length (Fig. 1d) and had several minute haematochrome grains and a single cup-shaped chloroplast, but no eye-spot. When liberated swarmers swam very actively and rapidly, and after few minutes they stopped movement, becoming round and shedding the flagella. They sometimes behaved as isogametes, although they were not successful in fusing with each other.

Stalked sporangia were also observed in some wild specimens (Fig. 2c, d). Those sporangia appeared only as terminal appendages, which were usually subspheroidal and were easily detached from a stalk-cell. The stalk-cell consists of a broad and subspheroidal proximal portion and a narrow and cylindrical distal portion. The stalked sporangia produced a considerable number of swarmers with four flagella of equal length, which were similar in both shape and size to those of the swarmer with two flagella. The liberation of swarmers was frequently observed in wild specimens collected in summer season.

2. Observations on cultured specimens

1) Colony

When the suspension of algal filaments was spread onto 3N BBM agar plates and inoculated under standard conditions, many colonies became visible to the naked eye after about one month. Two forms of colony, which were called form A and form B, respectively, or either of the two forms were observed on agar plates. The appearance of the two forms in cultures is summarized in Table 2. The form A formed orange, small spot colonies which developed more slowly on 3N BBM agar plate (Fig. 5a). On the other hand, the form B formed orange, coarse, broadly expanding fluffy colonies which developed more luxuriantly on agar plate containing 3N BBM (Fig. 5b).

2) Morphology of fiaments and cells

The two forms of this alga differ from each other in the morphology of both filaments and cells in cultures. A part of the results of cell size measurement in some isolates is shown in Fig. 4.

Filaments of the form A spread onto agar plate were richly and irregularly branched and formed many short branches, showing no differentiation of prostrate and erect systems (Fig. 5c, 6a). The branches were usually raised alternately to one another and usually originated from near the upper end of the parent cells. Young branches at first appeared as thin-walled protrusions, which swelled and gradually increased in size.

Individual cells were cylindrical to ellipsoidal, and showed some variation in both shape and size, ranging from 7.0 to 18.5 μm (average 10.8 μm) in length and from 4.5 to 12.0 μm (average 7.2 μm) in breadth. The size and shape of cells were similar to those of the wild specimens (Fig. 4), but the wall was smooth and did not show such grannular

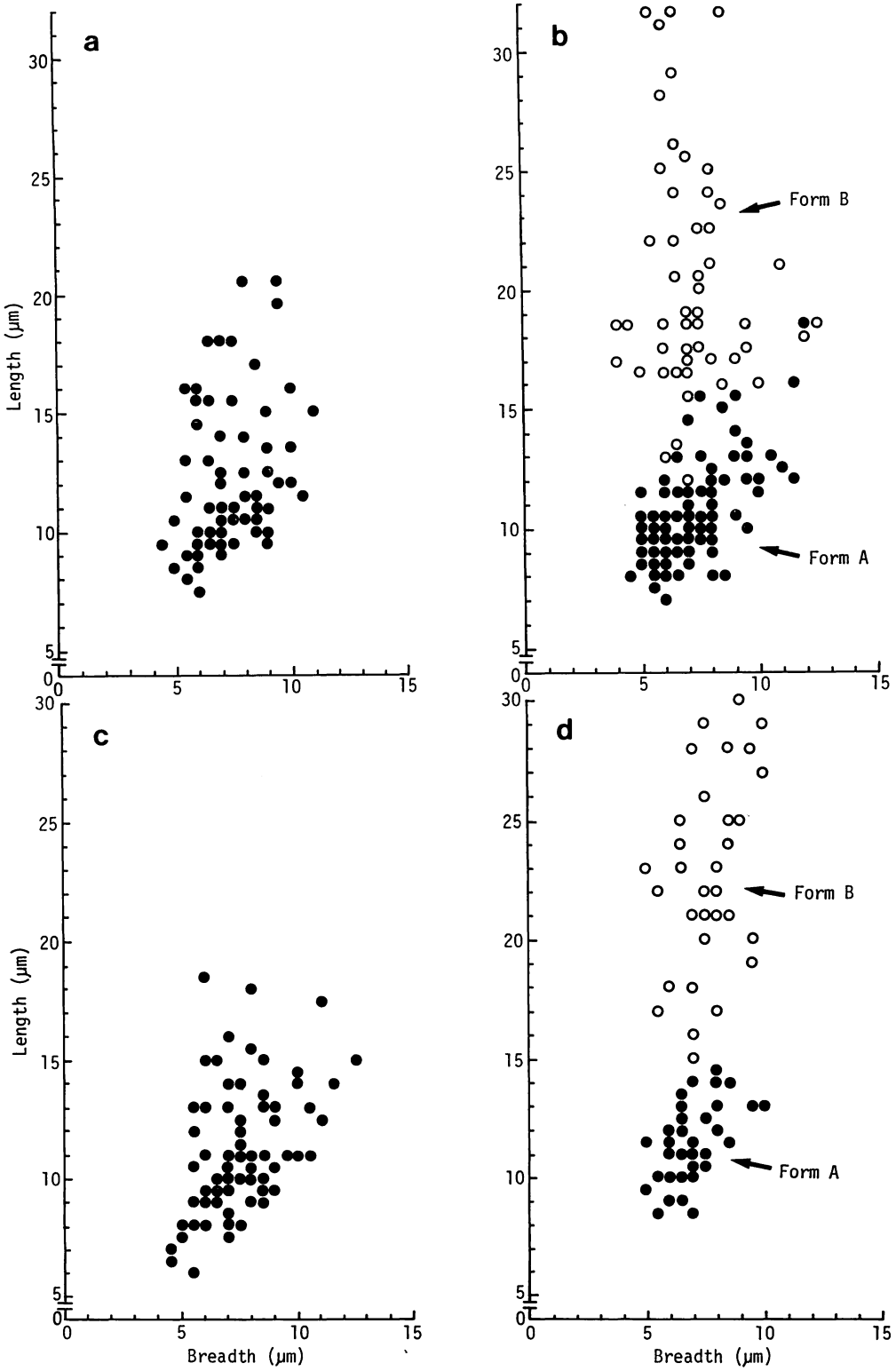

Fig. 4. Scatter diagram showing the cell size of *Trentepohlia lagenifera* from wild and cultured specimens. a. Specimen no. sh-164; b. Culture no. CCHU 2102 (Form A) and 2111 (From B); c. Specimen no. tn-223; d. Culture no. CCHU 2163 (Form A) and 2152 (Form B).

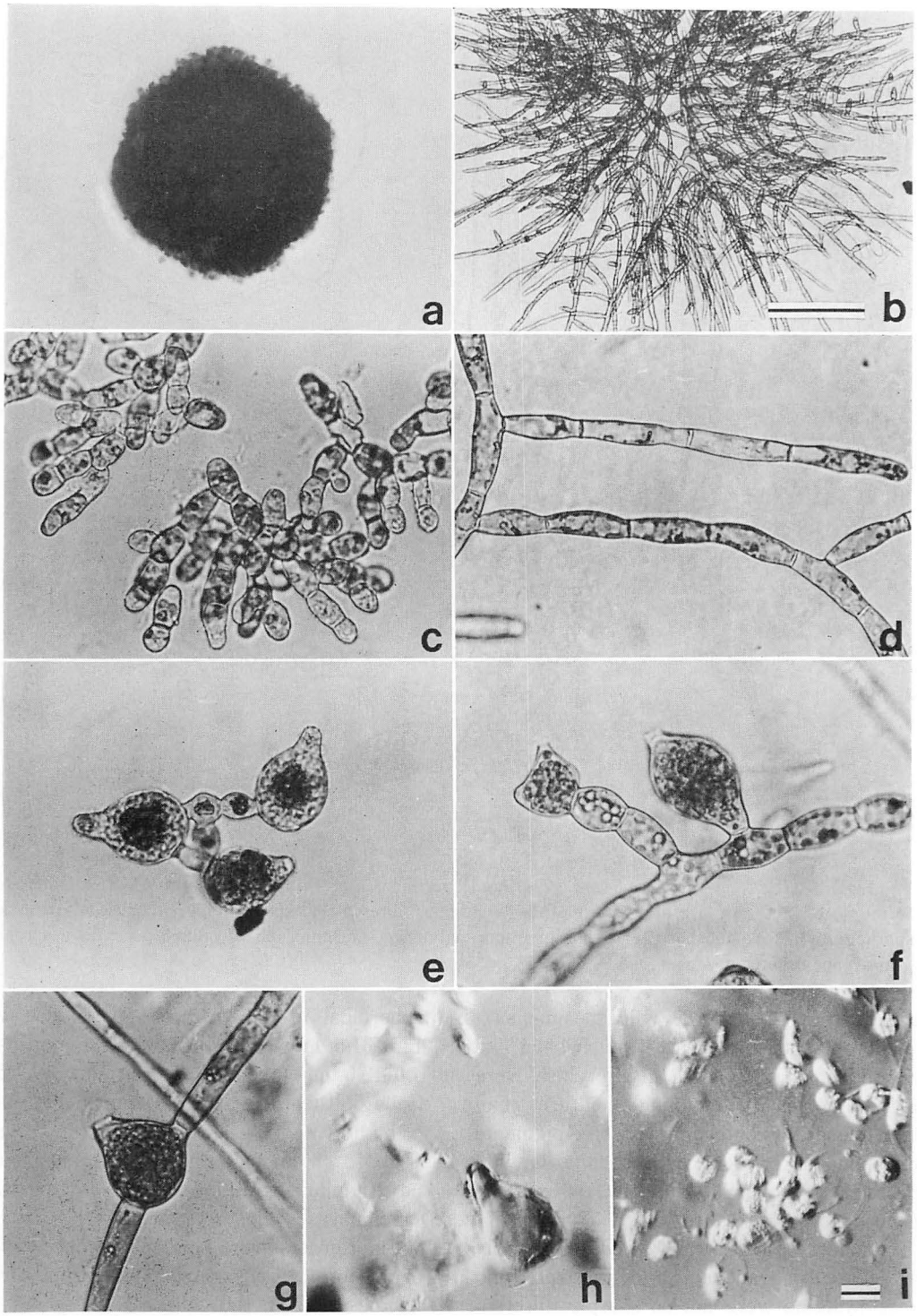

Fig. 5. *Trentepohlia lagenifera.* Cultured specimens. a, b. Colonies on agar plate (a. Form A; b. Form B); c, d. Filaments (c. Form A; d. Form B); e, f. Sessile sporangia (e. Form A; f. Form B); g. Mucilage secretion from ostiole of sporangium; h. Liberation of swarmers from sporangium; i. Biflagellate swarmers. Scale bar (100 μm) in b applies also to a; scale bar (10 μm) in i applies also to c-h.

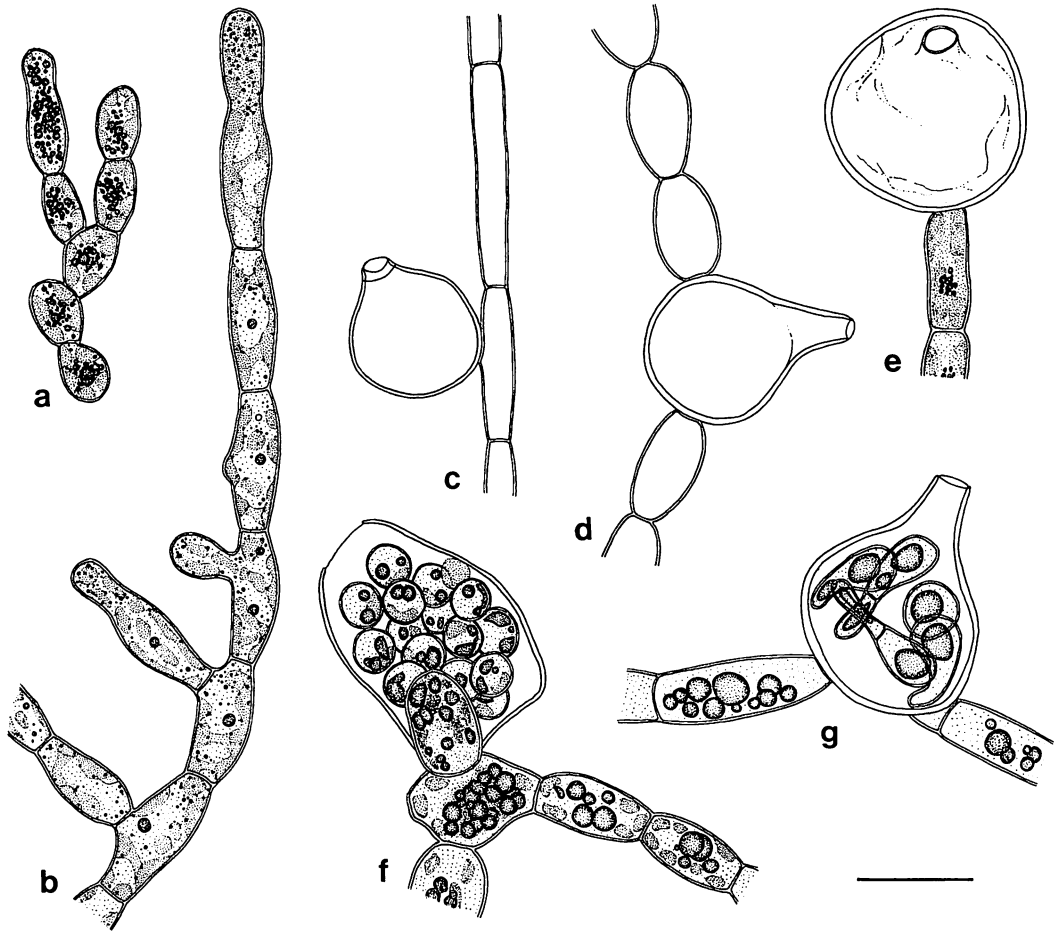

Fig. 6. *Trentepohlia lagenifera*. Cultured specimens. a. Filament of form A; b. Filament of form B; c. Sessile, lateral sporangium; d. Sessile, intercalary sporangium; e. Sessile, terminal sporangium; f. Aplanospore formation in a sporangium; g. Germination of aplanospores in a sporangium. Scale bar: 20 μm.

secretions as seen in the wild specimens.

Chloroplasts were parietal and ribbon like or broken up into small discs, which were visible more easily in younger cultures than older ones in which haematochrome pigments were abundantly produced in cells as in wild specimens. Pyrenoids and starches were not observed in these cells.

Filaments of the form B were irregularly and coarsely branched, and prostrate and erect systems were not so sharply differentiated (Fig. 5d, 6b). The process of branching was similar to that of the form A. Individual cells greatly varied in shape, being cylindrical to ellipsoidal, sometimes subspheroidal in old cultures. Cell size was 12.0-31.5 μm (average 19.8 μm) in length and 4.0-12.5 μm (average 7.5 μm) in breadth. Cells of the form B were longer than those of both the wild specimens and the form A (Fig. 4). The wall was smooth and showed no granular secretions as seen in the wild specimens.

Chloroplasts were similar in shape to those of the form A. Pyrenoids and starches were not observed in the cells.

3) Reproductive cells

Both the form A and the form B formed sessile sporangia of the same shape in culture (Fig. 5e, f, 6c, d, e). These sporangia arose

quite irregularly, being lateral, terminal or intercalary in position, and occurred usually singly and rarely in pairs. They varied in shape, being globose, subglobose and usually flask-shaped. Their size was 15.0-23.0 μm in diameter and larger than that of the wild specimen. The ostiole was colorless, longer than in the wild specimen and deliquesed at the tip to allow the swarmers to escape. The content of a sporangium was divided successively into 4, 8 and usually 16 cells in the ultimate. After the secretion of a little mucilage from the tip of the ostiole (Fig. 5g), swarmers escaped one by one from the ostiole of sporangium within about one minute after adding water (Fig. 5h-i). They contained several minute haematochrome grains with a single cup-shaped chloroplast and were similar to those of wild specimens in both shape and size.

Some swarmers unreleased from the sporangium were rarely observed. After a time they lost flagella and became globose, and developed into spheroidal aplanospores with a very thin cell wall in the sporangium (Fig. 6f). They enlarged and produced a knob-like protuberance which was later separated by a septum (Fig. 6g). The further development of these cells could not be observed in this study.

Neither stalked sporangia nor swarmers with four flagella were observed in the cultured specimens.

Discussion

As described above, the granular secretions on the cell wall were observed in wild specimens which were growing at the surface portion of a colony, but they were not observed in wild specimens from the under portion of a colony and growing at the shaded side of tree trunks and also in cultured specimens. From these results, we consider that those secretions are facultative productions under a certain growth condition.

HILDEBRAND (1861) showed a flask-shaped sporangium with a long ostiole in his description of *T. lagenifera*. PRINTZ (1939) also described a similar sporangium for this species in his monograph of the Trentepohliaceae. In our observations on wild specimens of this species, sessile, terminal and intercalary sporangia were flask-shaped and had a short ostiole (Fig. 1b, c, 2d). However, sporangia with a long ostiole were also observed though in a few cases in cultured specimens (Fig. 6d, g). CRIBB (1968) also reported the sporangium of the same shape with a long ostiole in this species which was isolated on nutrient agar from a tree trunk. The length of the sporangium ostiole in *T. lagenifera* seems to be variable with the difference of growth conditions.

In this study, two forms of sporangium, sessile and stalked (the latter only in the wild specimens), were observed. OLTMANNS (1922) has considered the sessile sporangium of the Trentepohliaceae as gametangium and SUÉMATU (1957) has reported that the intercalary sporangium was gametangium in *T. umbrina*. As to the nature of swarmers from such a sporangium, MAYER (1909) has considered them as gametes which lost sexual function and MAYER (1909) and FRITSCH (1935) have concluded that gametes were capable of germinating directly without fusion or giving rise to aplanospores. On the other hand, SUÉMATU (1951 1957) has observed a few instances of gamete fusion in *Cephaleuros virescens* and *Phycopeltis epiphyton*. SUÉMATU (1957) also reported the presence of many aplanospores in the gametangium of *Cephaleuros*. Moreover, he has reported the formation of hypnospores and filaments from the germinating aplanospores in *Trentepohlia aurea*. In cultured specimens of *Physolinum monile*, KHAN (1951) has reported that biflagellate swarmers became aplanospores and began to germinate inside the sporangium. We also observed biflagellate swarmers released from sessile sporangia. These swarmers showed the behavior as isogametes, although their fusion was not observed in this study. Some of them began to germinate in the sporangium and deveoloped a knob-like protuberance which was later separated by a septum, as reported by

KHAN (1951) for *Physolinum monile*. Unfortunately the further development was not observed in this study. Taking published data into consideration in addition to the present results, we are inclined to regard these swarmers as gametes.

Stalked sporangia of the Trentepohliaceae have been considered as zoosporangium by OLTMANNS (1922). MEYER (1909) also recognized the stalked sporangium as zoosporangium in which four-flagellate zoospores were produced. We agree with him in regarding these four-flagellate swarmers as zoospores. In this study, stalked sporangia were observed only in the wild specimens. This sporangium was easy to detach from a stalk-cell and produced four-flagellate zoospores (Fig. 2d). We suppose that easy detaching of a zoosporangium is useful for their wind-dispersal. Germination of zoospores was not observed in this study.

In the present study, two morphological forms were found in cultured specimens. The main differences between the form A and the form B were shown in colony form and cell size. The form A formed small spot colonies on agar plate and the cell size was similar to that of the wild specimens. On the other hand, the form B formed coarse, broadly expanding and fluffy colonies on agar plate. Cells of the form B increased in length and developed about two times or more longer than those of the wild specimens. The elongation of cell length in cultured specimens of *Trentepohlia* has been observed by SUÉMATU (1962) in *T. umbrina*. There were no conspicuous differences between the above-mentioned two forms in the shape and size of the sporangium. As shown in Table 2, there was found mixed state of the two forms or only either of the two forms on an agar plate. In wild specimens, however, we were not able to find any difference in the colony form and cell size. Characteristics of the two forms are considered in every respects to fall within the category of *T. lagenifera* that was circumscribed by HILDEBRAND (1861) and PRINTZ (1939) on the basis of wild specimens, but they still appear to be different taxa as far as they are recognized in our cultures. We consider that the two forms may be separated at variety level. In this study, however, we should hold off giving them any definite taxonomical position, because such differentiation into two forms was not detected in our wild specimens examined. Additional studies are needed to answer the taxonomical questions for these forms.

Table 2. Appearance of two forms in cultured specimens.

Specimen No. (HIRO)	Form A	Form B
sh-164	2102*	2111
sh-165	2100	—
sh-166	—	2064
sh-184	—	2046
sh-212	—	2098
sh-219	—	2133
sh-221	2147	—
tn-223	2163	2152

* Culture No. (CCHU).

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中野武登*・半田信司 *Trentepohlia lagenifera* (HILD.) WILLE (緑藻類，スミレモ科) の観察**

緑藻類，スミレモ科の *Trentepohlia lagenifera* について，野外および培養標本を基に観察を行った。野外標本のコロニーから藻体を分離，培養した結果，コロニーの形態と細胞の形態に2型のあることが明らかになった。Form A は，糸状体が密に分枝して，小塊状のコロニーを形成し，細胞が短い。Form B は，糸状体が長く伸び，分枝が少なく，粗なコロニーを形成し，細胞は前者より著しく長い。生殖器は，両型とも同じ形態を示した。これらの2型は，培養標本を基にする限りでは，変種として記載されるものと考えられるが，野外標本中にこれらの2型を見出すことができなかったため，本報告では，両型を単なる培養型として記載するにとどめた。(*730 広島市中区東千田町 1-1，広島大学理学部植物学教室，**733 広島市中区広瀬北町 9-1，広島県地区衛生組織連合会)