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Morphology, Reproduction and Occurrence of a Tropical Alga, *Tetrasporidium javanicum* Möbius *(Chlorophyceae)*

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From the Hydrobiological Field Station on the ponds of Blatná

Abstract — A macroscopic green alga *Tetrasporidium javanicum* Möbius 1893, reported hitherto only from Java and India, has been found in the fish-ponds Velký Pálenec and Smyslov near Blatná, Czechoslovakia. We have been able to complete the description of the alga with some morphological and cytological details and to elucidate the mode of reproduction, previously unknown. On the basis of laboratory cultures the complete morphological life-cycle from the zoospore to the adult thallus has been compiled. The alga belongs to the *Tetrasporales*, in the family *Asterococcaceae*. In nature it grows in shallow eutrophic waters enriched with organic matter; in culture, inorganic Bristol's solution with soil extract satisfies its nutritional requirements. *Tetrasporidium* is kept in the Culture Collection of Algae at the Department of Botany, Prague, under the number H 4101, isolated by Nováková 1964/1.

During phycological investigations of the ponds near Blatná in the summer 1964 the senior author found in the pond Velký Pálenec a peculiar alga, the appearance of which was strikingly different from the other common macroscopic algae living in the benthos. The fish-pond Velký Pálenec is situated near the Hydrobiological Field Station of the Faculty of Science and its algal flora has been examined regularly for more than 30 years (Fott 1929, 1933, 1954 etc.). It is extremely probable that the alga did not appear at the locality until 1964, as its slimy clusters, several cms in size, could not have been previously overlooked. In addition, the appearance of the alga is strikingly different from the skeins of associated algae (*Cladophora*, *Spirogyra*, *Stigeoclonium* and others), growing on submerged plants. In the spring of 1965 the alga was discovered also in the adjacent pond Smyslov.

First examination in the neighbouring laboratory revealed that the alga belongs undoubtly in the *Tetrasporales*, having non-motile cells of monadoid habit embedded in a mucilaginous matrix. Consulting Pascher's Süsswasserflora, *Chlorophyceae* 2, we found that the identification key led to *Tetraspora*; in particular, *Tetraspora lubrica*, having a perforated thallus, seemed to be the nearest species. However, the cells of a true *Tetraspora* are provided with pseudocilia and, since these were lacking in the alga under examination, we were obliged to reject our first identification and look for another designation.

Further investigations of the alga, performed on fixed and cultivated material in the Prague Phycological Laboratory, revealed its ontogeny and yielded further morphological and cytological details that made possible the correct identification. Consulting the papers dealing with palmelloid *Chlorophyceae*, we became convinced that the alga must be identical with *Tetrasporidium javanicum* described by Möbius (1898) from Java. The alga was

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subsequently recognised in India by IYENGAR (1932) who emended the diagnosis of MÖBIUS (based only upon the study of preserved material) and compiled a new description. Our own investigations, carried out on living material at the locality and later on laboratory cultures, proved with certainty that the alga from the fish-pond is identical with the tropical *Tetrasporidium*. We were also able to fill in the details missing from the descriptions of previous authors and to follow in culture the whole life-cycle from a zoospore to a mature macroscopic thallus. A good photograph of *Tetrasporidium javanicum* was taken by BOURRELLY (1958) of to the material collected by Professor RUTTNER in Indonesia. No details about the locality are known.

Morphology and the structure of the protoplast

Under natural conditions in the fish-pond, the alga appears as bright green clusters growing on submerged plants (*Potamogeton crispus*, *Elodea canad.*, *Batrachium aquatile*) and measuring up to 15 cm in length. Examined in the microscope under low magnification, globular cells may be observed, disposed in a mucilaginous, often folded, layer. In a mature thallus this mucilaginous layer exhibits a net-like appearance, having numerous, small, rounded or elliptical pores, or large irregular perforations (tab. XXVI : b). Small colonies, measuring only a few mm, are pear-shaped or pouch-like, consisting of well-defined superficial mucilaginous layer with vegetative cells embedded in it. At this stage the mucilaginous layer is solid, without perforations. Later on, as the colony grows, the one-layered vesicle enlarges and the mucilaginous layer becomes perforated. Through irregular growth, the smooth vesicle-wall becomes undulated and irregularly folded and colonies may reach a size of 2—5 (even 15) cm. Large colonies lose the pouch-like appearance and become irregularly folded and lobed; the one-layered arrangement of cells, however, always remains distinct (tab. XXVI : d, e,).

The vegetative cells of *Tetrasporidium* are broadly ellipsoidal to spherical, the ellipsoidal shape being, of course, their usual one. They possess a distinct cell-wall without projections. The long axis of cells is usually perpendicular to the plane of the mucilaginous layer. The cells are distinctly bipolar: at one end, which might be called „apical", 2 contractile vacuoles discharge; at the opposite end, a nucleus with a large nucleolus can be discerned without staining and without any cytological treatment. The length of the cells varies from 6,4 to 9,5 μ (tab. XXVI : g, h, i).

The chloroplast is relatively thin, parietal, not entirely covering the cell cavity, lobed at the margin, thickened laterally and enclosing a pyrenoid in the thickening. Sometimes a stigma can be observed in the anterior part of the chloroplast, especially in young, flagellated cells. The pyrenoid is rounded on one side, but distinctly flattened on the other. It is enclosed in a massive envelope of starch, composed of lenticular grains which cover both hemispheres of the pyrenoid. The pyrenoid is always lateral in position. No distinct starch grains (stroma starch) were observed in the chloroplast, but numerous small light-reflecting granules were present in the cytoplasm.

Reproduction and life-cycle

Most of the vegetative cells lying in the mucilaginous layer are flagellated. They bear two motionless flagella inserted at the anterior pole of the cell,

where the two contractile vacuoles occur. The flagella can be discerned only under high magnification with a good immersion objective. They may be quite short, straight or variously bent. These flagellated cells can acquire motility and swarm out as motile reproductive cells (tab. XXVIII : a—e).

The production of new cells in a colony occurs by division of the protoplast into 2 or 4 (rarely 8) portions that develop either into flagellated cells or into non-motile vegetative cells (autospores). It is very common for two autospores to be arranged in the mother cell in the same manner, that is to say, perpendicular to the mucilaginous layer. The autospores display the same inner structure of the protoplast (parietal chloroplast with a lateral pyrenoid, contractile vacuoles, etc.) as the mother cells. The mother cell-wall becomes thin during spore formation, changes into mucilage and soon disappears. The mucilage layer of the colony is without any visible structure.

The division of cells to produce autospores leads to enlargement of the colony from one cell to an extended vesicle, measuring a few centimeters in length. These divisions are not themselves reproductive in that they do not originate new colonies. The mature thallus, however, can disintegrate into small groups of autospores embedded in a common mucilaginous envelope and this small group of cells can give rise to a new vesicular (pouch-shaped) colony (tab. XXVIII : f, g). In the same manner, detached protrusions and portions of the thallus are capable of regenerating and growing into a new colony. It is thus possible to establish a culture of *Tetrasporidium* by separating off a small portion of the mucilaginous layer and transferring it to a new culture vessel.

The life-cycle of the alga usually starts in nature, as well as in culture, from the motile reproductive cell (tab. XXVIII : a—e). The latter originates from vegetative cells by dissolution of the mucilaginous layer, within which the cells are embedded, and by the acquisition of motility by the flagella.

The motile cells are always produced in small numbers; they do not arise by disintegration of the thallus. They differ from the zoospores of the other algae (*Chlorococcum*, *Stigeoclonium*, *Microthamnion*) in lacking phototactic sensitivity. Many algal zoospores form a green meniscus at the water's edge towards the light, and this may serve as clear proof of their occurrence. In *Tetrasporidium*, however, the motile cells display converse movement and become affixed irregularly either on the walls or on the bottom of the culture vessel. In many cases they prefer to settle down on the bottom, where the light intensity is lower. Small germlings of *Tetrasporidium* found growing on submerged plants in the pond give good evidence of motile reproductive cells in natural conditions (tab. XXVI : c).

In July 1964 the alga reached the peak of its occurrence in the pond and ceased to form new colonies. Towards the end of the month the tussocks of alga disintegrated and slowly disappeared without forming special resting spores. No sexual reproduction has been displayed by the alga either in natural conditions in the pond or in cultures.

The whole development of the alga could be followed in cultures, whereas in nature adult colonies towards the end of their vegetative life were mostly observed. The cultures thrived best in Erlenmayer flasks; the preparatory cultures were successful using enriched water from the locality. This was inoculated with a small portion of the thallus, since no germlings from the ponds were at our disposal. The inoculated portions enlarged to irregular colonies which produced motile reproductive cells, and these settled to the bottom of

the vessel. Subculturing was carried out by transferring small germlings, arising from these motile cells, into Bristol's solution, where the alga can reach a size of 4 cm (tab. XXV : a).

The motile reproductive cell,[1]) having lost its motility, produces a mucilaginous vesicle, by means of which it becomes attached to the substratum. Daughter cells, arising from the primordial mother cell, remained enclosed in the vesicle which enlarges with the increasing number of new cells. The mass of mucilage stretches by gelatinisation of the mother cell-walls of the continuously dividing cells. The shape of young vesicles is rather variable: ovoid, pear-shaped, elongated, oblong-ovate, spatulate, pouch-like, etc., straight or bent, but in all cases, whatever the form of the colony, the cells are disposed at its periphery forming a definite layer of mucilage. Owing to the irregular rate of cell divisions, perforations of various size occur in the older vesicles, varying from small holes to large apertures. In the last stage of colony development, the adult thallus becomes folded and irregularly clustered (tab. XXVI : a).

Locality and conditions of growth

The locality where *Tetrasporidium javanicum* was found is a eutrophic fish-pond, Velký Pálenec, belonging to the State Fish Farm, Blatná. The pond is situated a few metres from the Hydrobiological Field Station of the Faculty of Science, Prague, and since 1925 it has been the subject of hydrobiological observations. Like the other fish ponds of the drainage area, Lnáře-Blatná, it was constructed in the 16th century and since that time it has been used for carp farming. No doubt it was sited on infertile swamps and even now its bottom is siliceous sand and the zone of littoral macrophytes relatively narrow.

At first, the crop of fish was low and for this reason the pond has been manured regularly for more than 35 years. Inorganic manuring with lime and supperphosphate caused dense water-blooms of blue-green algae, especially of *Aphanizomenon flos-aquae*, the latter blooming in some years for the whole season of algal growth. The fish ponds coloured with *Aphanizomenon* are characterized by a striking simplication of the plankton community, not only in Czechoslovakia (Fott 1952, 1959) but also in Poland and Germany. In these ponds the nannoplanctonic algae disappear completely and the zooplankton is reduced to a few species, dominated by the large *Daphnias*. Although Velký Pálenec is a true *Aphanizomenon* pond with all the attributes of such, in some years *Aphanizomenon* has been replaced by another blue-green alga or by a different algal community. The irregularity of the *Aphanizomenon* blooming is caused by certain practices of pond management, e.g. by draining the pond in summer, by organic manuring with dung, by abundant growth of submerged plants, etc. In 1962 the pond was drained and sown with clover. This practice caused a slight waterbloom in the subsequent year, and even in 1964 *Aphanizomenon flos-aquae* discoloured the water till towards the end of July.

There are some data concerning the physiography of the pond in 1963, given in the diploma thesis of M. Jonášová (1964). The ara of the water level measures about 30 hectares. The mean depth is 1,44 m, the early morning temperature of the water surface changes from 20 to 24° C in July. During a sunny day, the temperature of the water at the surface can reach 26° C or even more. The pH changes during the day in accordance with the assimilatory activity of the water-bloom. It may reach pH 9,7 and in some cases even pH 11. The lowest value is pH 7. The mean value of alkalinity yields 1,52 mval, the mean amount of nitrogen is 1,54 mg/l (Jonášová 1964). One sees from these data that the habitat of *Tetrasporidium javanicum* is a eutrophic pond with an excess of major nutrients and with a great amount of organic matter derived from the ma-

[1]) The difference between a motile reproductive cell and a zoospore lies in their origin. Zoospores arise by the division of the protoplast inside the mother cell-wall into 2 to many portions which acquire their motility by forming flagella. The motile reproductive cell, however, originates from a motionless vegetative cell, the flagella of which become motile. In this manner the cell can leave the mucilaginous layer and, after settling, produce a new colony (Nováková 1964).

nuring. The localities in Java (MÖBIUS 1893) and India (IYENGAR 1932) seem to be of similar nature.

Taxonomy

Tetrasporidium javanicum MOEBIUS 1893 was described in a paper on the algal flora of Java, described from fixed material collected by Dr. F. Benecke near Semarang. The description emphasized the similarity to *Tetraspora lubrica*, not only as far as the net-like and perforated structure of the mucilage is concerned but also with regards to the appearance of the protoplast. MÖBIUS observed the dividing protoplast inside the mother cell-wall and presumed that gametes or zoospores might occur. The short latin diagnosis of the species runs as follow (l. c. p. 123):

„Thallus spongiosus,irregulariter perforatus,structuram et multiplicationem cellularum eandem, quam Tetraspora, praebet. Reproductio fit sporis (zoosporis aut gametis?) in cellula incrassata divisione succedanea senis denis evolutis, periplasmate multo in sporangio remanente. Diam. cellul. veget. $6-7\,\mu$, sporangiorum $20-25\,\mu$."

The alga was subsequently discovered by IYENGAR (1932), in small ponds in Madras formed during the rainy season (October—December); it was growing on submerged aquatic plants such as *Monocharia hastaefolia* PRESL. Another locality is the river N a g a r i in South India where the alga grows on sandy banks with *Hydrodictyon reticulatum*.[1])

IYENGAR having plenty of material, living and preserved, made a careful study of the alga and emended the short diagnosis of MÖBIUS. His description (l.c. 199) does not differ fundamentaly from our observations, but some details seem to have escaped him. In contradiction to IYENGAR, we were able to discern two non-motile flagella, embedded in mucilage, often indistinct or lacking. We also succeeded in detecting the modes of reproduction and the entire morphological life cycle of *Tetrasporidium* in culture.

There is no unity among the authors as far as the taxonomic position of the alga is concerned. MÖBIUS (1893) laid stress on the similarity with *Tetraspora*, WILLE (1909) and PRINTZ (1927) in Engler's Pflanzenfamilien placed it in the *Tetrasporaceae* under "wenig bekannte oder unsichere Gattungen", BLACKMANN et TANSLEY (1902) suggested referring the alga to *Tetrasporaceae*, while CHODAT (1902) considered it to be a species of the genus *Tetraspora*. IYENGAR himself (l.c.p. 198) emphasizes that the alga, having no pseudoflagella, cannot be included in *Tetrasporaceae* and therefore places it in the artificial family *Palmellaceae* (sensu LEMMERMANN 1915). He pointed out the great similarity with *Palmodictyon* from this family, whereas *Palmodictyon* is, in fact, a coccoid alga and in our opinion belongs to *Chlorococcales* (FOTT 1959). The same taxonomic position of *Tetrasporidium* is advocated by BOURRELLY (1958), who placed it in *Palmellaceae* in the vicinity of *Asterococcus*.

Our own consideration of the taxonomic position of *Tetrasporidium* is determined by a cellular habit that is undoubtedly tetrasporoid. The alga therefore belongs to *Tetrasporales* (sensu FOTT 1959). *Tetrasporales* exhibit the monadoid protoplast of their ancestors, *Volvocales*, but live in a non-motile state as do their descendants, *Chlorococcales*. *Tetrasporales* thus represent an evolutionary step between two grades of algal phylogeny, between the monadoid and coccoid habits. In the order *Tetrasporales* several families can be distinguished and among them the characteristics of the *Asterococcaceae* are in agreement with the description of *Tetrasporidium*. The family *Asterococcaceae* (FOTT 1966 in press) is defined in brief by the following characteristics: "Cells

[1]) This latter species is a regular member of the benthic community of algae growing at the locality in Czechoslovakia.

solitary or in colonies, living in simple or compound mucilaginous envelopes or in mucilaginous masses. Cells flagellated or not, but capable of swarming out of the mucilage as motile reproductive cells."

A further record of *Tetrasporidium javanicum* comes from China (JAO 1947). There is no drawing in his paper and the description is short and too general, mentioning none of the characteristic attributes of *Tetrasporidium* ("thallo ambitu variabili, plerumque lanceolato vel oblongo, longitudine usque ad 15 cm, latitudine usque ad 5 cm, interdum subovato vel irregulariter expanso; cellulis 5—12 μ latis, plerumque quaternis approximatis"). In addition, the occurrence of the Chinese alga in a clear rocky mountain stream is in disagreement with the ecological conditions of the localities known hitherto. For this reason we presume that JAO's alga is not *Tetrasporidium*. The only certain records of *Tetrasporidium javanicum* are from Java (MÖBIUS), India (IYENGAR) and our locality in Czechoslovakia. The alga is kept in culture in the Culture Collection of Algae at the Department of Botany, Caroline University of Prague, under the number H 4101, isol. Nováková 1964/1. Dried specimens originating from the locality are deposited in the Herbarium of the Prague Botany Department (PRC) and have been sent to the main herbaria in London (British Museum), Leningrad (Botaničeskij Institut ANSSR), Paris (Museum d'Histoire naturelle), Vienna (Naturwissenschaftliches Museum), Leiden (Rijksmuseum) and Berkeley, USA (Department of Botany at the University of California). A small number of exsiccata are available for distribution to anybody who asks for them.

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Explanations of the plates:

Tab. XXV: *Tetrasporidium javanicum*. — *a* vesicular colonies from culture vessels (natural size). — *b* folded border of a large colony from the pond; perforations of the mucilaginous layer not visible (100×). — *c* living cells at the edge of the colony; there are two non-motile flagella and two contractile vacuoles near their base; a large pyrenoid in the middle of the cell (1500×). — *d* cell with a pyrenoid enclosed by starch grains, forming two watchglass-shaped pieces. — *e* one

cell (right) with the nucleus focused, the other cell (left) with a distinct pyrenoid (3000×). — Tab. XXVI: *Tetrasporidium javanicum*. — *a* large colony in the form of a slimy mass with lobed protrusions, attached to the stem of *Potamogeton* (natural size). — *b* superimposed layer of cells with perforations and holes in the mucilage (wild material). — *c*, *d*, *e* oblong-ovate colonies showing arrangement of cells in the mucilaginous layer. — *f* colonies of various shapes and sizes (from culture vessels). — *g*, *h*, *i* vegetative cells, showing the inner structure: contractile vacuoles at one end, nucleus with nucleolus at the opposite end, pyrenoid in the lateral thickening of the chloroplast, small granules of lipids in the cytoplasm.

Tab. XXVII: Vegetative cells of *Tetrasporidium javanicum*. — *a* division of the protoplast into four aplanospores (autospores); the mother cell-wall dissolves slowly (wild material). — *b* formation of autospores in twos; young cells arranged in a layer, having the apical end (with vacuoles) outwards and the basal end (with nucleus) inwards with respect to the colony. — *c*, *d* rapid growth of autospores which finally fill the cavity of the mother cell-wall (material from culture). — *e* cell with eight autospores. — *f*—*m* flagellated non-motile cells, embedded in mucilage, with two equal flagella of various lengths and positions.

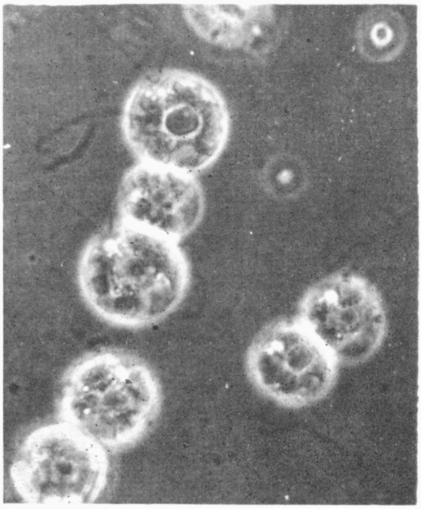
Tab. XXVIII: Reproduction of *Tetrasporidium javanicum*. — *a*—*e* motile reproductive cells. — *f*, *g* disintegration of the thallus into small portions, capable of growth into new colonies.



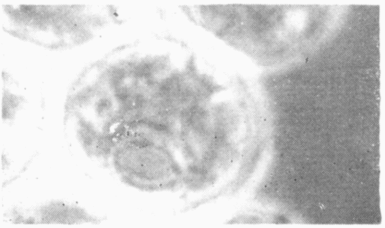
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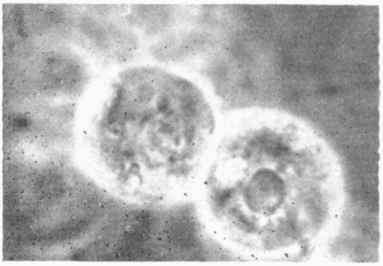
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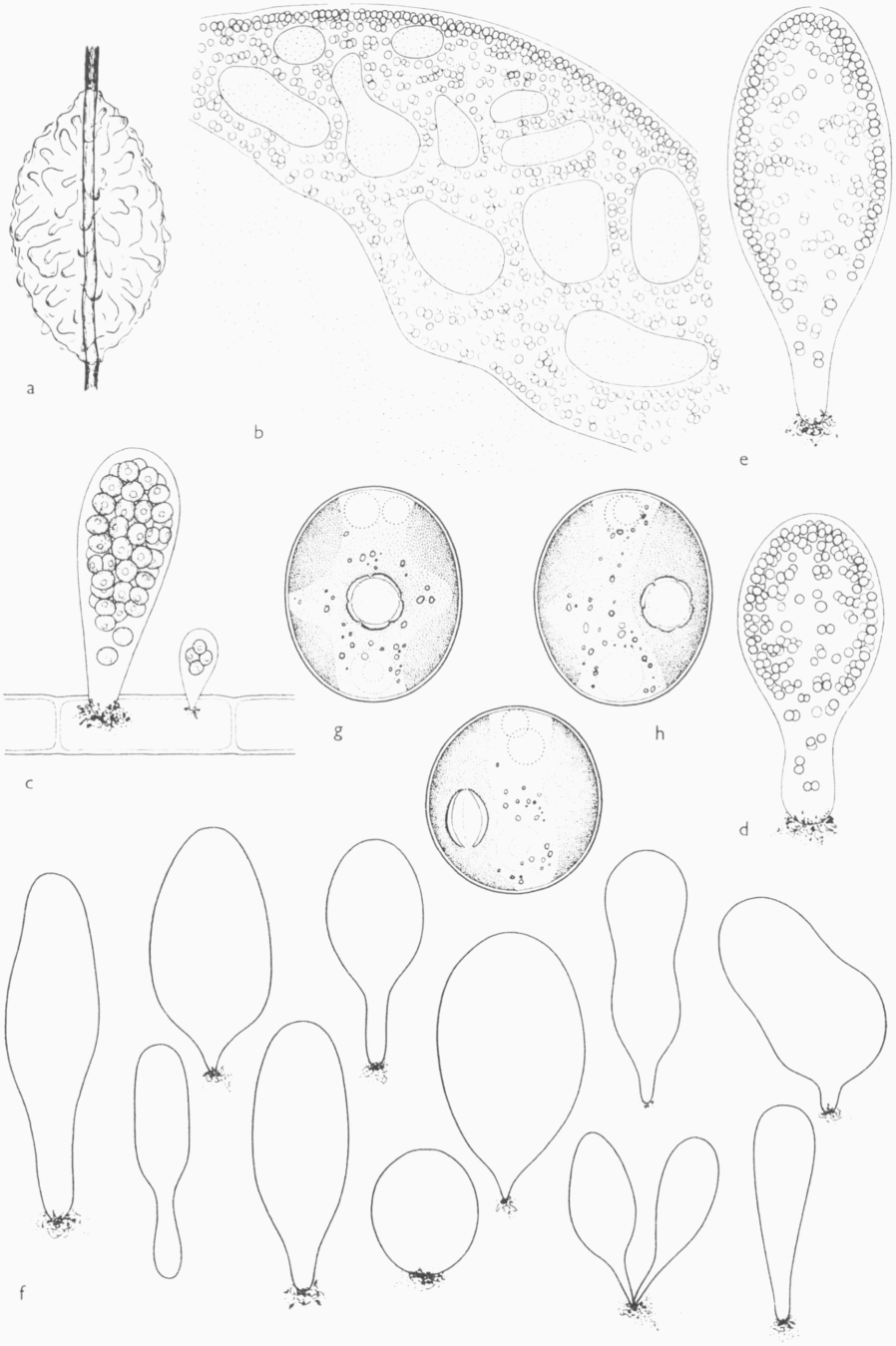


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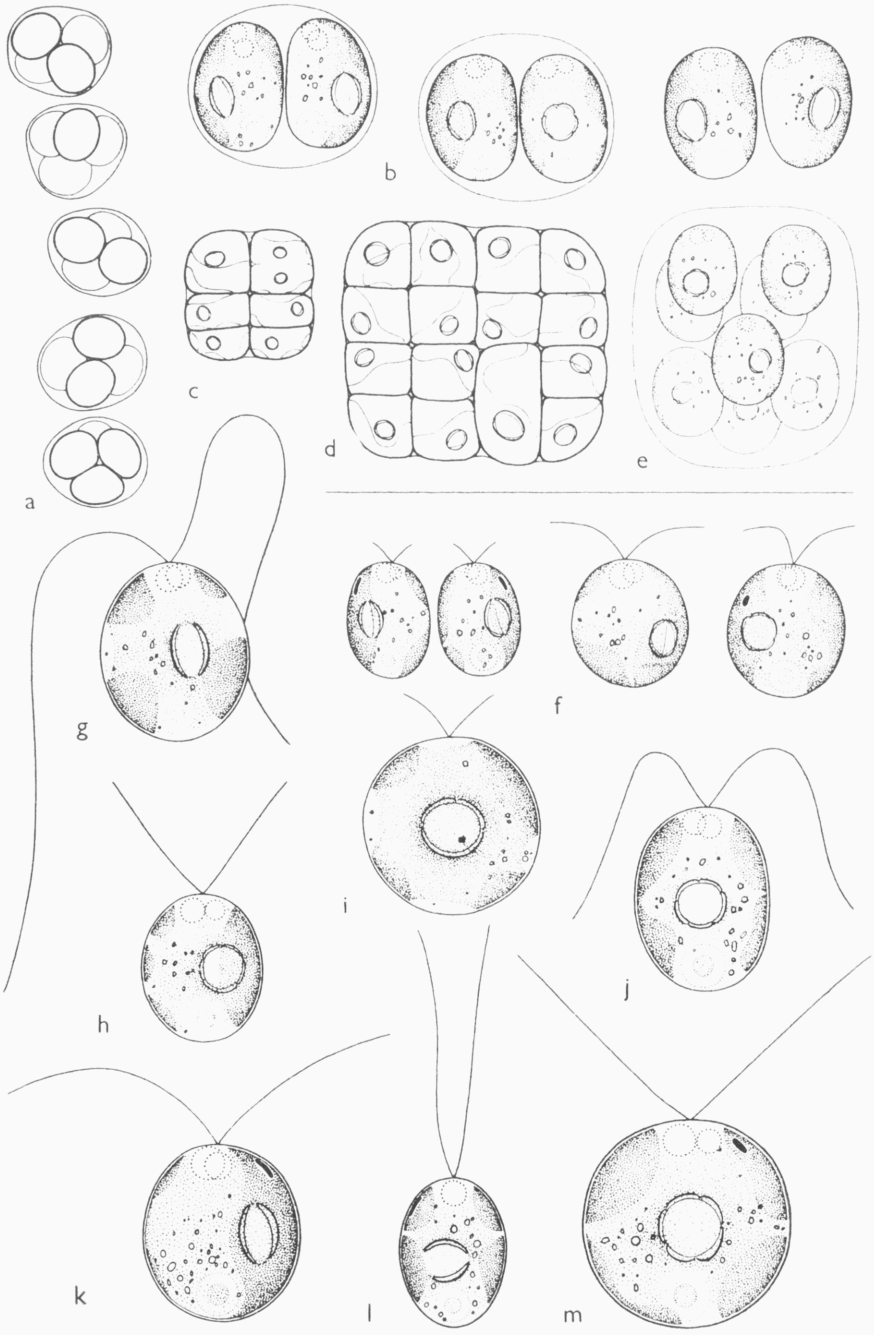


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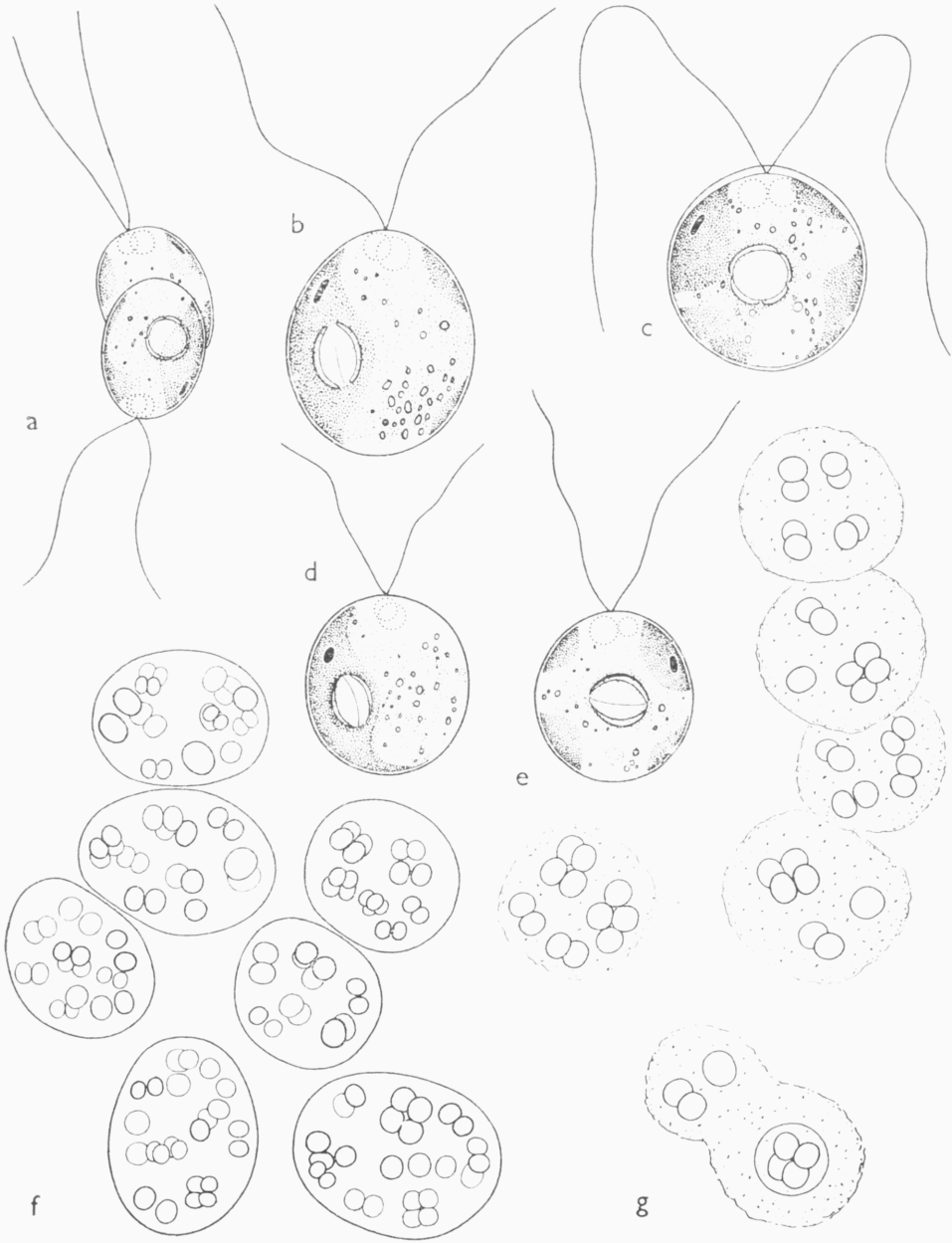
B. Fott, M. Nováková and T. Kalina: Morphology, reproduction and occurrence of a tropical alga, *Tetrasporidium javanicum* MÖBIUS *(Chlorophyceae)*



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