

Marvania aerophytica sp. nov., a new subaerial tropical green alga*

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A new species of the green algal genus *Marvania* is described from a tropical subaerial locality. Asexual reproduction by a special type of budding-like autospore formation is demonstrated. The differences between the newly described species and *Marvania geminata* HINDÁK are discussed.

Key words: *Marvania aerophytica* sp. nov., Chlorophyta, subaerial algae, budding, Malaysian Peninsula.

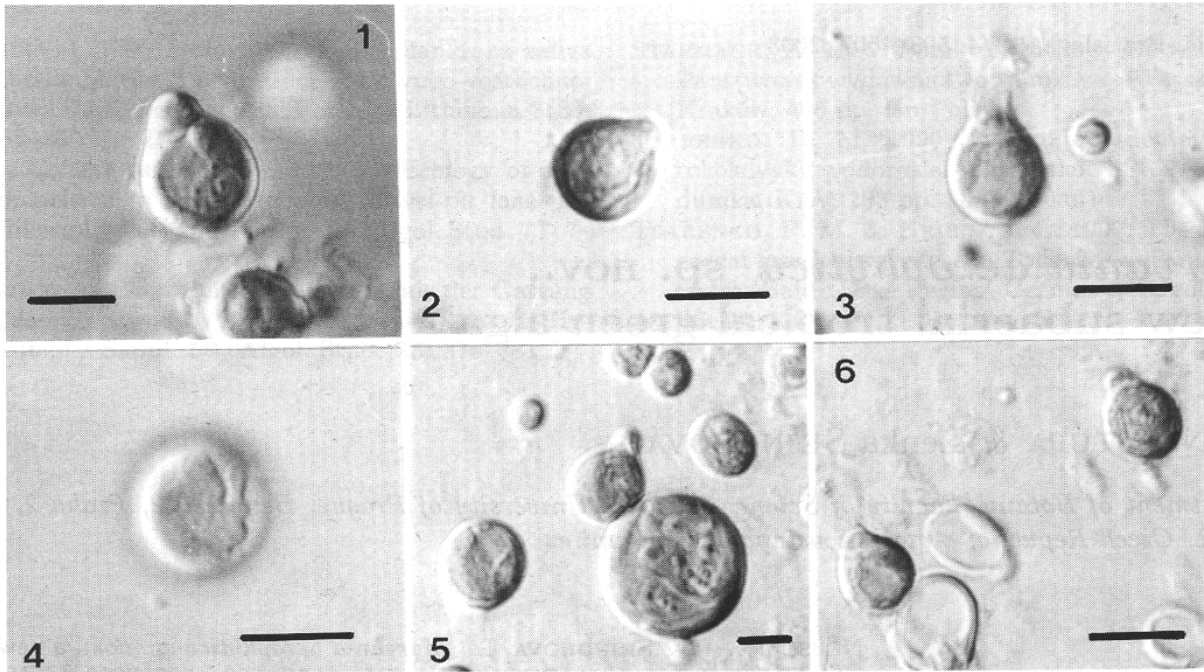
Introduction

The type species of the genus *Marvania*, *M. geminata* was described by HINDÁK (1976) from plankton of some localities in Southwest Slovakia. The alga was characterised primarily by its unique type of budding-like asexual reproduction of globular vegetative cells. REYMOND et al. (1986) studied the ultrastructure of cultured material from the type locality of *M. geminata*. They described details of the reproductive process and stated that the formation of a new cell wall of a daughter cell resembles a modified autospore formation. SLUIMAN & REYMOND (1987) investigated mitosis and cytokinesis of *M. geminata* and reported that daughter cells form a new wall layer at the new septum and under the mother cell wall. The cells separate after the old mother cell wall has broken at the level of the septum. Therefore, the authors considered *M. geminata* as a member of broadly defined order Chlorococcales accommodating green

algae capable of autospore production. However, for practical reasons HINDÁK (1996) included the monotypic family Marvaniaceae within the traditionally defined order Ulotrichales in his identification key of unbranched filamentous green algae.

YAMAMOTO et al. (2001) investigated several green algal strains labelled as members of the genus *Nannochloris* in culture collections. They found a budding-type of reproduction in the strain CCAP 251/1b labelled as *Nannochloris coccooides* NAUMANN. The strain was isolated from a freshwater locality in Cambridge, England (TOMPKINS et al., 1995). TSCHERMAK-WOESS (1999) determined strain CCAP 251/6, labelled originally as *Nannochloris atomus* BUTCHER and isolated from plankton of the York River estuary, Virginia, USA, as *Marvania geminata*. An investigation of actin gene phylogeny of *Nannochloris*-labelled strains led YAMAMOTO et al. (2001) to the conclusion that typical autospore formation can be regarded as

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Figs. 1-6. *Marvania aerophytica*. 1, 2 reproducing cells; 3 the adult cell after repeated reproduction; 4 the detail of chloroplast margin in the adult cell; 5 vegetative cells in different stage of reproduction; 6 reproducing cells. Scale 7 μm (Figs 1-3), 10 μm (Figs 4, 6) and 5 μm (Fig. 5).

ancestral to budding-like type of reproduction and vegetative division.

In the course of an investigation of the sub-aerial algal flora of some epiphytic biotopes of the Malaysian Peninsula a coccal green alga was isolated which is described here as a new species of *Marvania*.

Material and methods

Algal material was isolated from the bark of *Pandanus* sp. growing in secondary rainforest in the area of Hulu Kelantan, Peninsular Malaysia, geographical coordinates $5^{\circ}13'10''\text{N}$ and $101^{\circ}48'00''\text{E}$, altitude 450 meters above sea level. Strains were cultivated both on agar-solidified and liquid BBM (BISCHOFF & BOLD, 1963) and in biphasic cultures (PRINGSHEIM, 1954) at temperatures of 13°C and 25°C and under illumination of about 2500 lux (light source: Tungstram 36W F33, cool white).

For transmission electron microscopy, cells were fixed in 2% glutaraldehyde in 0.05M phosphate buffer, postfixed in 1% osmium tetroxide in phosphate buffer and 1% uranyl acetate in methanol. The cells were dehydrated in a graded series of ethanol and transferred to butanol and embedded in Spurr's low viscosity resin (SPURR, 1969). Sections were poststained with uranyl acetate and bismuth oxynitrate and examined with a PHILIPS T300 TEM.

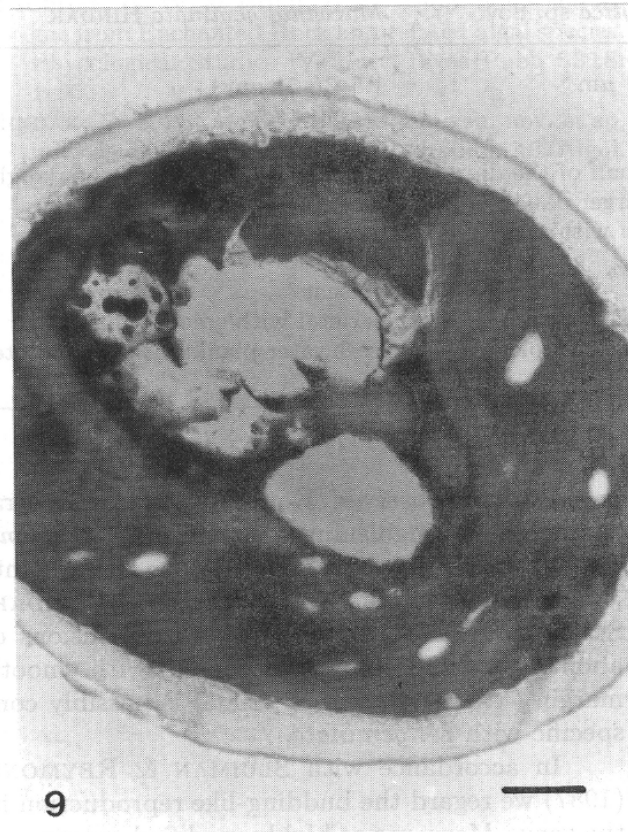
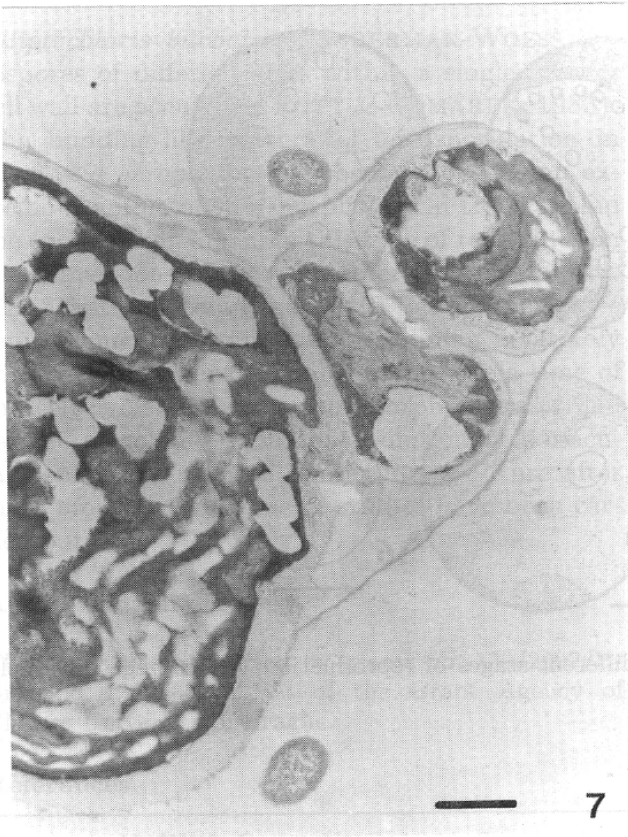
The investigated strain was deposited at the Collection of Algae of Charles University of Prague, Czech Republic (CAUP), accession number H 7301.

Results and discussion

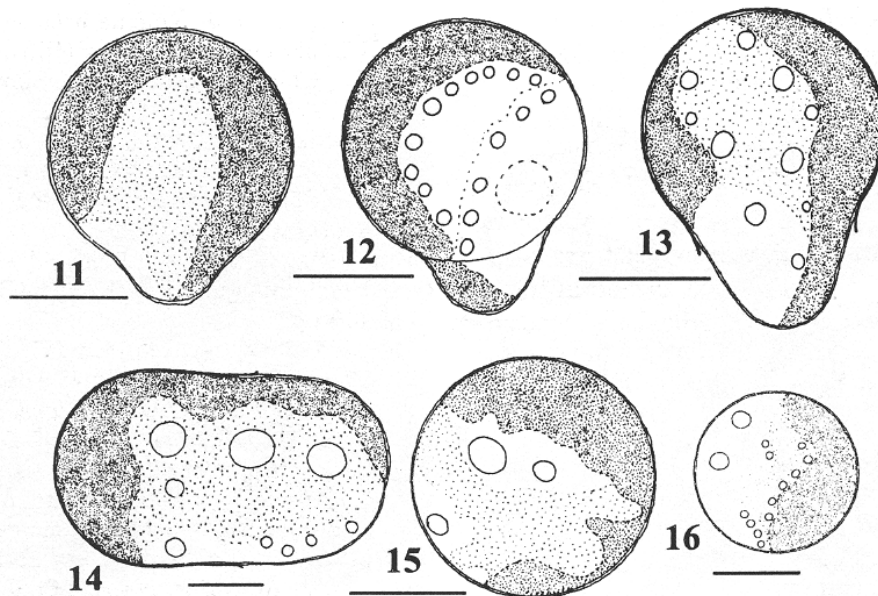
Marvania aerophytica NEUSTUPA et ŠEJNOHOVÁ sp. nov.

Diagnosis: Cellulae sphaericae, (2,5-)3,5-9(-13.5) μm diametro. Membrana cellulae laevis. Chloroplastum parietalis, sine pyrenoide. In protoplasto hyalino globulae presentes. Propagatio per autosporas eximiae effecta, germinationi similes videntur. Cellulae juveniliae 2.2-3.2 μm diametro. Iconotypus: figura nostra 12.

Vegetative cells globular, (2.5-)3.5-9(-13.5) μm in diameter. Cell walls smooth. Chloroplasts single, parietal, without a pyrenoid and with deeply lobed margins in adult cells. Cytoplasm usually containing hyaline globular vacuoles, occasionally arranged in a row along the chloroplast margins (Fig. 12). Reproduction takes place by a special type of autospore formation that resembles budding. Mother cells are producing a cytoplasmic protuberance containing part of the chloroplast (Figs 2, 11, 13). Following mitosis and cytokinesis daughter cells are released immediately by rupture of the mother cell wall at the level of septum. Young daughter cells are globular, with simple parietal chloroplast, 2.2-3.2 μm in diameter. Daughter cells were retained within the common mother cell wall (Fig. 7) on rare occasions, especially in rapidly



Figs. 7-10. *Marvania aerophytica*. TEM sections. 7 reproducing cells; 8 the detail of young cell after cell division; 9 vegetative cell; 10 the reproducing cell, note the thickened cell wall in the original cell. Scale 1 μm (Figs 7, 10) and 0.5 μm (Figs 8, 9).



Figs. 11–16. *Marvania aerophytica*. 11–14 the cells in different stages of reproduction; 15, 16 vegetative cells. Scale 3 μm (Figs 11, 12, 14–16) and 5 μm (Fig. 13).

Table 1. Comparison of two *Marvania* species.

	<i>Marvania aerophytica</i> sp. nov.	<i>Marvania geminata</i> HINDÁK
Dimensions of the vegetative cells	(2.5–)3.5–9(–13.5) μm	1.5–3(–5) μm
Cell wall	smooth	granulated
Pseudofilaments	absent	present
Final dimensions of daughter cells escaping from the mother cell wall	usually less than half of the dimensions of the larger daughter cells that remain within the mother cell wall	equal to dimensions of the daughter cells remaining within the mother cell wall
Mucilage	absent	present
Chloroplast	parietal with lobed margin	parietal with smooth margin
Autecology	subaerial epiphytic in tropical rainforests	freshwater plankton in temperate zones

growing cultures. Cell walls of mother cells are thickening subsequently with repeated formation of daughter cells within the same cell (Figs 10, 13, 14). Sexual reproduction and production of zoids were not observed.

Iconotype: Fig. 12.

In its peculiar type of reproduction the investigated alga is somewhat similar to both *Marvania geminata* and strain CCAP 251/1b labelled as *Nannochloris coccooides*. In the case of *M. geminata*, where the morphology and reproduction of original strain was investigated in detail (HINDÁK, 1976; REYMOND et al., 1986; SLUIMAN & REYMOND, 1987), a similar pattern of the formation of daughter cells within the mother cell wall as in our

organism was observed. However, there are several characters distinguishing our alga from *M. geminata* (Tab. 1). Hence we propose that it represents a new species of the genus *Marvania* HINDÁK. Strain CCAP 251/1b with its cell dimensions of about 2 μm and simple chloroplast with smooth margin (YAMAMOTO et al., 2001) is possibly conspecific with *M. geminata*.

In accordance with SLUIMAN & REYMOND (1987) we regard the budding-like reproduction in the genus *Marvania* as highly modified autosporulation. Typical autosporulation (e.g. in *Chlorella kessleri* FOTT & NOVÁKOVÁ) is characterised by forming of 2^N number of identical autospores regularly situated within the mother cell wall. In some species such as *Chlorella luteoviridis* CHODAT or

Elliptochloris bilobata TSCHERMAK-WOESS autospores of different sizes within a single mother cell wall are occurring (FOTT & KOMÁREK, 1983). The budding-like pattern of autospore formation in *Marvania aerophytica* can be regarded as an extreme example of this morphological trend within the coccal green algae. In this type of reproduction the dimensions of two daughter cells are always significantly different with the larger autospore reproducing repeatedly and remaining invariably within the mother cell wall for the whole time of the cell cycle. Whether this morphological trend can be correlated with the evolution of particular genes may become clearer in the future after more molecular systematic studies have been carried out.

Acknowledgements

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