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THE MORPHOLOGY OF *WOLFFIA ARRHIZA*: A SCANNING ELECTRON MICROSCOPIC STUDY[1]

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Abstract

The morphology and the vegetative reproduction of the samllest angiosperm *Wolffia arrhiza* Winm. were observed under the scanning electron microscope. The vegetative body of frond of *W. arrhiza* is a boat-shaped structure about the size of a pinhead (0.5 mm). It has a flat surface, on which stomata are arranged in parallel rows. A deep reproductive pocket extends halfway to the interior of the frond, leaving an opening at one end. On the lining of the reproductive pocket distal to its opening is a group of cells, through whose meristematic activity new fronds are formed. The developing frond grows in size and finally is pushed out of the pocket, temporarily forming a dumbbell-like structure. While the daughter frond is still attached, the primordia for new fronds appear within the mother frond as well as the daughter frond.

Introduction

Duckweeds (*Lemnaceae*) are the smallest flowering plants. Their convenient size, ease of culture, fast growth, and homogeneous population resulting from budding have made them favorable objects for biological investigations (cited in Hillman, 1961; Maheshwari and Chauhan, 1963; Trawavas, 1970; Swader and Stocking, 1971; Cleland and Ajami, 1974).

The vegetative body of a duckweed is typically a green flattened structure (frond) consisting largely of chlorenchymatous cells. Some species bear rhizoids on the ventral side of the frond.

Most species of duckweeds propagate vegetatively by budding. New buds arise from the meristematic cells located in reproductive pockets. Morphogenesis of floral buds and cell ultrastructure of *Wolffia* (a genus representing the smallest duckweeds) were reproted by Rimon and Galun (1968) and Anderson *et al.* (1973). This work concerns external morphology as shown by the scanning electron microscopy (SEM) and the sequence of events in budding.

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MATERIALS AND METHODS

The plant - The duckweed *Wolffia arrhiza* Winm. was isolated from a rice paddy in central Taiwan. It is one of the five species of Lemnaceae recorded from Taiwan. The culture was maintained on a modified Hoagland solution (Maheshwari and Seth, 1966). and kept at room temperature (ca. 25°C). The plants received 16 hours of illumination at 2000 ft-c daily. Under these conditions, the number of separable fronds doubled every 2 days.

Sample preparation for SEM-Several methods, including the popular glutaraldehyde-OsO_4 fixation, were tried. We found that for this aquatic plant with air chambers, FPA (30% formalin : propionic acid : 50% ethanol = 5 : 5 : 90)could achieved quick penetration and cause least distortion of the frond. Therefore, the duckweeds were fixed in FPA overnight, dehydrated through ethanol series. Pure ethanol was finally replaced with isoamyl acetate. The samples were then dehydrated by the critical point method, coated with 200–300 Å of gold, and observed under a JEOL JSM-15 scanning electron microscope at an accelerating voltage of 15 kV (Falk *et al.*, 1971).

Anatomical work - The samples were fixed in FPA similarly, dehydrated through an alcohol series to which t-butyl alcohol was added in increasing concentrations up to 100, infiltrated with and embedded in paraffin, cut to 8–10 um thick, stained in hematoxylin, and photographed using a Leitz compound microscope (Feder and O'Brien, 1968).

OBSERVATIONS AND DISCUSSION

Wolffia arrhiza has a boat-shaped frond covered with polygonal epidermal cells; each frond measures ca. 0.5 mm long and 0.3 mm wide. On the flat dorsal surface are 4 to 5 rows of linearly arranged stomata (Fig. 1, 2). Each stoma is bound by two slender guard cells whose wall is thickened on one side (cf. Brown and Johnson, 1962). No subsidiary cells are present (Fig. 3). Stomata become differentiated on a daughter frond prior to its emergence from the mother frond (Fig. 2).

A large opening which is encircled by a tier of long narrow cells leads to a "reproductive pocket" located inside the frond; through this opening, frequently a young bud is seen to emerge (Fig. 2,4). If the culture is undisturbed, the new (daughter) frond usually remains attached until it reaches full size, thus the pair assumes a dumbbell-like appearance (Fig. 5).

Transverse sections were made to gain insight into the sequence of bud development. The result is shown in Figures 7 and 8, which can be compared with the SEM view in Figure 6. Before a daughter frond (IIa) was pushed out, the primordia (IIb, IIc) for the next two fronds already made their appearance within the pocket of the mother frond (I). Concurrently, the daughter frond (IIa) was making three new fronds (IIIa, IIIb, IIIc) of its own. This pattern of vegetative reproduction was first mentioned by Rimon and

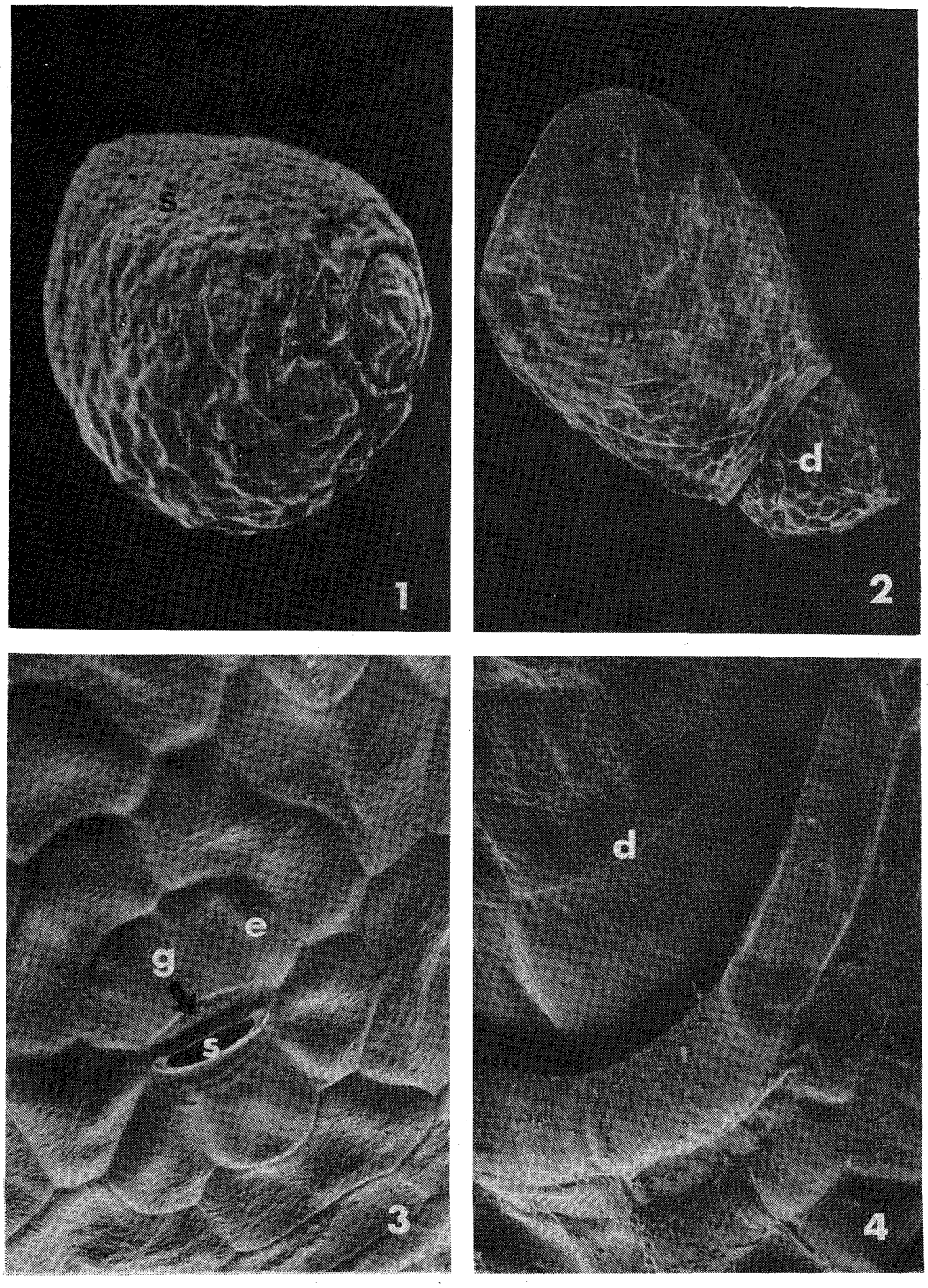

Fig. 1 - 4. General morphology of *Wolffia arrhiza*. 1. A SEM view of a frond, showing the opening to reproductive pocket and stomata (s). x100. 2. A plant with an emerging daughter frond (d). x75. 3. Epidermal cells (e), guard cells (g), and a stoma (s). x1000. 4. An enlarged view of the opening to reproductive pocket, with a portion of a daughter frond (d). x500.

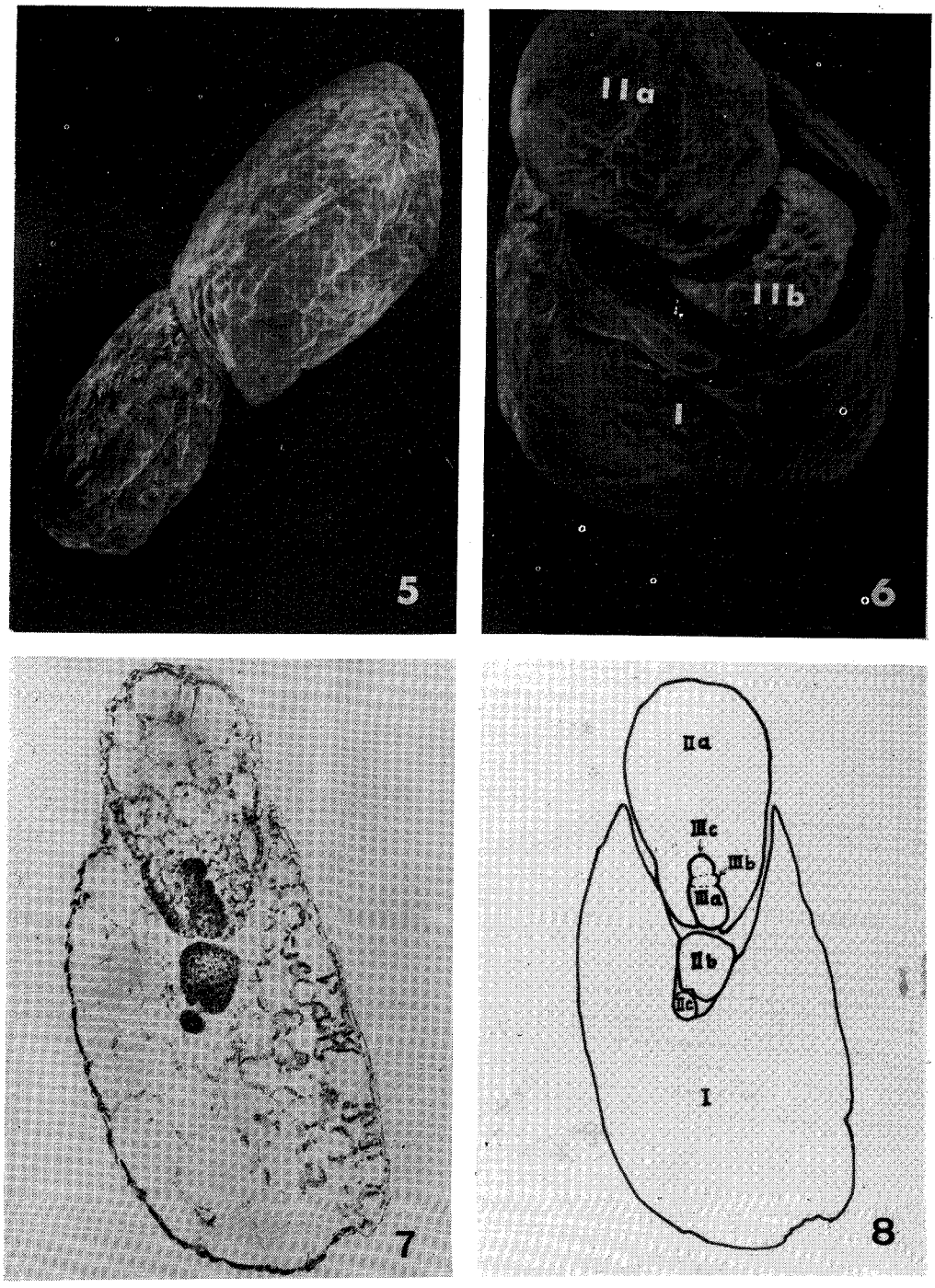

Fig. 5-8. Budding in *W. arrhiza*. 5. A plant with a nearly mature new frond attached. x75. 6. A mother frond (I) with two successively formed daughter fronds (IIa, IIb). x120. 7. A transverse section showing the sequence of bud development. x100. 8. A diagramatic representation of Fig. 7. x100.

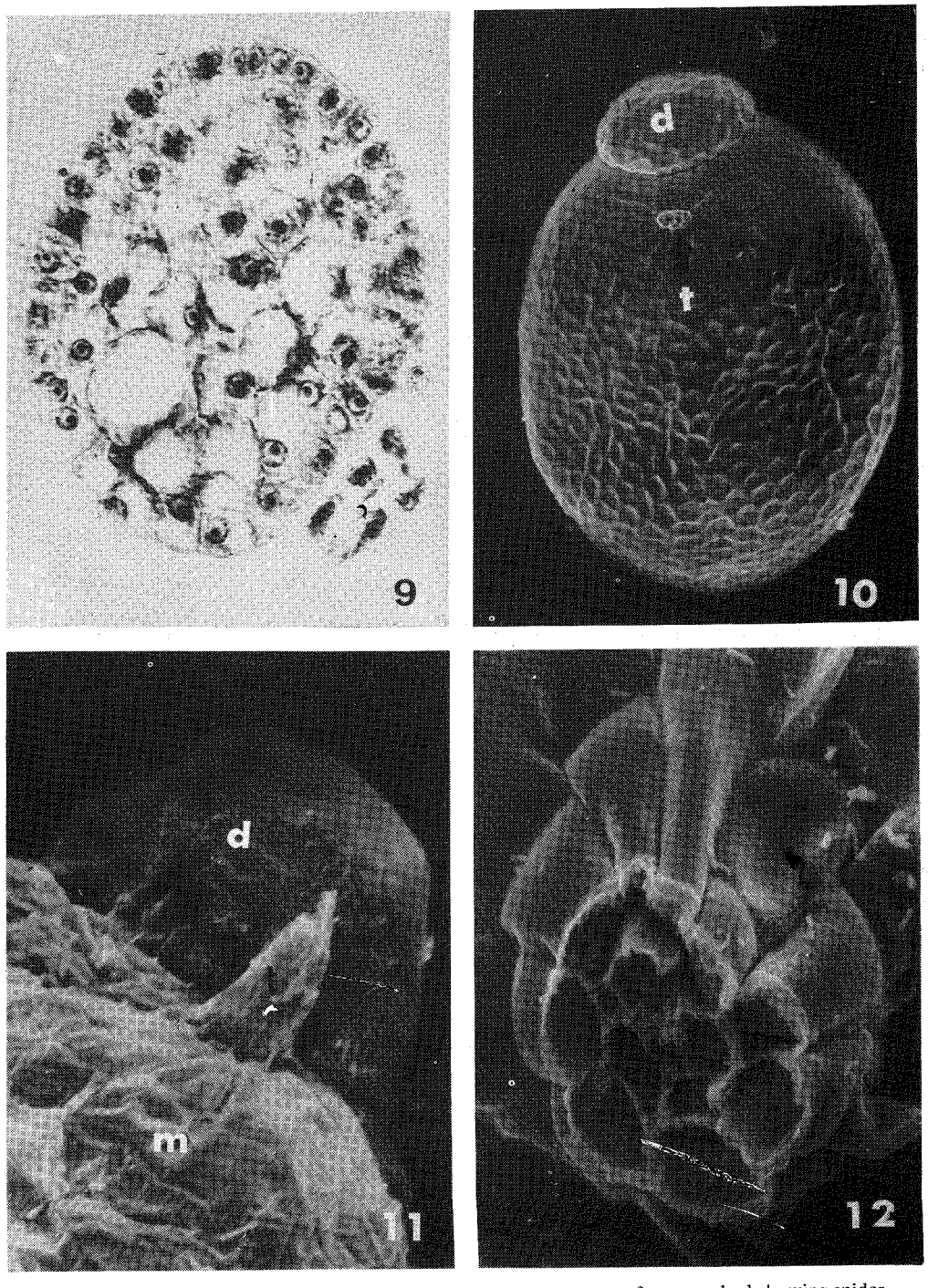

Fig. 9-12. A young bud and a "trichome" of *W. arrhiza*. 9. Anatomy of a young bud showing epidermal cells with dense cytoplasm, large nucleus with a conspicuous nucleolus, and highly vacuolated chlorenchymatous cells. x200. 10. A frond (ventral side) showing where the "trichome" is attached. x120. 11. An enlarged view of a "trichome" (t), portions of mother frond (m) and daughter frond (d) are seen. x2300. 12. Fine structure of a "trichome" with tip broken. x1000.

Galun (1968) for *W. microscopica.* We now present a pictorial representation of this unique morphogenesis exemplified by *W. arrhiza.*

The cells of new fronds have dense cytoplasm, large nucleus with one conspicuous nucleolus (Fig. 9). Growth of new fronds at later stages is probably accomplished by cell expansion, since the number of cells did not increase; a comparison of a mature frond in Fig. 7 with a young frond in Fig. 9 shows that both plants have 8 to 9 cell thick. As cells enlarge, a huge central vacuole makes its appearance in the chlorenchymatous cells, and the cytoplasm is pushed to the cell periphery (Fig. 7).

Finally, *W. arrhiza* is distinguished from *W. microscopica* by lack of a rhizoid (Daubs, 1965). However, we have observed a very short "trichome" on the ventral side of the frond (Fig. 10, 11); this structure is inconspicuous and easily broken when the plant is processed for microscopy. This heretofore unknown outgrowth consists of 13 cells at its base, and perhaps measures two cell long (Fig. 12); it probably represents a vestigial rhizoid serving no function.

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無根萍形態及其無性生殖之研究

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無根萍是最小的開花植物，本篇文章利用掃描電子顯微鏡研究其外表形態及無性生殖方式，並做石臘切片觀察其分生組織之發生及發育情形，無根萍只具有一個生殖腔，成熟幼體逐漸從生殖腔突出，以致分開成爲獨立之個體。其分生組織之分化情形與其他浮萍相似，幼體在母體之生殖腔時，母體之分生組織已具有另二子代之芽體，而幼體之分生組織也同樣開始分化具有子代之芽體。