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地钱生殖托形态结构新观察及其对有性生殖的适应

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摘 要: 用显微观察技术对苔类植物地钱(*Marchantia polymorpha*)生殖托的形态结构进行了研究, 本研究的新观察包括: (1)生殖托具有明显的背腹分化, 被认为是叶状体为适应有性生殖而高度特化的直立枝; (2) 雌托盘的 9~11 个指状裂瓣中边缘两个稍不同, 除了两个边缘裂瓣外的其他裂瓣间具雌苞。托柄具有背腹之分, 雌托柄背面具光合组织, 并向两侧扩展形成纵沟, 雌托柄的腹面具 2 条被鳞片重叠覆盖相互平行的纵沟, 内具假根; (3)与雌托柄不同, 雄托柄外观平滑, 背面无光合组织及纵沟。观察发现, 伞状的雌托能滞留水分, 并沿雌托柄的纵沟缓慢释放连续的水流, 游动精子沿着纵沟内的水流到达雌托下面的颈卵器。上述观察表明地钱生殖托的结构是对有性生殖的一种适应, 这有助于我们理解地钱受精作用的机理。

关键词: 地钱; 生殖托; 雄托; 雌托

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New Observations on the Morphology and Structure of *Marchantia polymorpha* Gametophores in Sexual Reproduction Adaption

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Abstract: The morphology and structure of the gametophores of the liverwort *Marchantia polymorpha* were studied using light microscopy. New observations included: (1) The gametophores had distinct dorso-ventral differentiation and were highly specialized upright branches of the thallus bearing sex organs. (2) Two marginal ones of 9–11 rays of the female receptacle were distinguished by their slightly different sizes compared with others. There was no involucre between the two marginal ones. The dorsal region of the archegoniophore stalk was comprised of photosynthetic tissue, which extended laterally and formed two narrow grooves under the extended photosynthetic tissue. The ventral region of the stalk consisted of two-parallel rhizoid furrows half-wrapped by overlapping scales. (3) In contrast to the female archegoniophore, the antheridiophore stalk was smooth and no photosynthetic tissue or grooves were formed in the dorsal region. The umbrella-shaped female receptacle retained water, which could be released slowly along the grooves to form a tiny water flow, through which the spermatozoids swam to the archegonia. The morphology and structure of the *Marchantia* receptacles were adaptations to sexual reproduction, and can help us to understand the mechanism of fertilization in *Marchantia*.

Key words: *Marchantia polymorpha*; Gametophore; Antheridiophore; Archegoniophore

The gametophytes of *Marchantia polymorpha* L. exhibit dorso-ventral differentiation and are dioecious. The female plant produces archegoniophores (female receptacles) and the male

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plant produces antheridiophores (male receptacles) at maturity. Major events of *Marchantia* sexual reproduction such as the development of sexual organs and embryos and the formation of sporophytes have been studied previously[1-4]. Ultrastructural aspects of spermatogenesis and oogenesis in *Marchantia polymorpha* have also been investigated[5-8]. Although sexual reproduction in *Marchantia* has been researched for more than a century, some questions remain unresolved. For example, how do sperm cells reach the female receptacle atop the elongated stalk. The present investigation revealed that the transport of sperm may be related to the structure of the stalk and female receptacle. The morphology and structure of *Marchantia polymorpha* gametophores are reported here in detail; these observations can help to understand the mechanism of sexual reproduction in this species.

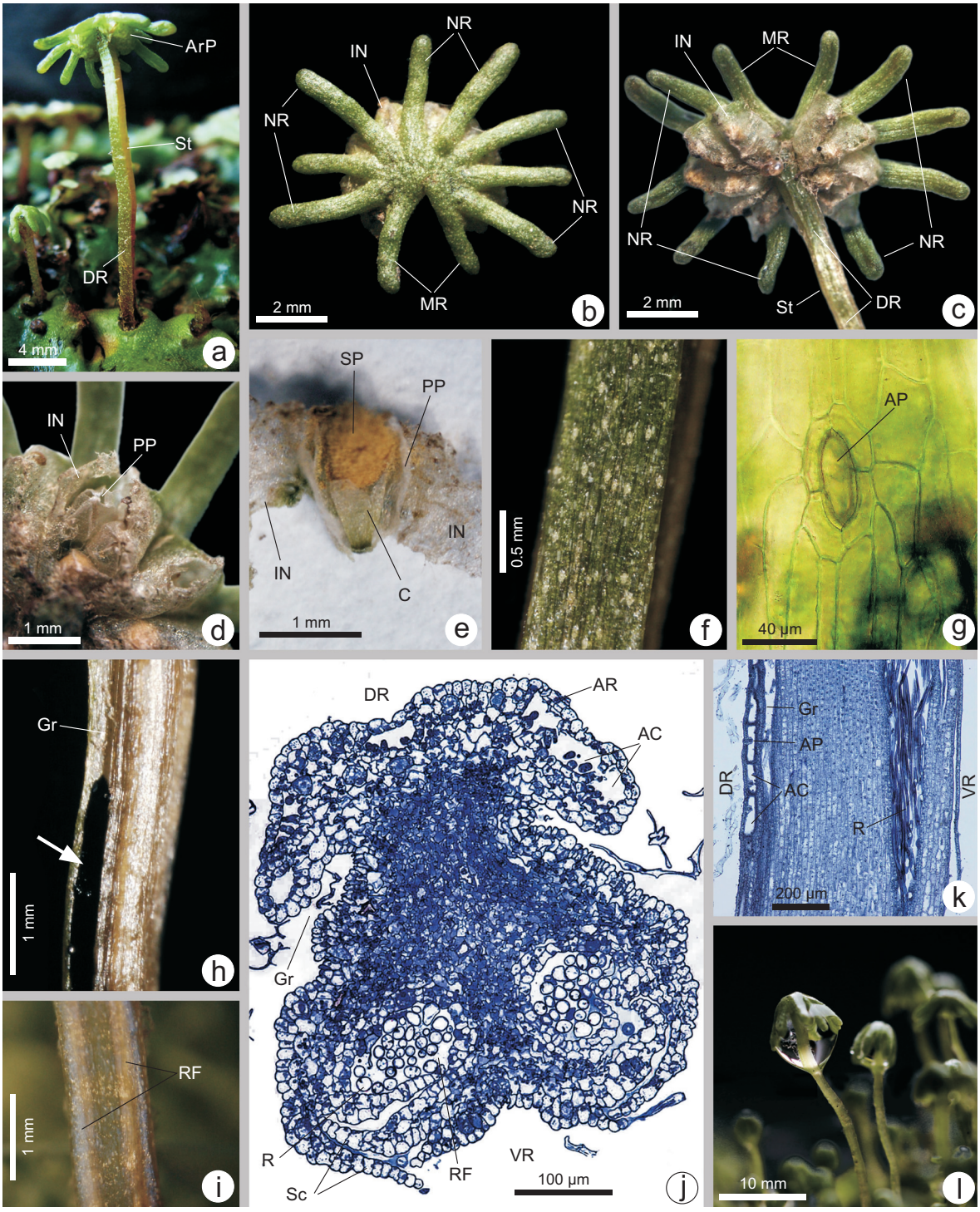
1 Materials and Methods

Living *Marchantia polymorpha* L. plants were collected from the botanical garden of the Shanghai Normal University. Gametophytes with male or female gametophores were cultured in a pot covered with plastic film on a shaded north-facing balcony. Young to mature gametophores were observed with a Nikon SMZ-1500 stereomicroscope and photographed for morphological investigation. For the structural (anatomical) investigation, the gametophores and stalks were cut and fixed with 3% glutaraldehyde in 0.1 mol/L phosphate buffer at room temperature for 6–12 h. The specimens were subsequently washed three times with the same buffer and fixed in osmic acid (2% aqueous solution) for 2 h and washed again with the same buffer three times. After dehydration in a graded acetone series, the specimens were infiltrated with a mixture of acetone and Spurr's resin (SPI-Chem, USA), and then embedded in pure resin. Specimens were thick sectioned with glass knives. The sections were stained with Toluidine blue, observed and photographed using a Nikon E-800 microscope.

2 Results

2.1 Archegoniophore

Archegoniophores are highly specialized reproductive organs with distinct dorso-ventral structure. The archegoniophore stalk is more or less square-shaped in appearance. A dorsal region, two lateral regions and a ventral region was distinguished clearly (Fig. 1: a, f, i, j; Fig. 2: a). The dorsal region facing towards the thallus was equivalent to the upper surface of the horizontal thallus. The ventral region facing away from the thallus was equivalent to the lower surface of the thallus (Fig. 1: a; Fig. 2: a). The umbrella-shaped female receptacle consisted of 9–11 rays, which were distinguished into two marginal rays and 7–9 normal rays (Fig. 1: b, c; Fig. 2: b). The two marginal rays faced towards the dorsal region of the stalk, and no involucre was produced between the two marginal rays (Fig. 1: b, c; Fig. 2: b). In contrast, involucres were produced between each of the adjacent pairs of normal rays other than the two marginal rays (Fig. 1: b, c; Fig. 2: b). Dissection of the mature receptacle showed that each involucre contained 3–4 pocket-shaped pseudoperianths (Fig. 1: d). Each pseudoperianth contained a sporophyte protected by an archegonial jacket (calyptra) when young, but the sporophyte extended out of the calyptra due to the elongation of the sporophyte stalk at maturity (Fig. 1: e, Fig. 2: c). Hence, the sporophyte was protected by the calyptra, pseudoperianth and involucre during maturation (Fig. 2: c). Although a group of archegonia arose in each involucre, only 2–3 sporophytes grew up.



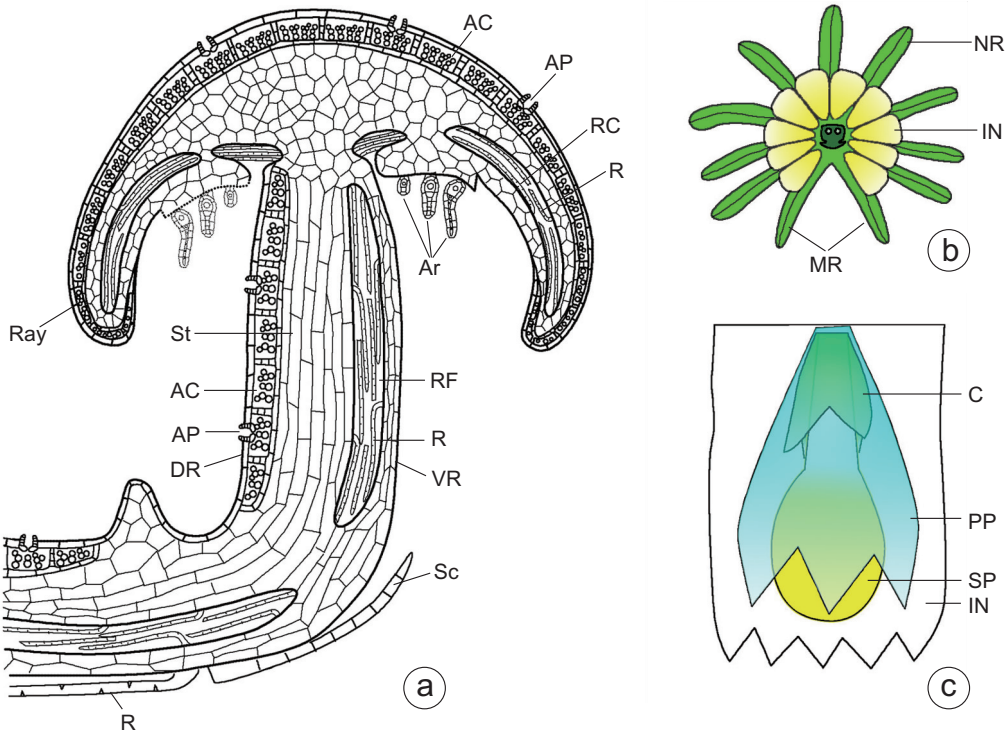
a. Archegoniophore (ArP) with a long stalk (St). Dorsal region (DR) of the stalk facing towards the thallus; **b**. Apical view of female receptacle showing two marginal rays (MR), normal rays (NR), and involucres (IN); **c**. Ventral view of archegoniophore, showing no involucre produced between the two margin rays. Upper surface of stalk (St) in dorsal region (DR); **d**. Involucre (IN) opening, and pseudoperianth (PP) seen inside; **e**. Dissection of involucre (IN), showing pseudoperianth (PP), calyptra (C), and sporophytes (SP); **f**. Dorsal region of stalk, showing green photosynthetic tissue; **g**. Air pore (AP) on upper epidermis of dorsal region; **h**. Lateral side of stalk, showing groove (Gr). Ink (arrow) flowed along the groove; **i**. Ventral region of stalk, showing two rhizoid furrows (RF); **j**. Transverse section of stalk, showing air chambers (AC), air pore (AP), and groove (Gr) in dorsal region (DR); rhizoid furrows (RF), scales (Sc), rhizoids (R) in ventral region (VR); **k**. Longitudinal section of stalk through dorsal region (DR) and ventral region (VR); l. Umbrella-shaped archegoniophore retaining large drop of water.

Fig. 1　Archegoniophore of *Marchantia polymorpha*

The dorsal region of the stalk was composed of photosynthetic tissue (Fig. 1: f). Air pores were seen in the upper epidermis of the dorsal region (Fig. 1: g). Transverse and longitudinal sections showed that the chambers and photosynthetic filament cells were formed inside. Each chamber possessed an air pore in the central part of the upper epidermis (Fig. 1: j, k). The side view of the stalk and transverse section showed that the photosynthetic tissue extended laterally and formed a narrow groove under the extended tissue (Fig. 1: h, j). Some multicellular hairs arose in the groove (Fig. 1: j). Adding black ink to the base of a stalk in a sloping position showed that the ink flowed upwards along the groove (Fig. 1: h, arrow). In the ventral region of the archegoniophore stalk, two rhizoid furrows were clearly recognized (Fig. 1: i). A transverse section of the stalk showed that the rhizoid furrow was not enclosed, but half-wrapped by overlapping scales (Fig. 1: j). The longitudinal section showed a distinct dorso-ventral differentiation of the stalk (Fig. 1: k). The rays of the female receptacle were also dorso-ventrally differentiated. Each ray was formed by a half enclosed thallus. The seam in the lower surface of the ray could be clearly seen (Fig. 1: c; Fig. 2: b).

2.2 Antheridiophore

The antheridiophore had a reddish elongated stalk and no distinct dorso-ventral appearance (Fig. 3: a). Magnified observations showed that the dorsal region possessed no green photosynthetic tissue or air chamber differentiation (Fig. 3: b, d). In the ventral region of the antheridiophore stalk, two rhizoid furrows could be clearly recognized by their light color (Fig. 3: c). The transverse section of the stalk showed that the rhizoid furrow was not yet enclosed, but wrapped by overlapping scales, and many rhizoids could be seen within it (Fig. 3: d, e). The disk-shaped



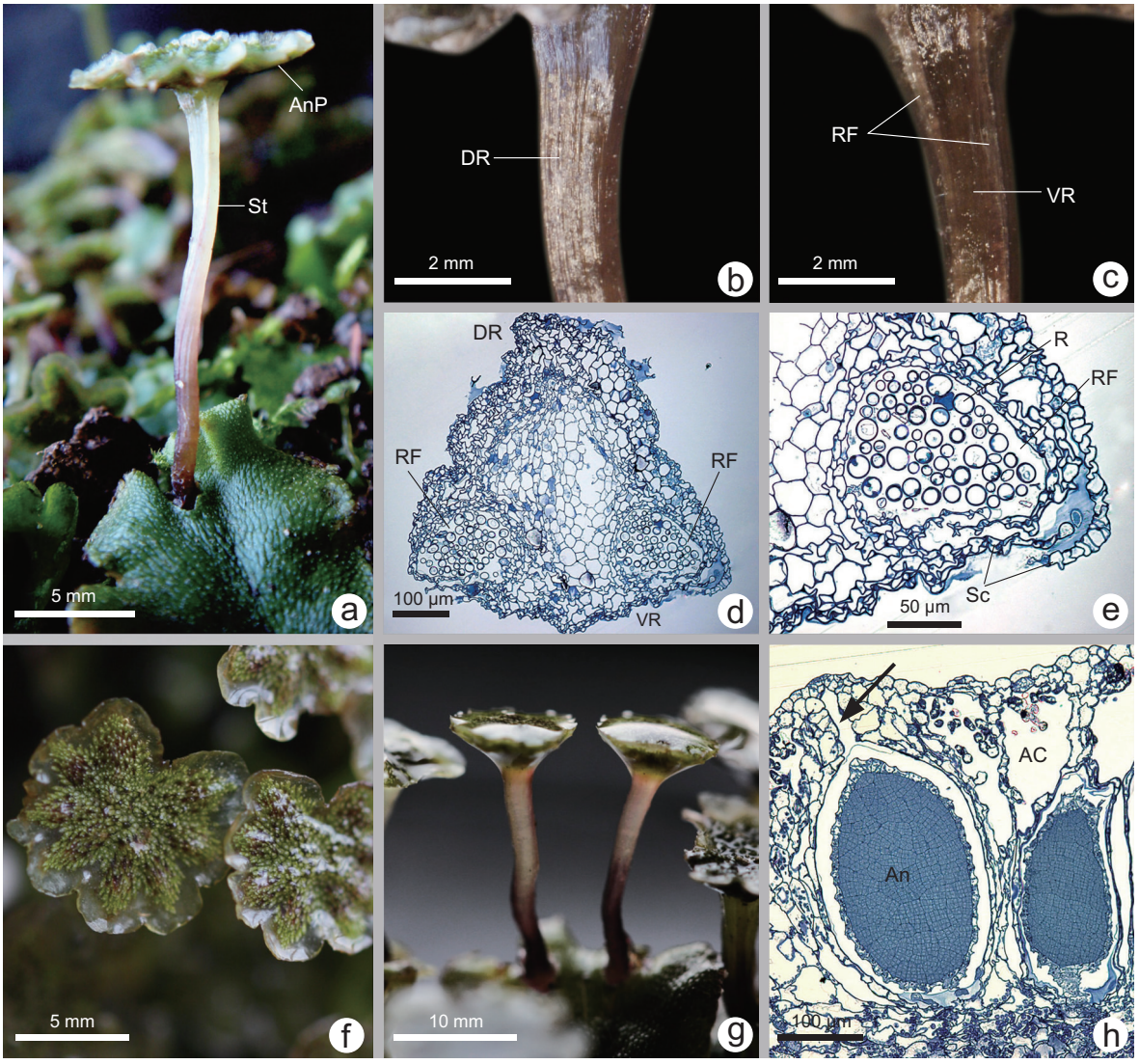
a. Archegoniophore with a long stalk (St), showing air chambers (AC), air pore (AP) in upper surface of the receptacle and dorsal region (DR) of the stalk; archegonia (Ar) at the base between the rays. Rhizoid furrow (RF) and rhizoids (R) in the stalk and rays, Sc, scale; **b**. Ventral view of the female receptacle showing the margin rays (MR), normal rays (NR) and involucres (IN); **c**. Involucre (IN), containing pseudoperianth (PP), calyptra (C), and sporophyte (SP).

Fig. 2 Schematic diagram of archegoniophore

male receptacle usually consisted of 6－8 lobes that resembled flower petals in appearance (Fig. 3：f). The slightly depressed upper surface of the male receptacle could hold rain water, which was favorable for sperm release (Fig. 3：g). The male receptacle consisted of many chambers, each of which contained an antheridium (Fig. 3：h). The matured antheridia released the sperm cells through the cap cell and the sperm travelled of the antheridial chamber through the channel (Fig. 3：h, arrow).

2.3　Observations on the fertilization process

To see whether the sperm could swim to the female receptacle up the elongated stalk, an unfertilized female receptacle was selected. (We chose a female plant with an archegoniophore, but with no male plants nearby). Water was then sprayed on the female plant to simulate rain. As a result, a large water drop was suspended under the female receptacle (Fig. 1：l). At the same time, we added a drop of water on the upper surface of the male receptacle (Fig. 3：g). If



a. Antheridiophore (AnP) with a long stalk (St); **b**. Dorsal region of stalk; **c**. Ventral region of stalk, showing two rhizoid furrows (RF); **d**. Transverse section of stalk, showing two rhizoid furrows (RF) in ventral region (VR); **e**. Magnification of part of Fig. d, showing rhizoid furrow (RF), scales (Sc) and rhizoids (R); **f**. Apical view of male receptacle; **g**. Slightly depressed upper surface of antheridiophore retaining water. **h**. Longitudinal section of antheridiophore, showing antheridia (An) in air chamber (AC); Arrow indicates opening of antheridium chamber.

Fig. 3　Antheridiophore of *Marchantia polymorpha*

there were mature antheridia in the male receptacle, sperm were rapidly released and formed a slightly whitish sperm suspension. The sperm suspension was absorbed with a pipette and added onto the base of archegoniophore stalk. Ten minutes later, a drop of water just under the female receptacle was absorbed with a new pipette and checked for sperm cells under a microscope. Results showed that the drop of water contained sperm, and we deduced that sperm can reach the female receptacle through continuous tiny water flow in the groove of the stalk.

3 Discussion

Although many studies on the structure and development of gametophyte, sex organs, embryos and sporophytes have been conducted on *Marchantia* species[1,7-15], the morphology and structure of the gametophores have not been described in detail previously. We investigated the morphology and structure of the female and male receptacles and their stalks, and helped to clarify the mechanism of sexual reproduction in *Marchantia polymorpha*.

3.1 Structure and function of the archegoniophore

The present investigation showed that the archegoniophore was a specialized upright branch of the thallus adapted for sexual reproduction. Although the stalk of the archegoniophore became upright, it possessed obvious dorso-ventral differentiation. The dorsal region of the stalk, with differentiation of photosynthetic tissue, was equivalent to the upper surface of the thallus. The ventral region of the gametophore stalk, with rhizoid furrows, was equivalent to the lower surface of the thallus midrib. Haupt[12] also determined that the gametophores in *Reboulia*, *Asterella* and *Conocephalum* represented specialized upright branches of the thallus. Based on the features, we suggested that the female receptacle rays were a palmately branched system of specialized thalli. The two marginal rays were, in fact, marginal branches of the palmate thallus. This explains why no female reproductive organs were formed between the two marginal rays. The heterogeneity of the rays in *Marchantia polymorpha* was first described here. This interpretation has important significance for understanding the function of the female gametangiophore.

Haupt[12] indicated that the female receptacle in *Marchantia* reached its greatest degree of specialization and that the archegonial receptacle consisted of rays alternating with groups of archegonia. However, the function of the specialized female receptacle was rarely mentioned or discussed. The present investigation showed that the umbrella-shaped receptacles had the ability to accumulate water (Fig. 1: l), which flowed slowly downwards along the elongated stalk. Microscopic observation and ink-water experimentation showed that the water flowed along the grooves along the two sides of the stalk (Fig. 1: h). It can be inferred that the specialized structure of the archegoniophore has an important function in sexual reproduction. The continuous tiny water flow provided a medium for sperm to swim to the archegonia in the female receptacle.

3.2 Structure and function of the antheridiophore

The morphology and structure of the antheridiophores differed greatly from those of the archegoniophores. The male receptacle possessed no rays, but rather had 6-8 shallower lobes. It had a slightly depressed upper surface, which could hold a little water and was favorable for sperm release. Although the male stalk was dorso-ventrally differentiated like the female stalk, there was no distinct difference between the dorsal and ventral surfaces in appearance. The male stalk had no green photosynthetic tissue in the dorsal region. In addition, no groove was formed in the male stalk. The structural features of the *Marchantia* antheridiophore were undoubtedly adapted for the release and transport of sperm. As the sperm

cells were released, they dropped rapidly to the soil and swam to the female receptacle.

3.3 Archegonia, sporophytes and the tissues outside of them

Haupt[12] also determined that female receptacles in *Reboulia*, *Asterella* and *Conocephalum* were commonly hemispherical or conical and more or less lobed. Each lobe represented a separate growing point from which either one or several archegonia arose. In *Marchantia*, the archegonial receptacle did not have lobes, but instead consisted of a number of rays. The present investigation showed that no archegonia were formed between the two marginal rays. Numerous multicellular hairs arose from between the two marginal rays when the female receptacle was young. The hairs undoubtedly enhanced the ability to retain water, which was advantageous for sperm reaching the archegonia.

The present observations showed that sporophyte development and involucre formation were as described in previous work[12,16]. The archegonia were naked before fertilization; and only paraphyses existed between archegonia. The naked archegonia and paraphyses were favorable for fertilization. After the egg was fertilized, the archegonial jacket developed into the calyptra, within which the embryo grows. Then, an additional envelope; i. e., the pseudoperianth, arose outside the fertilized archegonium. At the same time, an involucre arose around the archegonia. All these tissues served to protect the young sporophyte.

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