# Gametophyte Development in Adenophorus (Grammitidaceae)

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## ABSTRACT

Gametophytes of 5 species of *Adenophorus* (Grammitidaceae) were raised from spores in sterile culture. Three species represented the subgen. *Adenophorus*, the other 2 subgen. *Oligadenus*. Most of the salient developmental details conform to those found previously to be characteristic of the family. The species of subgen. *Oligadenus* are exceptional in lacking an initial, prolonged filamentous growth phase. Therefore, the individual gametophytes normally result in a single gametophytic plate. The consequence of this difference is considered in view of the overall reproductive strategies of the 2 subgenera.

## INTRODUCTION

Although the taxonomy of ferns has by long tradition been based primarily on the characters of the sporophyte, there is no special reason why the gametophyte should not also display characteristics of value in systematic syntheses. Various investigators have found such characters helpful in delimiting supraspecific categories (Atkinson & Stokey 1964, Atkinson 1973). Because the taxonomy and interspecific relationships of the Hawaiian genus *Adenophorus* have been worked out in some detail on the bases of morphology and anatomy (Bishop 1974), it seemed of value to study the gametophytes of several available species and to compare the results with previous observations in the family.

## MATERIALS AND METHODS

Spores of 5 species of *Adenophorus* were cultured: *A. tamariscinus*, *A. tripinnatifidus*, and *A. hymenophylloides* of the subgenus *Adenophorus*; *A. pinnatifidus* and *A. oahuensis* of the subgenus *Oligadenus*. Also, wild gametophytes of *A. tamariscinus* were found and studied. Spores were sown on agar plates (Klekowski 1969) and maintained under continuous, fluorescent illumination (ca. 140 foot-candles) at room temperature (ca. 22° C.). The cultures were observed microscopically at various time intervals, and representative specimens were mounted on slides for further study. The cultures were continued for 10 months.

## OBSERVATIONS

For this discussion *A. tripinnatifidus* has been selected as representative of the subgenus *Adenophorus*, *A. pinnatifidus* of *Oligadenus*. *Adenophorus tamariscinus* was found to be essentially identical in gametophyte development to the former, *A. oahuensis* to the latter. Details of *A. hymenophylloides* differential to those of *A. tripinnatifidus* will be mentioned where appropriate. The most typical (i.e., most frequent) developmental series has been used in either case for the illustrations (Figs. 1–2). Aberrations are explained in the text.

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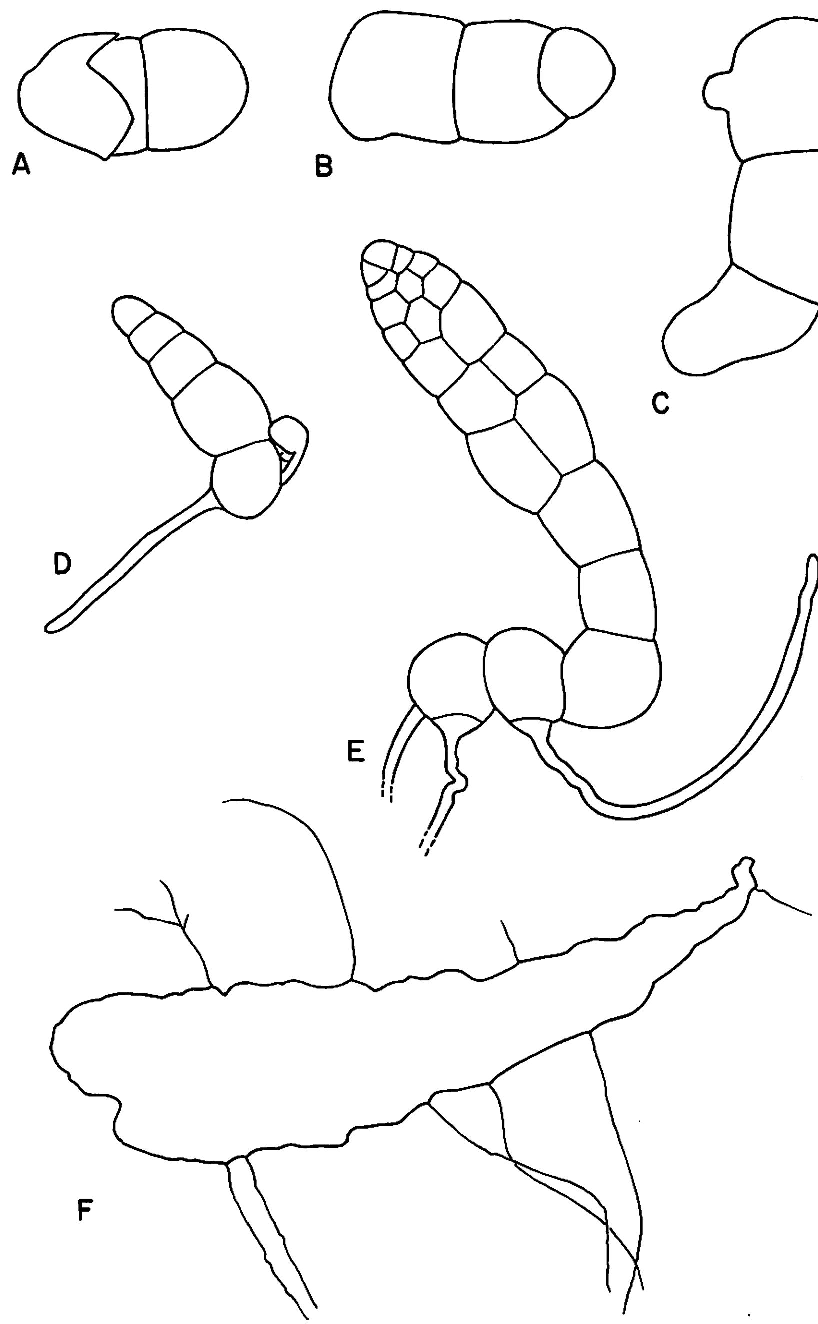


Fig. 1. Gametophyte development of *Adenophorus pinnatifidus*. **A,** 3 days. X385. **B,** 7 days. X385. **C,** 10 days. X385. **D,** 15 days. X210. **E,** 30 days. X210. **F,** 150 days. X50.

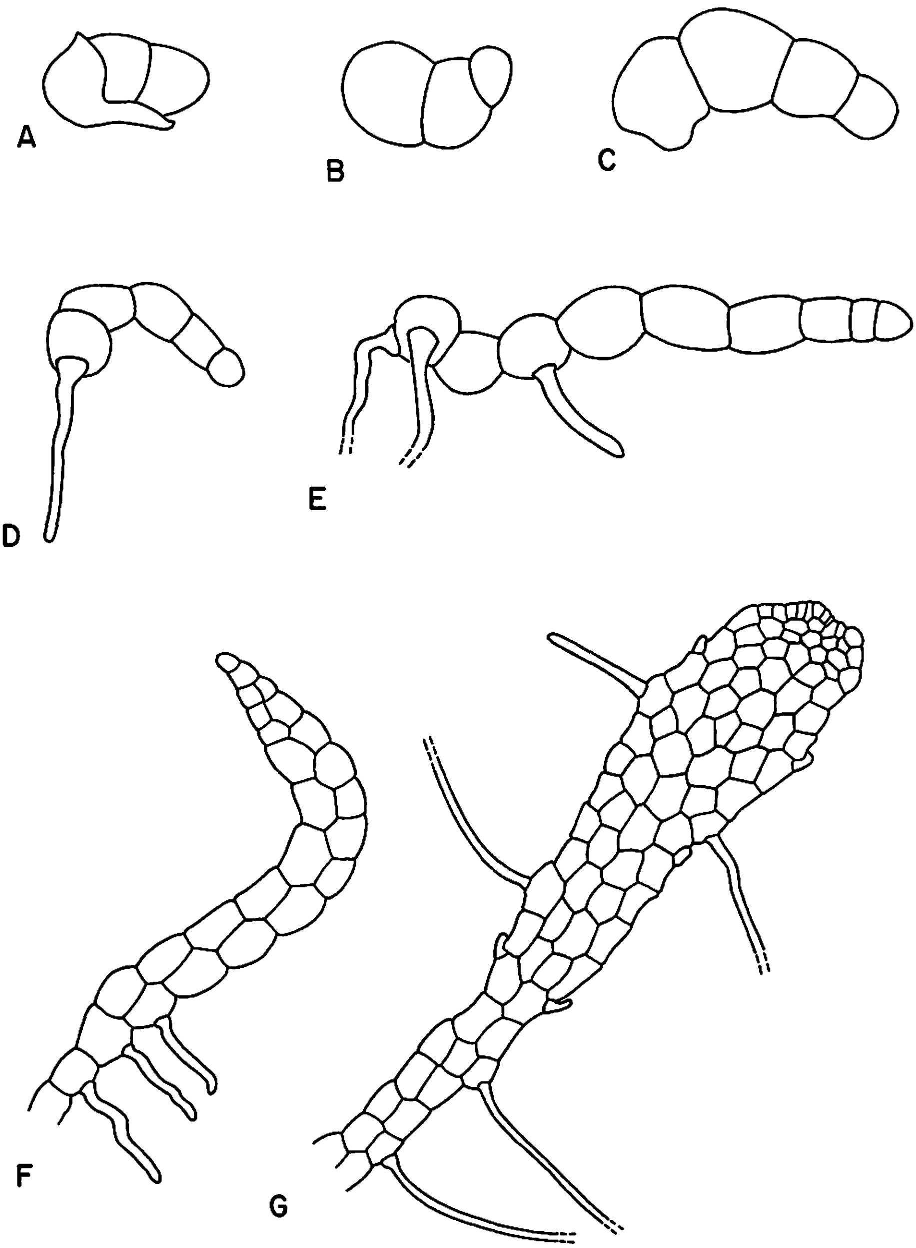


Fig. 2. Gametophyte development of *Adenophorus tripinnatifidus*. **A,** 3 days. X385. **B,** 7 days. X385. **C,** 10 days. X385. **D,** 15 days. X210. **E,** 30 days. X210. **F,** 80 days. X93. **G,** 110 days. X93.

The spores of *Adenophorus* are green, trilete, globose, and minutely sculptured. Germination begins on the 2nd day after sowing and is essentially complete by the 4th day. Immediately upon germination cell division occurs, resulting in 2 equal, green daughter cells, 1 of which—still partially retained within the spore wall—may be termed the basal cell. By the 7th day cell division has usually occurred again, normally by the unequal division (budding) of the apical cell only. Occasionally both cells divide, producing a young gametophyte with 2 growing points. In normal development the 4th cell is added apically by the 10th or 11th day, the 5th cell at the end of 2 weeks. The new cells formed by these divisions are usually not added rectilinearly but at slight angles to the more proximal cells, so that the young filament becomes distinctly arcuate. The initial cells of *A. pinnatifidus* are appreciably larger than those of *A. tripinnatifidus*. Species of subgenus *Oligadenus* exhibit larger spores than those of subgenus *Adenophorus*, so that these larger initial cells possibly reflect the larger spore size.

### Plate Development: Subgenus *Oligadenus*

The subsequent development of most gametophytes differs markedly in the 2 subgenera. Plate formation is usually evident in *A. pinnatifidus* by the 4th week. The 1st longitudinal divisions normally occur in the 1 or 2 subapical cells. As apical growth continues, the apical region also becomes longitudinally divided and at length constitutes a zone of meristematic cells. The gametophytic plate is at first spathulate with a rounded apex. Later the apex is truncate or emarginate. Lateral, unicellular trichomes are not present, but some of the older gametophytes exhibit conspicuous digitate, multicellular, uniseriate or biseriate projections from the margin (Figs. 3C, 3D). With the exception of the small percentage of spores that produce 2 (rarely 3) filaments from the basal cell, 1 spore results in a single gametophytic plate and therefore, presumably, a single sporophyte.

### Plate Development: Subgenus *Adenophorus*

At the end of 1 month most of the gametophytes of *A. tripinnatifidus* consist of a single filament of 8–12 cells. Some have initiated branch filaments by this time. A few show longitudinal cell divisions and thus plate initiation. Most, however, continue in a wholly filamentous stage for some time. Five-month-old gametophytes of *A. hymenophylloides* are small dense mats of much-branched, beadlike filaments (Fig. 3A). Comparable stages of *A. tripinnatifidus* are similar but somewhat less densely branched. Plate formation is evident in *A. tripinnatifidus* in some distal filaments of most gametophytes by the end of the 3rd month. The sequence of plate development is similar to that of *A. pinnatifidus* except that initially in many cases more cells of the uniseriate filament divide longitudinally, forming a longer biseriate plate stalk. The older plates exhibit marginal, unicellular trichomes that are distally directed. These are particularly conspicuous in *A. hymenophylloides* (Fig. 3B). The cells of the gametophytic plate are of approximately the same size in each subgenus (Figs. 4C, D). The filaments are fragile and easily fragmented. They appear collectively to undergo indeterminate growth, with individual filaments differentiating into plates from time to time.

Wild gametophytes of *A. tamariscinus* have been seen at 3 localities in the Koʻolau Mountains of Oʻahu: Poamoho, Kipapa Gulch, and Kahana Valley. Those at Poamoho and Kipapa Gulch were growing on the introduced *Leptospermum scoparium*, those at Kahana Valley on a necrotic trunk of *Metrosideros polymorpha*. These gametophytes were colonial and quite elongate (Fig. 4E). Many exhibited platelike outgrowths either on the lateral margin or apically (Fig. 4F). Very little cushion development was apparent around the young sporophytes, and none showed evidence of a midrib.

### Rhizoids, Gametangia, Leaf Development

In both subgenera, the 1st rhizoid of the young gametophyte regularly develops from the basal cell at about the 4-celled stage. Initially colorless, the rhizoid soon becomes dark brown

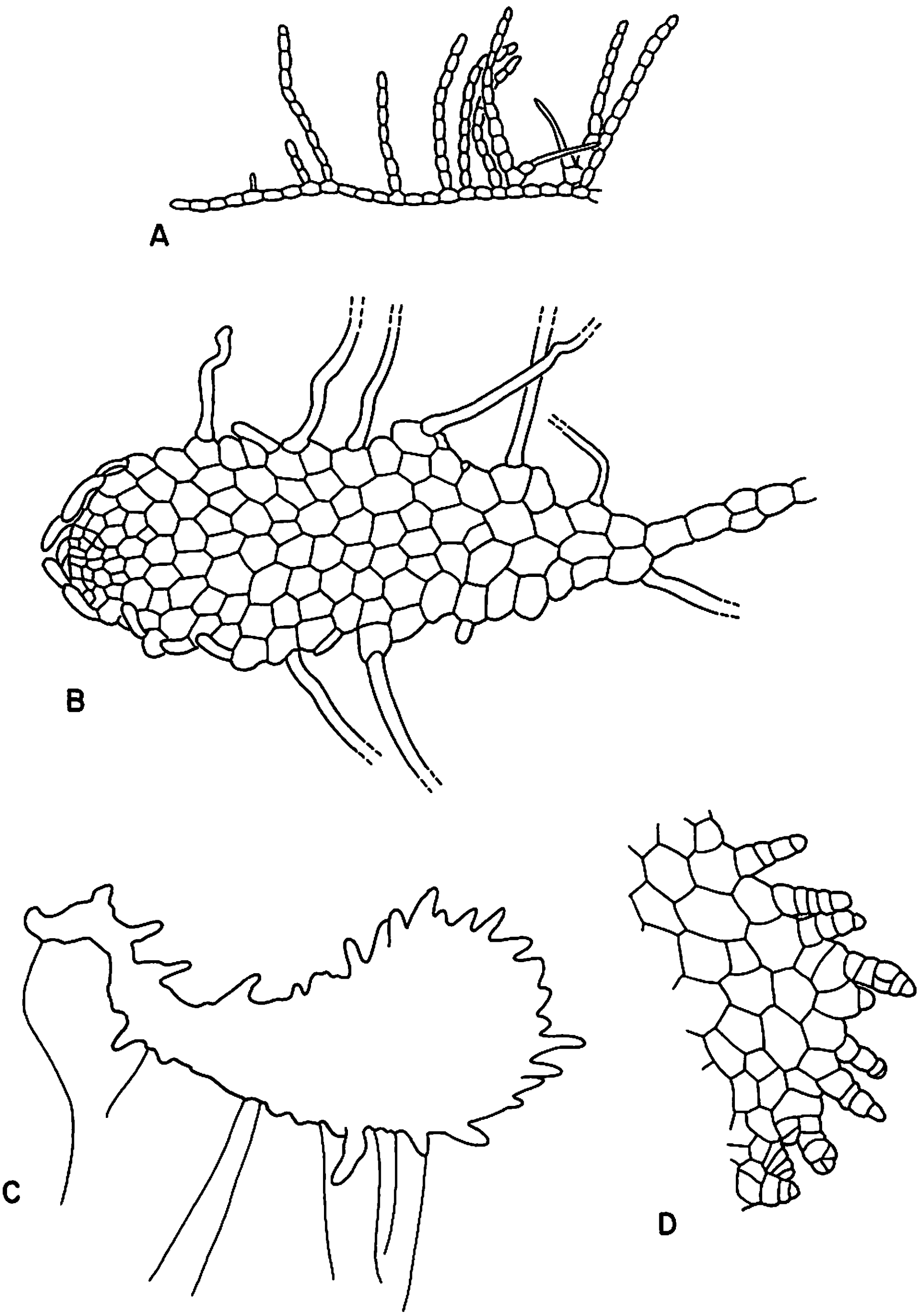


Fig. 3. Details of *Adenophorus* gametophytes. **A,** *A. hymenophylloides.* 150 days. X50. Portion of branched filament. **B,** *A. hymenophylloides.* 150 days. X93. Developed gametophyte plate. **C,** *A. pinnatifidus.* 150 days. X50. Gametophyte with digitate projections. **D,** detail of Fig. 3C. X93.

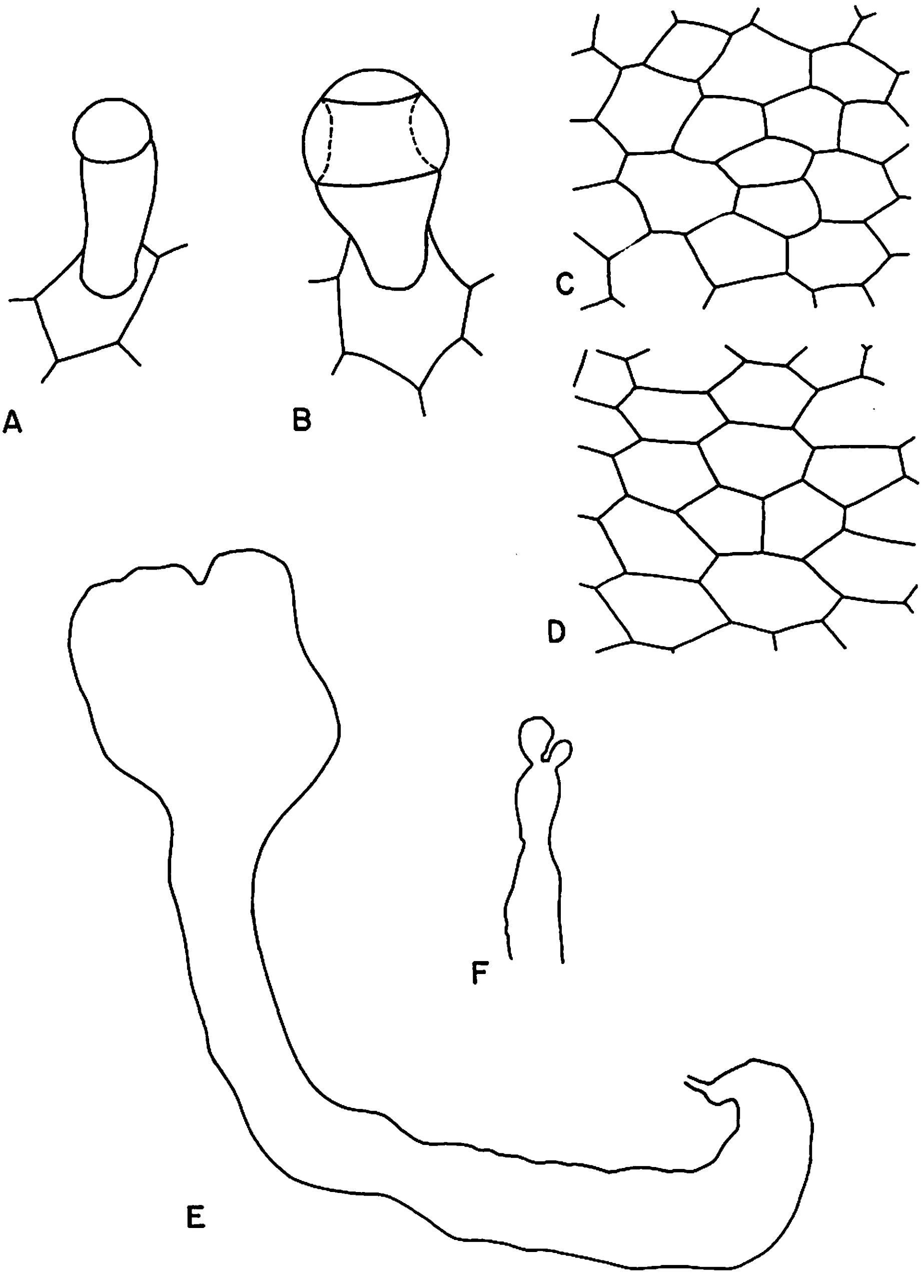


Fig. 4. Details of *Adenophorus* gametophytes. **A,** *A. pinnatifidus*. X385. Young antheridium. **B,** *A. pinnatifidus*. X385. Mature antheridium. **C,** *A. tripinnatifidus*. X210. Cells of gametophyte plate. **D,** *A. pinnatifidus*. X210. Cells of gametophyte plate. **E, F,** *A. tamariscinus*. X25. Wild gametophytes (rhizoids not shown).

and sharply delimited from the green subtending cell. Similar rhizoids later develop elsewhere on the filaments or marginally on the plate. Rarely they may be present on the ventral side of the plate. The rhizoids are usually simple, but occasionally are variously branched.

Antheridia appeared on cultured gametophytes of *A. pinnatifidus* after 4 months (Figs. 4A, B). These occur ventro-medially and are basally directed. The mature antheridium consists of an elongate stalk cell, a ring cell, a single cap cell, and the androgametic tissue. No antheridia were found in equally aged cultures of species of subgenus *Adenophorus*; however, wild gametophytes of *A. tamariscinus* bore antheridia that, although badly distorted by fixation, appeared to be essentially like those of *A. pinnatifidus*. Archegonia were not present on cultured gametophytes, nor were they discernible on the wild material.

The sequential events of frond development in *A. tamariscinus* were observed in the young sporophytes from Kipapa Gulch. The 1st leaf is simple, entire, spathulate, and 1.5–2.0 mm long. It is provided with laminar glands as in the adult leaf. The costa is simple or slightly forked apically. Usually the 2nd and sometimes the 3rd leaves are similar to, but slightly larger than, the 1st. Occasionally the 2nd or more generally the 3rd leaf is obliquely bilobed, and the vascular supply is similarly furcated. This leaf is ca. 3 mm long. Successive leaves are increasingly larger and have a progressively greater number of lateral lobes. The costa of the young, simply pinnatifid leaves is conspicuously flexuous. Three to 5 such leaves are developed. When a leaf attains a length of ca. 15 mm, some evidence of secondary lobing is displayed. The next leaf after this one is essentially bipinnatifid and successive fronds merely exhibit an increase in size and development.

## DISCUSSION

Stokey and Atkinson (1958) studied the gametophyte development in culture of 27 Jamaican species of the Grammitidaceae. Irregularities in details of growth were frequent in their work, both interspecifically and intraspecifically, but certain features were found to occur in all species and were considered characteristic of the family. These included: equal or subequal division of the spore cell; slow development of the gametophyte; a prolonged phase of filamentous growth; belated appearance of the 1st rhizoid; brown rhizoids, which on the plate are generally marginal; elongate-cordate gametophyte plate; and the long basal cell of the antheridia. All these characters are found in *Adenophorus*. However, the usual absence of an indeterminate filamentous growth phase found in the subgenus *Oligadenus* was not explicitly reported in any of the species studied by Stokey and Atkinson (1958). Moreover, the growth rate of *Adenophorus* species, although very slow in comparison to most ferns, was generally faster than in their material. This could be attributable to the continuous light under which my cultures were grown; Stokey and Atkinson do not state the culture conditions employed in their work.

At least with respect to the species studied, the gametophytes of the subgenera of *Adenophorus* differ in 2 major respects. (1) The plate of subgenus *Adenophorus* bears unicellular, usually marginal trichomes, which are absent in subgenus *Oligadenus*. These trichomes are reminiscent of the sessile, laminar glands characteristic of the sporophyte of the former subgenus. (2) Gametophytes of subgenus *Oligadenus* usually produce a single gametophytic plate; those of subgenus *Adenophorus*, by virtue of their indeterminate filamentous phase, may produce many. On considering the reproductive biology of the various species, this latter difference seems to be correlated with the presence of vegetative root buds in subgenus *Oligadenus* and their absence in subgenus *Adenophorus*. Green spores have a significantly curtailed period of viability when compared to nongreen spores (Lloyd & Klekowski 1970), so that whatever the adaptive advantage of chlorophyllous spores may be, the incidence of successful diasporal dispersal must be reduced. The production of several to many sporophytes from a single instance of successful spore germination could be interpreted as providing a means of reproductive compensation, especially in species whose individual plants—and therefore spore output—are small. I find it remarkable that such compensatory mechanisms are exhibited in the genus *Adenophorus* in 1 subgenus by the gametophyte and in the other by the sporophyte.

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