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GAMETOPHYTES AND SUBGENERIC CONCEPTS IN LYCOPODIUM[1]

JAMES G. BRUCE
Department of Botany, University of Michigan, Ann Arbor 48109

ABSTRACT

A critique of the Freeberg and Wetmore work on cultured *Lycopodium* gametophytes of *L. selago, L. flabelliforme,* and *L. cernuum* is presented. All three gametophytes are shown actually to be *L. cernuum* based on morphological and anatomical features of their sporophytes. A reassessment of characters in the genus demonstrates the taxonomic validity of the three groups proposed as subgenera within *Lycopodium.*

CONSIDERABLE DISAGREEMENT has been generated over the generic taxonomy of *Lycopodium*, the clubmosses. A number of separate genera have been proposed even within the last twenty years (Holub, 1964; Vasconcellos and Franco, 1967). Other authors have argued forcefully for the traditional maintainance of a single genus (Wilce, 1972), and the situation is still fluid.

One of the more important pieces of taxonomic evidence has been the remarkable diversity of form discovered in the gametophytes of *Lycopodium.* Bruchmann (1898), in his monumental study of *Lycopodium* gametophytes, erected five categories based on the diversity of types found by him and others. The various types are represented by particular species, *L. clavatum* (Type 1), *L. complanatum* (Type 2), *L. selago* (Type 3), *L. inundatum* (Type 4), and *L. phlegmaria* (Type 5). Bruchmann (1898) further proposed that five genera be named to correspond with this diversity, but he did not name these. Unfortunately, only a few species of *Lycopodium* are known from their gametophytes so that an assignment to a taxonomic unit based solely upon the type of gametophyte is impractical. However, it is interesting to note that all gametophytes described since Bruchmann's (1898) work have in fact fitted into one or the other of his categories.

Later workers (Rothmaler, 1944; Boivin, 1950) found the different gametophytic types to be taxonomically useful. In fact, Rothmaler (1944) used them in part in his recognition of four genera, *Lycopodium* (Type 1 gametophyte), *Diphasium* (Type 2), *Lepidotis* (*Lycopodiella*) (Type 4), and *Huperzia* (Types 3, 5), and this treatment was later adopted in the Flora Europaea.

However, the gametophytic evidence was called into question by subsequent laboratory studies. The remarkable report by Freeberg and Wetmore (1957) that gametophytes of certain *Lycopodium* species, when grown under identical cultural conditions, became strikingly similar to each other has been particularly disquieting. They purportedly found that in the absence of any associated endophytic fungi the cultured gametophytes of what they designated as "*L. complanatum* var. *flabelliforme*" and "*L. selago*" were surficial, photosynthetic, and morphologically almost indistinguishable from both the wild and cultured gametophytes of *L. cernuum.* This fact has been used to suggest that gametophytic form is simply a consequence of growth conditions, and that the remarkable diversity of form is therefore of little systematic value. As Wagner (1974) points out, "If the distinctive gametophytes of two lycopod 'genera' can be grown artificially under conditions that make them develop into the gametophyte-type of a third 'genus,' then one may well question the value of the gametophyte in the taxonomy of clubmosses."

Recently Whittier (1975) studied the gametophytes of *Lyocopdium obscurum.* He was successful in culturing these saprophytic gametophytes in a medium free of their endophytic fungi. These gametophytes, however, retained their normal morphology in spite of absence of the fungi. He placed some of the cultures in the dark and others in the light. Only the spores in culture which were kept in the dark germinated and produced gametophytes. Upon exposure to light the gametophytes, which were initially cultured in the dark, became pale green after several months. No concomitant change in morphology was reported. The largest gametophyte grown by Whittier was reasonably typical of wild *L. obscurum* as it possessed a thickened, disk shape. Although Whittier's work (1975) does not test directly the conclusions of Freeberg and Wetmore (1957) and Freeberg (1957), his findings suggest strongly the need to reevaluate their findings.

Several features of the Freeberg-Wetmore work are quite unexpected, and I list these below. (*Lycopodium* taxonomy adopted here is based on

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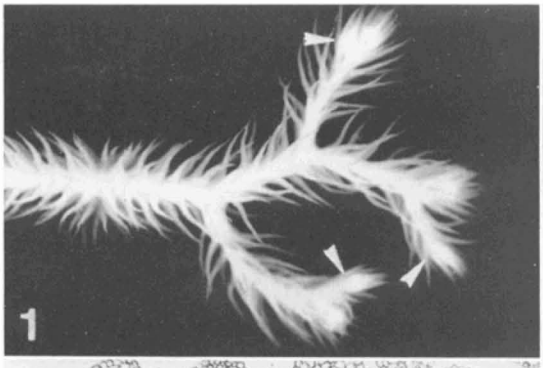
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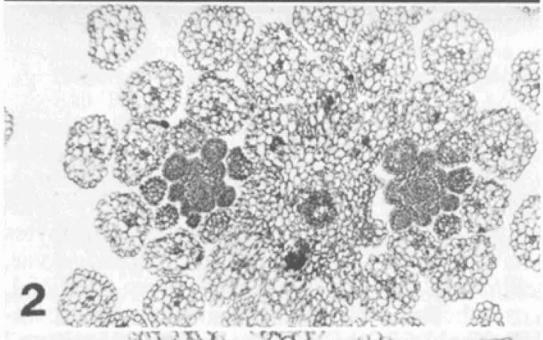
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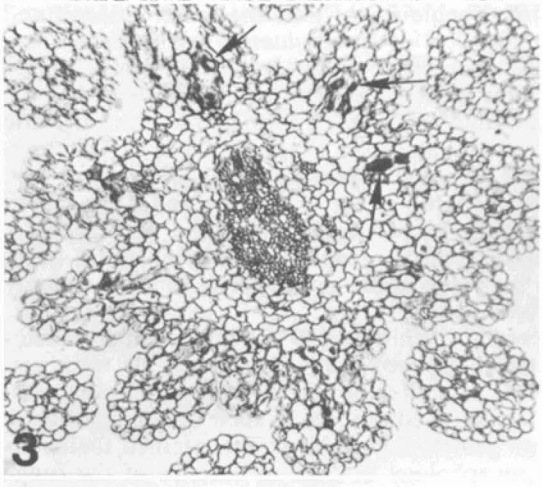
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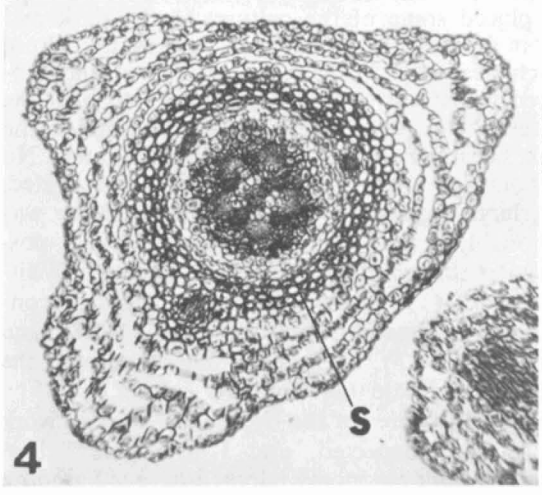
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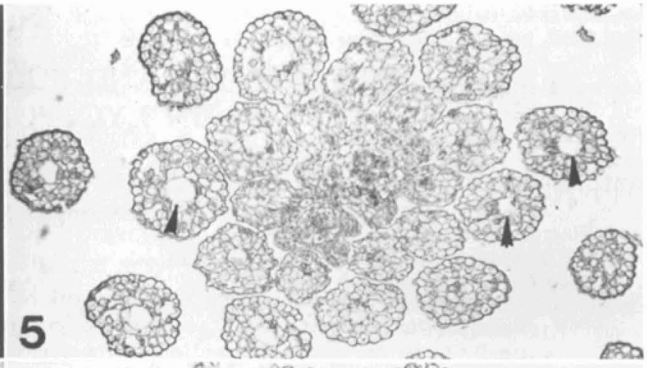
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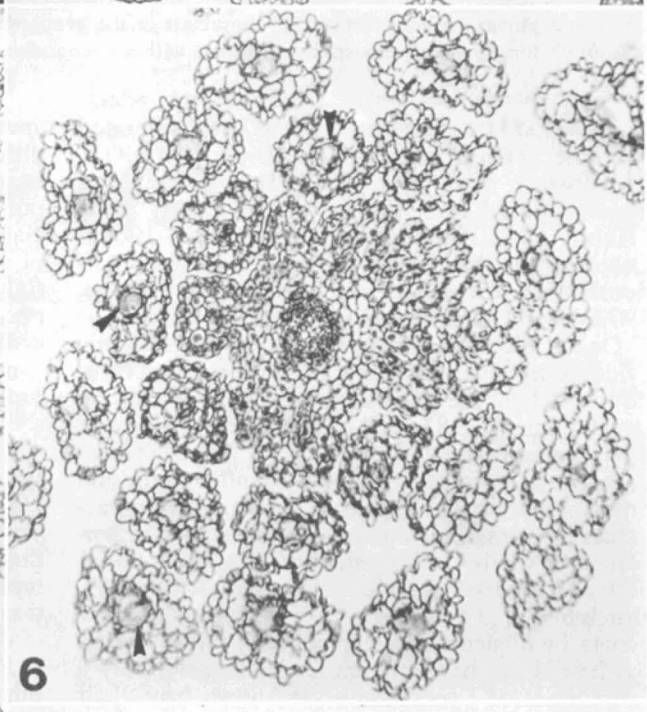
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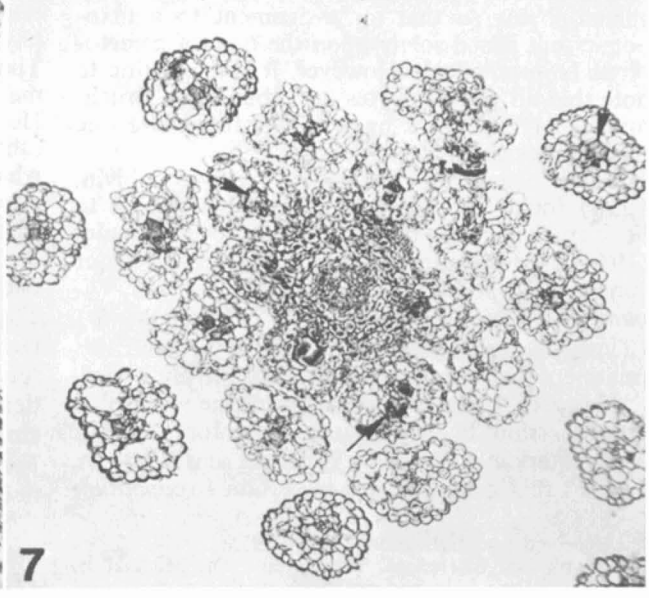
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Wilce, 1972, viz., subgenus *Lycopodium* including *L. complanatum* var. *flabelliforme*, referred to as *L. flabelliforme* below, subgenus *Lepidotis* including *L. cernuum*, and sugenus *Urostachys* including *L. selago*.)

GAMETOPHYTE

1. The general organography of the reported cultured gametophytes of both *L. selago* and *L. flabelliforme* is similar to both cultured and wild *L. cernuum*, but is different from wild *L. selago* (Bruchmann, 1898) and wild *L. flabelliforme*.[1] The overall form as well as the production of multicellular lobes in and around where the sex organs are produced is quite like the situation found in *L. cernuum* as described by Treub (1884).

2. *L. selago* may be green and surficial in the wild condition as shown by Bruchmann (1898) and, when it is, it does not produce the distinctive multicellular lobes reported in the cultured material.

3. The well-defined meristem present in the wild gametophytes of *L. selago* and *L. flabelliforme* is absent in the cultured specimens of these two species. *L. cernuum* lacks such a meristem in the wild state as well as in culture.

4. Short-necked archegonia are characteristic of subgenus *Lepidotis* which includes *L. cernuum*. The details of the presumed *L. flabelliforme* archegonium (Freeberg and Wetmore, 1957) show the outer tiers of neck cells sloughed off. This condition is similar to the condition described for *L. cernuum* (Treub, 1884) and *L. inundatum* (Goebel, 1887; Bruce, 1972). Indeed, the resemblance is strengthened by the presence of thickening in the walls of the lower tier of neck cells apparent in the photograph of the presumed *L. flabelliforme* archegonium. This is pointed out specifically by Treub (1884) for *L. cernuum*, and this condition can also be observed in *L. inundatum* (Bruce, 1972). It is not known, however, to occur in *L. selago* or in any of the described materials of *L. complanatum*.

5. Freeberg and Wetmore (1957) did not note the occurrence of multicellular, uniseriate paraphyses in cultured *L. selago*. These structures—unknown in other lycopods—are diagnostic for all known gametophytes of subgenus *Urostachys*, which includes *L. selago*.

SPOROPHYTE

1. The aciculate leaves of all three cultivated taxa are closely similar to wild *L. cernuum* and unlike the juvenile leaves of *L. selago* and *L. flabelliforme*, both of which have flatter, more blade-like laminae.

2. Young sporophytes of what was identified as *L. flabelliforme*, even when they were maintained in culture for a considerable period of time, never developed the reduced leaves characteristic of wild sporophytes.

3. In Freeberg's (1957) interpretation both *L. selago* and *L. flabelliforme* were believed to produce conspicuous protocorms in culture. Such embryonic structures as far as we know are restricted, however, to the subgenus *Lepidotis* in the wild state and, except for a reference to a "reduced protocorm" in *L. phlegmaria* by Treub (1886), they are unknown in subgenus *Urostachys* or subgenus *Lycopodium*. Even in *L. phlegmaria*, however, the situation was exceptional, and may have represented simply an anomaly.

Freeberg and Wetmore (1957) and Freeberg (1957) interpreted most of these unexpected results as consequences of the absence of the endophytic fungi and growth of the gametophytes in light. I would like to suggest an alternative hypothesis which can account for the observed changes in a more parsimonious manner. Simply stated, my conclusion is that all materials successfully cultured by Freeberg and Wetmore were actually *L. cernuum*.[2] This obviates the elaborate explana-

[1] Structural details of the wild gametophytes of *L. flabelliforme* have not been described in the literature. The gametophytes are, however, similar to those described for the very closely related and often synonymized *L. complanatum*. Indeed, Wilce (1965) has suggested that some descriptions of gametophytes may have been erroneously attributed to *L. complanatum* and may actually be *L. flabelliforme* or *L. tristachyum*. One specimen from Massachusetts, which I have examined, is very probably *L. flabelliforme* because it was associated with a colony of *L. flabelliforme*; *L. tristachyum* was not in that area, and, as Wilce (1965) states, *L. complanatum* is unknown from Massachusetts.

[2] Freeberg and Wetmore (1957) also cultured *L. obscurum* but the gametophytes did not grow beyond two or three millimeters in size and no sex organs were produced.

←

Fig. 1–7. **1.** Young sporophyte of cultured *L. "selago"* which shows anisodichotomous branching (arrows). × 4. **2.** Transection of cultured *L. "selago"* which illustrates two anisodichotomous branches (densely stained axes to the right and left of the main central axis). × 17. **3.** Transection of cultured *L. "selago"* which clearly shows mucilage canals in basal portions of leaves and outer stem cortex (arrows). × 30. **4.** Transection of first sporophytic axis of young, wild *L. flabelliforme* with conspicuous sclerenchymatous band (S) and no veinal mucilage canals. × 32. **5.** Transection of mature branch of small, wild *L. cernuum* which shows veinal mucilage canals (arrows). Comparison of this figure with 2, 3, 6, and 7 shows remarkable similarity between the three cultured "species" and wild *L. cernuum*. × 30. **6.** Transection of cultured *L. cernuum*, veinal canals (arrows) easily apparent. × 32. **7.** Transection of cultured *L. "flabelliforme"* which shows veinal mucilage canals (arrows). × 25.

tions required by the several seemingly extraordinary departures from wild-type morphology for the saprophytic species. Essentially all anomalous features of the cultured plants thought to be *L. selago* and *L. flabelliforme* are resolved by assuming that they are in fact actually *L. cernuum*.

MATERIALS AND METHODS—Dr. Freeberg has been so kind as to supply sporophytic materials from his experiments of all three species and gametophytic materials of two species, *L. flabelliforme* and *L. cernuum*. These had been preserved in FPA and were sent in three separately labeled vials. Specimens were photographed, and portions of the sporophytes were embedded and sectioned using standard paraffin techniques. Additionally, juvenile sporophytes and gametophytes of both *L. flabelliforme* and *L. lucidulum* were obtained through the generosity of Dr. George J. Wilder, and Dr. Warren H. Wagner kindly provided juvenile sporophytic plants of *L. cernuum*.

RESULTS—Isodichotomous branching, in which the shoot divides into two equal axes, characterizes the subgenus *Urostachys*. This produces the very symmetrically forked plants of *L. selago* and its relatives. Anisodichotomous branching, where one of the two branch shoots overtops the other, is restricted to subgenus *Lepidotis* and subgenus *Lycopodium*. However, in the photograph of one of the sporophytes of *L. "selago"* from culture, anisodichtomous branching can be seen (Fig. 1). In addition, in the sectioned material of cultured *L. "selago"* two anisodichotomous branchings are immediately apparent (Fig. 2).

In the course of studying certain anatomical features of *Lycopodium*, mucilage canals of two distinct types were found in various members of the genus (Bruce, 1976). One type, the basal canal, is restricted to the strobilus where it occurs in the sporophylls and outer cortex of the strobilar axis. This type is found in both subgenus *Lepidotis* and subgenus *Lycopodium*. The other type, the veinal canal, occurs in both sporophylls and vegetative leaves where it lies adjacent to the abaxial side of the vein. This type is known only from subgenus *Lepidotis*. Subgenus *Urostachys* was found to lack any sort of mucilage canals.

Thus, the vegetative leaves of both subgenus *Urostachys*, which includes *L. selago*, and subgenus *Lycopodium*, which includes *L. flabelliforme*, lack mucilage canals. This finding meant that the alternative hypothesis of contamination by *L. cernuum* in the Freeberg and Wetmore (1957) work could be tested directly.

All three of the cultured "species" were found to possess veinal mucilage canals associated with the vegetative leaves (Fig. 3, 6, 7). This is a characteristic of the sporophytes in subgenus *Lepidotis* which includes *L. cernuum*. I have also examined wild materials of mature *L. selago* as well as juvenile wild materials of *L. lucidulum*, a close relative of *L. selago*, and found no mucilage canals. Additionally, juvenile wild materials of *L. cernuum* possess conspicuous mucilage canals. Finally, juvenile wild material of *L. flabelliforme* showed no mucilage canals (Fig. 4).

Examination of the juvenile wild plant of *L. flabelliforme* from Massachusetts shows a conspicuous sclerenchymatous band in cross-section (Fig. 4). This band lies just outside of the stele much as is found in mature plants of *L. flabelliforme* (Roberts and Herty, 1934). This band is absent from the cultured *L. "flabelliforme"* (Fig. 7). Comparison of the sections of the three cultured "species" (Fig. 3, 6, 7) with a section of a smaller branch of wild mature *L. cernuum* (Fig. 5), however, reveals that the three cultured "species" are indistinguishable from the wild *L. cernuum*.

DISCUSSION—The present information concerning both histological features and branching characters of the sporophytes, combined with the points raised earlier, strongly suggests contamination of the *L. selago* and *L. flabelliforme* cultures with the spores of *L. cernuum*. The possibility of a contaminant other than *L. cernuum* was considered highly improbable because the cultured *L. cernuum* attained reproductive maturity and the other two species closely resembled it.

It is still possible that *Lycopodium* gametophytes may extensively alter their morphology in the absence of their endophytic fungi or when grown under unusual light regimes. However, this is not yet demonstrated to be the case, as the basis for this conclusion is shown to be untenable. Unless future work contradicts this the gametophyte continues, therefore, to be a valuable source of systematic data in formulating the taxonomy of *Lycopodium*. It can no longer be assumed that the form of a gametophyte is simply an ecological manifestation resulting from the level to which its spore is dispersed in the soil prior to germination (Freeberg and Wetmore, 1957).

With this in mind, the number of features of value in delimiting groups in *Lycopodium* is considerably increased. Rothmaler (1944), Chowdhury (1937), Boivin (1950), Wilce (1972), Øllgaard (1975), Bruce (1976), among others, have contributed useful information. This and other information is summarized in Table 1. Some of these features are based on very few species and may require future revision. These data, in my opinion, warrant the recognition of these three subgenera of *Lycopodium*. All are distinct, although subgenus *Lepidotis* and subgenus *Lycopodium* are more similar to each other than either is to the subgenus *Urostachys*. The characters involved are both sporophytic and gametophytic and include features of both vegetative and reproductive organs. In fact, nearly every aspect of the

TABLE 1. *Summary of subgeneric characteristics in Lycopodium*

Character	Subg. *Lycopodium*	Subg. *Lepidotis*	Subg. *Urostachys*
SPOROPHYTE			
Major branching	Anisodichotomous	Anisodichotomous	Isodichotomous
Shoot type	Horizontal indeterminate and upright determinate	Horizontal indeterminate and upright determinate	Either all indeterminate or all determinate
Root emergence	Direct	Direct	Long, descending in cortex
Root stele at origin	Polyarch	C-shaped	C-shaped
Root hair initial division	Oblique	Straight?	Oblique
Sporophyll blade attachment[a]	Pseudopeltate	Pseudopeltate	Sessile or nearly so
Sporophyll stalk	Present	Present	Absent or nearly so
Sporangial stalk	Thick	Thick	Thin?
Sporangial position	On sporophyll stalk	Axillary or on sporophyll stalk	Axillary
Sinuosity of side walls of sporangial cells.	Sinuate	Straight	Sinuate
Thickness of side walls of sporangial cells	Thin	Thin	Thick
Lignification of side walls of sporangial cells	Lignified	Unlignified	Lignified
Veinal mucilage canals	Absent	Present or absent	Absent
Basal mucilage canals[a]	Present	Present	Absent
GAMETOPHYTE			
Shape	Carrot or convoluted button	Tuberous with multicellular lobes	Cylindrical with radial or bilateral symmetry
Nutrition	Holosaprophytic	Hemisaprophytic	Holosaprophytic
Position in substrate	Below surface	Surficial	Below surface
Archegonial length	Long	Short	Long
Archegonial neck longevity	Persistent	Ephemeral	Persistent
Wall of basal tier of neck cells	Thin	Thick	Thin
Paraphyses	Absent	Absent	Present
CHROMOSOMES			
Number	$x = 23, 34$	$x = 35, 78$	$x = ?\ 44, 45$
SPORES			
Surface ornamentation	Reticulate	Rugulate	Foveolate-fossulate

[a] The pseudopeltate condition of the sporophyll and the basal mucilage canal are probably different expressions of the same character (Bruce, 1976).

life cycle is taxonomically important. It is interesting in this regard to draw attention to the discussion by Sneath and Sokal (1973) of classifications based on different portions of life cycles of organisms. They have suggested that similarity or congruence between classifications of this sort will be greater the higher the rank of the taxa involved. In *Lycopodium*, where a broad array of characters from each of the stages of the life cycle supports the division of the genus into three units, a taxonomic elevation of the subgenera to generic rank would appear reasonable. However, Wilce (1972, p. 79) has argued strongly for the maintainence of a single genus based largely on the ease with which the group is recognized as "a relatively non-arbitrary clustering of related elements." This, perhaps, is the best and most practical approach at present, not only for the reasons given, but also because of the considerable amount of new information of taxonomic importance in *Lycopodium* which has become available only recently. Much of this information will need to be digested and evaluated in order to arrive at a consensus. Taxonomic arguments have the peculiar distinction in science in that they are based in part on judgement and tradition, and an opinion which

reflects thoughtful consideration of both the facts and historical perspective is the one most likely to survive.

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