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Morphology and development of floral shoots and organs in certain Zannichelliaceae

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Development of reproductive shoots and associated organs in *Vleisia aschersoniana, Althenia filiformis, Lepilaena bilocularis* and *L. cylindrocarpa* (Zannichelliaceae, *sensu* Dumortier) has been examined using an epi-illumination technique to provide photographic documentation. Floral shoots share a similar basic developmental pattern. The vegetative shoot is terminated by a unisexual flower, but growth is continued from the axils of leaves immediately below so that fairly regular sympodia develop. *Vleisia* is most variable in the expression of this pattern. The flowers are simple, consisting of a single stamen or three carpels (one in *Vleisia*) and show marked similarity in development. Short scale-like appendages, reminiscent of a perianth, develop at the base of the stamen in *Althenia* and *Lepilaena.* An outgrowth at the tip of the connective in *Lepilaena bilocularis* and two at its middle part in *Vleisia* are initiated at late stages of stamen development. Carpels are subtended by membranous tepal-like appendages that are initiated at the same time as the carpel primordia. Each carpel primordium becomes peltate and develops a bitegmic ovule on the adaxial portion of the carpel wall which in turn overgrows the ovule and ultimately forms a long thin style with either a funnel-shaped (*Vleisia, L. cylindrocarpa*), peltate (*L. bilocularis*) or feathery (*Althenia*) stigma. Relationships with other Alismatales are discussed.

KEY WORDS: — Alismatiales — Zannichelliaceae — *Althenia* — *Lepilaena* — *Najas* — *Ruppia* — *Vleisia* — floral morphology — morphogenesis.

CONTENTS

INTRODUCTION

The Alismatales (Helobiae) has long been recognized as a rather heterogeneous group of aquatic monocotyledons, but with certain aggregations which seem more natural. A morphological transitional series between the Potamogetonaceae (*Potamogeton*) and Najadaceae (*Najas*), via *Ruppia* and *Zannichellia,* based primarily on features of floral development has been proposed recently by Posluszny & Sattler (1976a). Limited previous work on floral anatomy (Uhl, 1947; Singh, 1965) supports this morphological series but there are evident points of discontinuity. Furthermore, an approach which is admittedly typological and based on limited samples does omit consideration of variation. For example, although the flower of *Zannichellia palustris* does share some developmental characteristics with *Potamogeton* and *Ruppia,* its simplicity and the separation of sexes make the suggested links very tenuous. In addition, since there are other genera in the Zannichelliaceae, comprehensive comparative statements cannot be made with conviction in the absence of information about floral morphology in these taxa also. With this objective in mind, we present information for four other species within the Zannichelliaceae (*Vleisia aschersoniana, Althenia filiformis, Lelipaena bilocularis* and *L. cylindrocarpa*). Together with the previous study of *Zannichellia palustris* (Posluszny & Sattler, 1976a) we now have comparative information for all four genera in Zannichelliaceae *sensu* Dumortier (Tomlinson & Posluszny, 1976), which allows a discussion of their relationship to each other and to related members of the Alismatales.

The plants examined grow totally submerged and have thread-like stems, narrow linear leaves and unbranched roots. Pollen is globose and pollination hydrophyllous. Though there has been much discussion as to whether we are dealing with 'flowers' or much reduced 'inflorescences' in this family (Uhl, 1947; Sculthorpe, 1967) the term flower is used in its non-interpretative sense for the very simple unisexual units, composed of a single stamen with various combinations of pairs of one to four microsporangia in the male and one, three or more carpels in the female.

MATERIALS AND METHODS

All the material examined was supplied as fluid-preserved specimens by various correspondents and from various sources listed below:

Vleisia aschersoniana (Graebner) Tomlinson & Posluszny: Loc River, running into Table Bay, near Milnerton, South Africa. (Mrs P. Reinecke, August 1968.)

Althenia filiformis Petit: Mares des Onglous, near Sete Hérault, France. (Dr de Solignac, April 1969.)

Lepilaena bilocularis Kirk ex Petrie: Kaituna Lagoon, Canterbury, New Zealand. (Dr L. B. Moore, December 1965.)

Lepilaena cylindrocarpa (Körn.) Ascherson: (female flowers only) Boomer Marsh, Tasmania, Australia. (Dr W. M. Curtis, November 1947.)

Specimens originally fixed in either FAA or 70% ethanol were stained in alcoholic acid fuchsin, dissected and examined entirely immersed in 100%

ethanol with a Leitz epi-illumination attachment on a Wild photomicroscope, following the technique of Sattler (1968). Young floral stages in these species are so small (some less that 100 μm in length) that they had to be mounted in black silicone cement in order to orientate them for photography. Serial sections of certain shoots were prepared by standard histological techniques and stained in Safranin and Delafield's haematoxylin. Analysis of such sectioned shoots was aided by using the camera-lucida cinematographic technique described by Zimmermann & Tomlinson (1966). This anatomical study provided information about the vascular system and served to complement morphological study.

Information is presented here as black-and-white drawings (Figs 1 to 5) which show gross organography, supplemented by interpretative diagrams (Figs 6 to 10). Developmental details are shown in the photographic plates.

MORPHOLOGICAL OBSERVATIONS

Vleisia aschersoniana

Although originally described by Graebner (in Ascherson & Graebner, 1907) as a species of *Zannichellia*, the many morphological features by which this taxon is distinct have been summarized by Tomlinson & Posluszny (1976) in awarding it generic status.

Organography

The sympodial rhizome system, with scale leaves, as first described by Reinecke (1964), gives rise to at least two types of flowering shoots: firstly and most commonly, a monopodial shoot, which is initially vegetative, distally produces axillary flowering branches which are themselves sympodia (Fig. 7C, D); secondly, a short proximal shoot, terminated by a female flower, develops lateral branches giving a succession of monopodial shoots similar to those described above (Fig. 7A, B). The monopodial and initially vegetative shoot has distichously arranged leaves, each leaf subtending an axillary vegetative branch enclosed in a sheathing adaxial prophyll which lacks vascular tissue. When the shoot becomes reproductive, sympodial flowering branches develop in the axils of the leaves of the main shoot, which continues its vegetative monopodial growth. The axillary reproductive shoot usually terminates in a male flower, and like the axillary vegetative shoot, is enclosed by a sheathing prophyll. Renewal growth of the axillary shoot continues from the axil of a leaf below the main flower. This renewal shoot may develop in one of three ways: (i) as a short shoot, producing one or two leaves with further lateral meristems in their axils, before terminating in a female flower (e.g. the lower axillary complex in Plate 2A); (ii) as a monopodial shoot with further sympodial flowering branches in its leaf axils, or (iii) as a wholly vegetative shoot. Most of the material we examined was made up of monopodial shoots with a high proportion of male flowers. Other patterns (noted by Reinecke, 1964) are common but our material was insufficient to clarify whether or not these represent transitional stages of an ordered pattern which develops as the plant matures. One regular feature of the shoot system is that the plane of distichy of each successive branch order is at right angles to the plane of distichy of the

Figure 1. *Vleisia aschersoniana.* Habit of portions of flowering shoots. Isolated male and female flowers to the left.

subtending leaf, as is shown in Fig. 7B, D; Plates 1A, B and 2A, C. Despite the variability of shoot construction in this genus, the strong tendency to sympodial growth is evident, while differences between shoots are largely qualitative.

The male flower consists of one stamen with eight microsporangia arranged in four pairs (Figs 1 and 7). At the base of the connective are two outgrowths (Fig. 7; Plate 1H) directly opposite each other.

The female flower consists of one uni-ovulate carpel surrounded by a membranous envelope (Fig. 1). The style is long and thin, ending in a funnel-shaped stigma (Fig. 1; Plate 2J). More comprehensive descriptions of mature male and female flowers are provided in the accounts of Ascherson & Graebner (1907) and Reinecke (1964).

Organogenesis

A lateral meristem first initiates the sheathing adaxial prophyll primordium (L_s) by marginal growth which soon girdles the entire lateral apex. At about this stage the apex of the lateral shoot may begin to differentiate as a floral meristem (usually male) while the lateral meristem (*V*) of the next higher branch order appears in the axil of a reduced leaf (*L*) formed subsequent to the prophyll. This sequence is clearly shown in Plate 1B-F. This higher shoot may or may not immediately become floral, as we have noted in the organography.

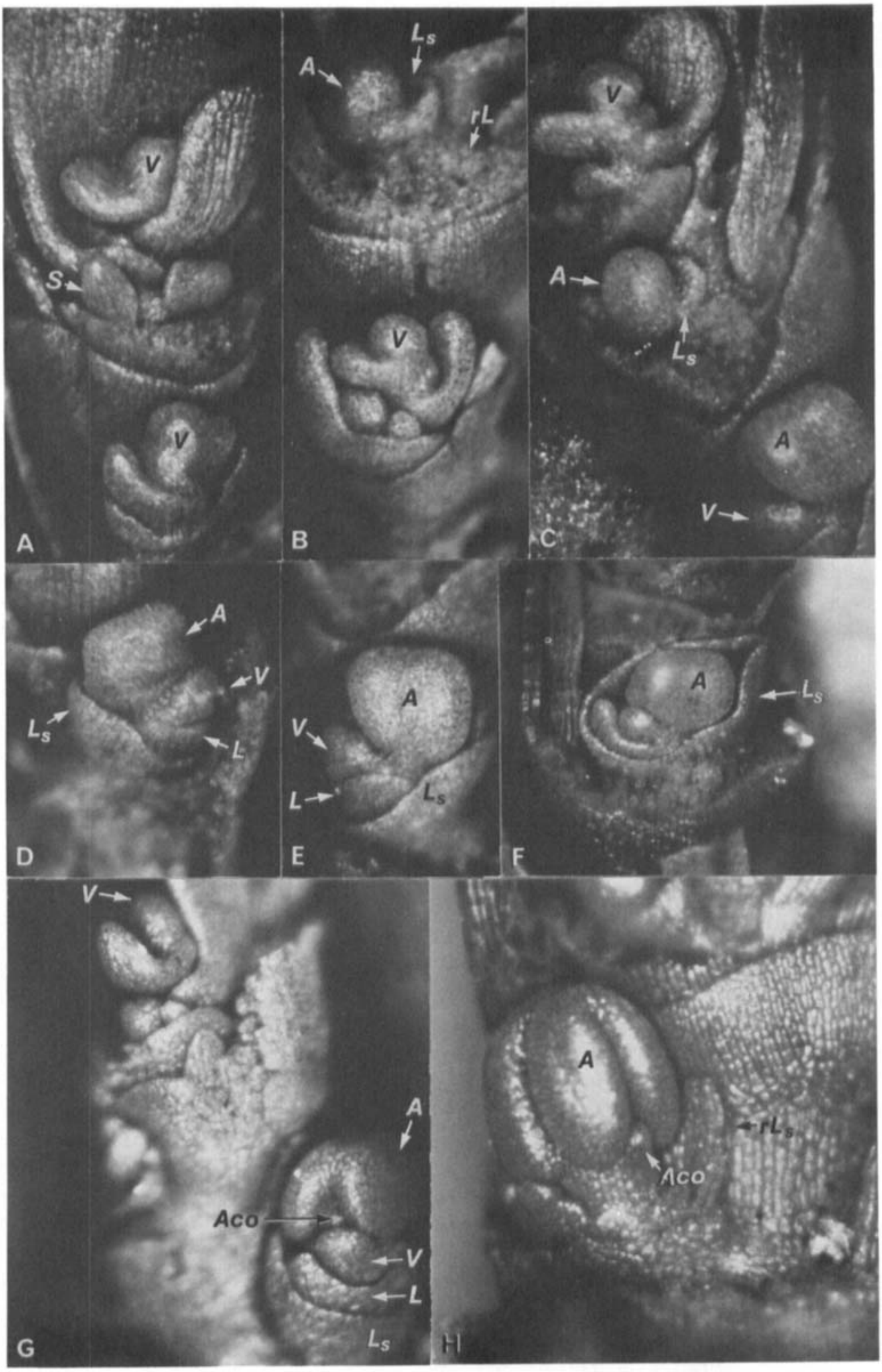
If any axis terminates in a male flower, the apex becomes broad and almost square (*A* in Plate 1D-F), indicating the initiation of the four regions that will each form a pair of microsporangia. Subsequently when the developing microsporangia are clearly distinguishable, two outgrowths (*Aco*) are initiated laterally on opposite sides of the stamen connective, i.e., between each abaxial and adaxial pair of microsporangia (Plate 1G, H). These outgrowths remain small (Plate 2C) and are subsequently obscured by the developing pairs of microsporangia (Plate 2D). As the stamen enlarges, a prominent apical extension of the stamen connective (*Ae*) differentiates (Plate 2A-D).

The unicarpellate female flower usually develops at the apex of the lateral branch (which has already produced one or two leaves) arising below the male flower (Plate 2A, C). It in turn can be overtopped by later-formed branches of higher order. The carpel primordium is at first dome-shaped (Plate 2A) but rapid upgrowth of tissue around the periphery of the primordium soon produces the peltate structure on which the ovule primordium is initiated (Plate 2E). At this stage, when the carpel primordium is becoming peltate, the primordium of the sheathing membranous envelope (*E*) is initiated (Plate 2E, F) as an outgrowth in the plane of distichy of the leaves below. This unilateral outgrowth soon girdles the entire base of the carpel (Plate 2G, H). A broad, peltate stigma develops at the apex of the gynoecial wall soon after the enclosure of the ovule (Plate 2I) and the style is extended by intercalary growth. Towards maturity the margins of the stigma turn inwards, producing its characteristic funnel-shape (Plate 2J).

Althenia filiformis

Organography

The shoot system in this species is more strictly organized than in *Vleisia,* as described by Prillieux (1864), with a clear distinction between a rooted, scale-bearing, regularly branched, sympodial rhizome and erect foliage- and flower-bearing axes without roots (Fig. 2). In the vegetative phase of the erect shoot, leaves (Fig. 2) are distichous with a lateral branch developed in the axil of each foliage leaf. These lateral branches are each enclosed basally by a membranous, non-vasculated, sheathing prophyll. When the erect shoot becomes reproductive (Fig. 3) it terminates as a flower (either male or female) but develops a lateral renewal shoot in the axil of each of the last two or three leaves formed (Fig. 8). The renewal shoots in turn each produce a single, reduced, leaf or bract and also end in a flower. This pattern is maintained until about three flowers have been produced with successive flowers in the axils of successive bracts, before all meristematic activity stops. Since all flowers and their associated bracts are in the same plane, the system approaches that of a simple cincinnus. The axes of this reproductive complex are distinguished by the absence of basal sheathing prophylls (Fig. 8).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The male flower consists of a single bisporangiate stamen which includes, at the base of the filament, three minute scales (Fig. 3). The female flower consists of three uni-ovulate carpels, each of which is subtended by a membranous toothed tepal (Fig. 3).

Organogenesis

Floral inception is indicated by the apex becoming dome-like and ceasing to produce leaves. At this time the lateral meristems in the axil of each of the last two or three leaves also begin to be recognizable as floral apices by changes in their shape. This occurs before they form the sheathing prophylls one sees in the lateral branches of vegetative shoots (Plate 3A-C).

The apical meristem of the future male flower becomes asymmetric by enlargement of the portion that will become the microsporangia (Plate 3B-D). At this stage the three primordia (*B*) of the minute scales are initiated at the base of the developing male flower (Plate 3C, D, F). They are initiated as elongated primordia and show dorsiventral symmetry, but their growth rate is slow and they never rise above the base of the microsporangia (Plate 3G, I).

The female floral meristem initiates the primordia of the three membranous tepals that will each subtend a carpel (Plates 3E and 4A), at about the same time it initiates the three growth centres of the future carpels themselves (Plates 3E and 4B). Each carpel primordium more or less synchronously develops a peltate outgrowth at its periphery (Plate 3H). After enclosing the single bitegmic ovule which soon develops, the carpel wall begins to differentiate a long style terminating in a peltate stigma (Plate 4B-E). The membranous tepal subtending each carpel also develops rapidly and is at first bilobed apically (Plate 4B, C). Towards maturity when it almost totally sheaths the axillant carpel, the tepal apex becomes unevenly serrated (Plate 4D, E).

Lepilaena bilocularis

Organography

The shoots are probably dimorphic, with a distinction between creeping, sympodially-branched and rooted rhizomes which bear only scale-leaves, and erect, foliage-bearing flowering shoots. However, we have had insufficient material to establish if there is any regularity in the system. Leaves are arranged distichously throughout the vegetative state, with lateral branches developing in the axils of most leaves. Each branch apex initially develops a prophyll which

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Plate 1. *Vleisia aschersoniana.* Side views of monopodial shoots with young axillary sympodial male shoots. All magnifications x140.

A. Vegetative apex (*V*) producing distichously arranged foliage leaves. A lateral vegetative branch is seen developing below. B. A young male branch (*A*) in the axil of foliage leaf (*rL*). C. Two stages of male branch development. A very young stage just below the main apex and an older stage, already developing a renewal apex (*V*), at the lower right hand portion of figure. D, E, F. Various views of young staminal branches, each showing the development of the terminal stamen (*A*), renewal apex (*V*) in the axil of the first formed foliage leaf (*L*) and sheathing prophyll (L_S) beginning to girdle the base of the branch. G, H. Two stages in the initiation and development of the staminal connective outgrowth (*Aco*) which forms on both sides of the connective between the developing pairs of the microsporangia.

Figure 2. *Althenia filiformis.* Terminal portions of rhizomes with developing aerial shoots in the axils of each scale leaf. Isolated foliage leaves to the right.

remains nonvasculated, becomes membranous and encloses its own branch axis at maturity (Fig. 9; Plate 5C). As an erect shoot becomes fertile, the vegetative meristem is transformed into either a male or female floral bud. The renewal shoot in the axil of the uppermost leaf in its turn also terminates in a flower after a short period of vegetative growth, but without forming a sheathing prophyll (Fig. 9).

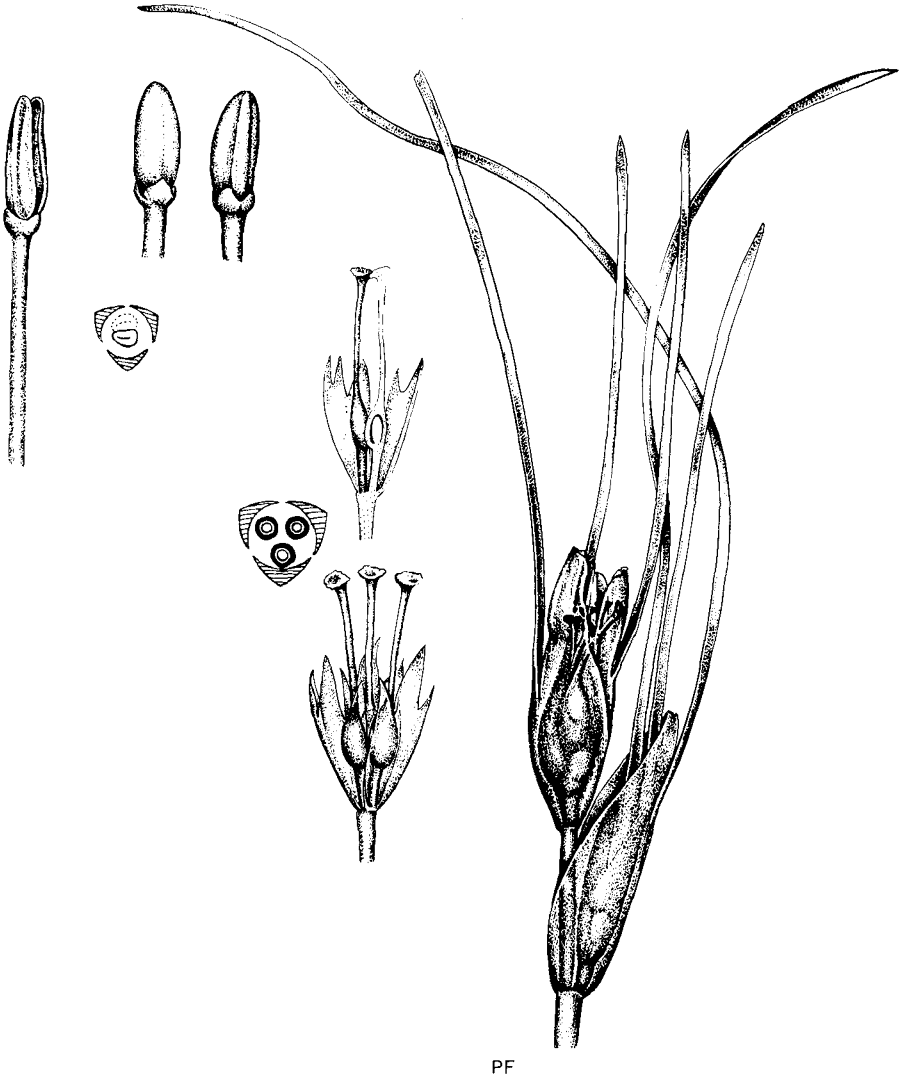
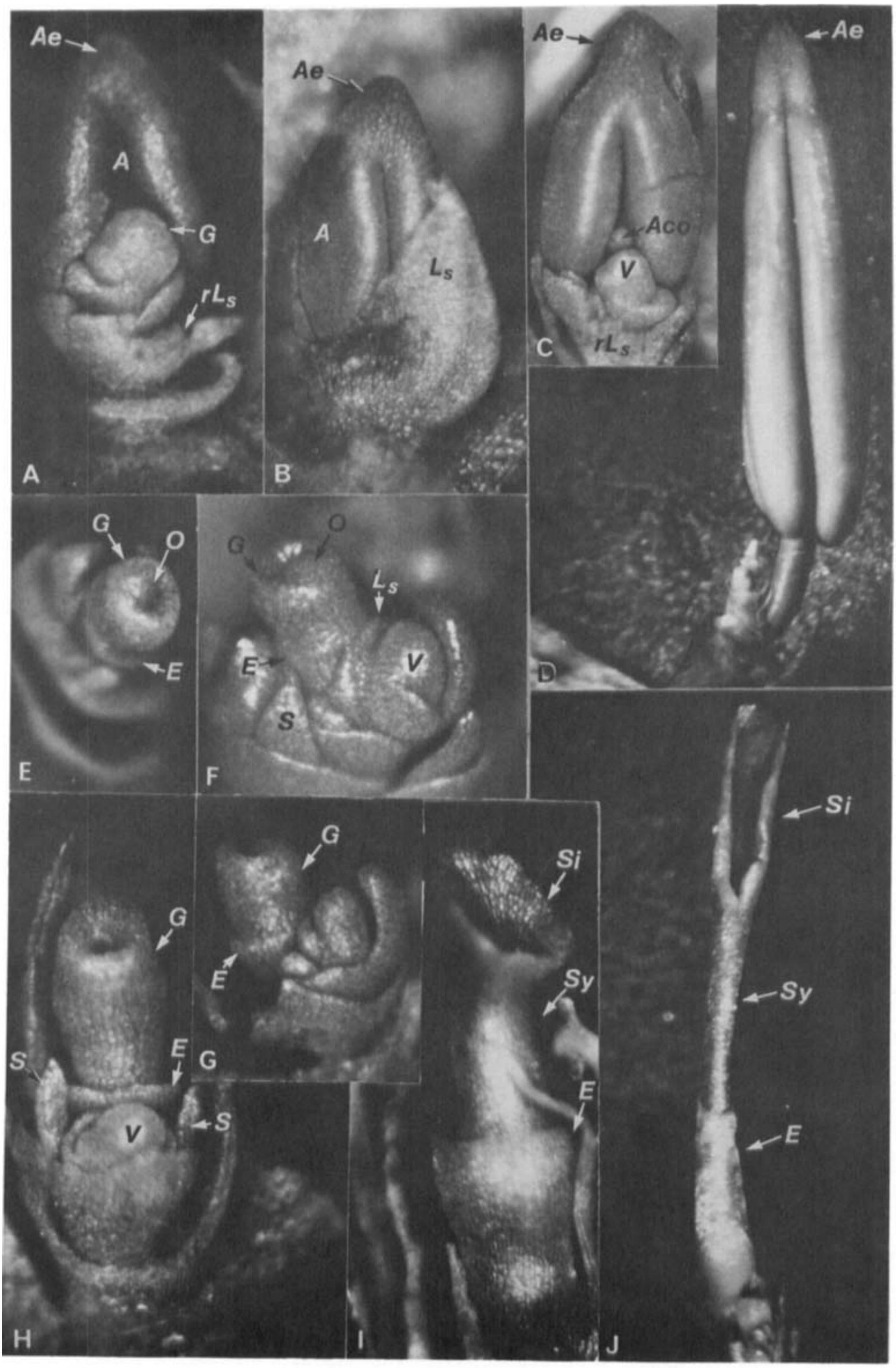


Figure 3. *Althenia filiformis.* Terminal portion of flowering shoot with various views of male and female flowers.

The female flower consists of three uniovulate carpels, each subtended by an entire, or more or less bilobed, membranous tepal. The style ends in a feathery stigma (Fig. 4). The male flower is a single stamen with four microsporangia in two pairs and three basal scale-like bracts. At maturity a long pointed outgrowth (Ae) extends from the tip of the connective (Fig. 4).

Organogenesis.

Differentiation of the male flower (transformed from the vegetative apex, Plate 5A, B, D, E) is initiated by dorsiventral expansion of the apex. Very early in development three separate tepal primordia are initiated below the male floral bud (Plate 5E). These are at first broad, but ultimately become scale-like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and fuse by marginal growth to form the cupule which envelopes the mature stamen (Plate 5F-I). Anther development itself involves the formation of the two pairs of microsporangia (Plates 5G, I) and is completed by the apical extension of the anther connective (Plate 5H-J).

In the early stages of development, the female flower forms three carpel primordia, initially visible as separate outgrowths. Tepal primordia are initiated simultaneously with carpel primordia (Plate 6A). The three carpel primordia are radially symmetrical at first, but through rapid marginal growth become peltate (Plate 6B, C). An ovule is soon initiated on the adaxial (ventral) portion of each developing carpel wall (Plate 6C). As the carpel wall encloses the ovule, its further growth produces the stigma and style (Plate 6D-F). Subsequently, in turn, a broad peltate stigma becomes feathery by marginal outgrowth (Plate 6G). The developing tepals become bi-lobed distally, a feature which is lost as they mature, becoming membranous with an unevenly serrate apical margin (Plate 6F, G).

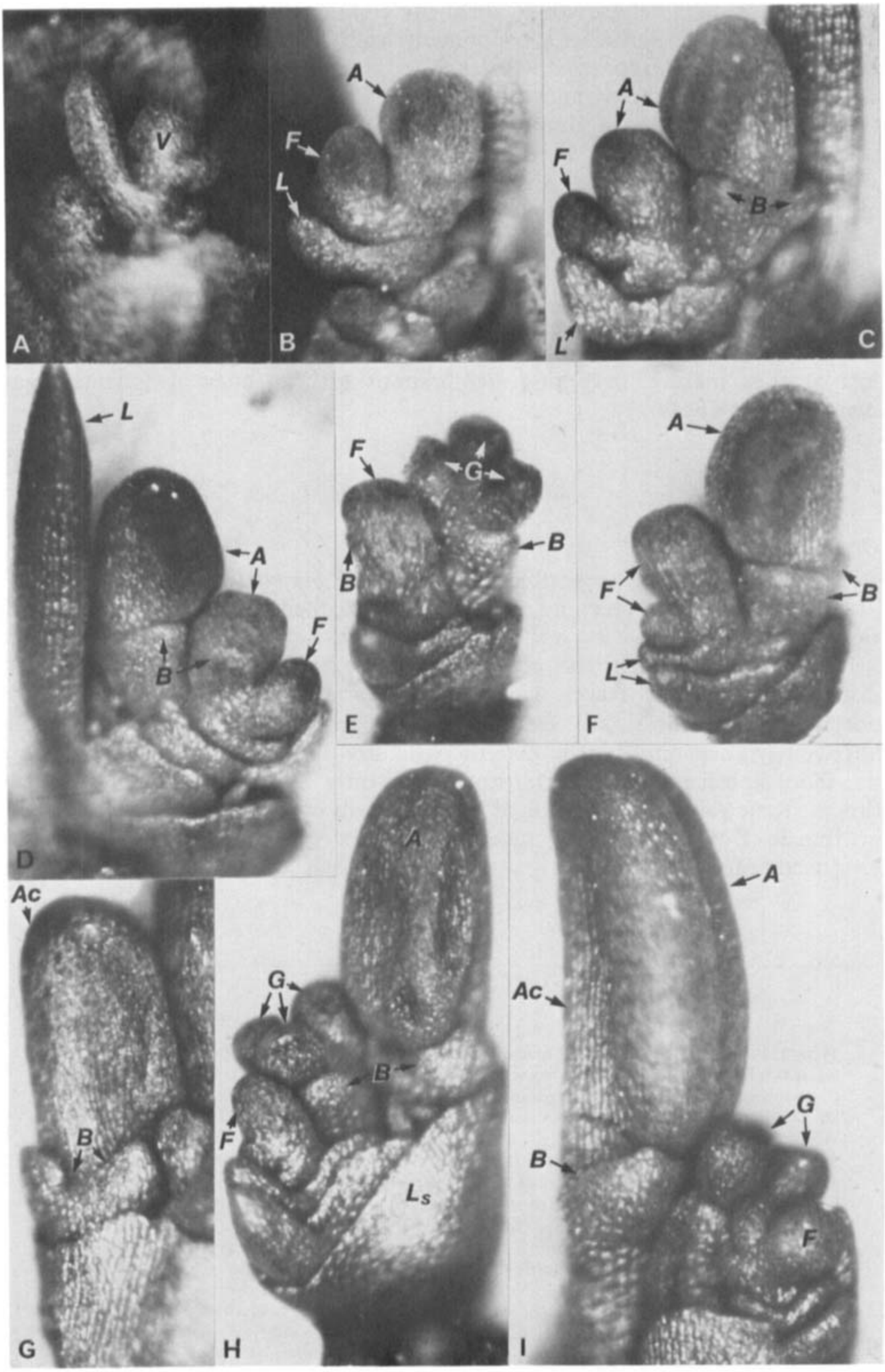
Lepilaena cylindrocarpa

Organography

The shoot system resembles that of *L. bilocularis* with a probable dimorphism between creeping, sympodially-branched, rooted shoots and erect flowering shoots. However, our material has again been insufficient to make the necessary observations. The erect shoot is vegetative at first and produces distichously arranged leaves. Lateral branches apparently lack a membranous prophyll. Flowering shoots are unisexual, suggesting that plants are dioecious but we have had only female material to study. In the reproductive condition, the shoot apical meristem is transformed directly into the tri-carpellate female flower. Renewal shoots, developed from the axils of lower leaves also terminate in female flowers. Further successive higher order flowering shoots are produced, each usually bearing a single foliage leaf and no prophyll (Fig. 10).

Plate 2. *Vleisia aschersoniana.* All magnifications x140 except for D and J (x32).

A-D. Late stages in male branch development. Side views of stamens showing the development of staminal tip extension (*Ae*). A. Sheathing prophyll (*Lg*) has been removed to show sympodial female branch just initiating dome-shaped carpel primordium (*G*). B. Similar to A, but with sheathing prophyll (*Lg*) intact. C. Developing staminal connective outgrowth (*Aco*) can still be seen at this stage between the pair of microsporangia. D. Nearly mature male flower. E-J. Early to late stages in female branch development. E. Top view of young carpel primordium (*G*), just initiating its ovule (*O*). Girdling primordium of membranous envelope (*E*) can also be seen. F. Slightly oblique top view of a short, proximal female branch which usually develops at a rhizome node. Terminal single carpellate female flower is at a similar stage as that in E. The renewal apex (*V*) to the right is just initiating its first two foliage leaf primordia. G. Side view of a similar stage as in F. Note the girdling primordium of the membranous envelope (*E*). H. Slightly oblique top view of carpel at a stage after the ovule has been enclosed. The carpel tube, though, is still open. I. Side views of carpel with already well developed stigma (*Si*) and style (*Sy*). Membranous envelope (*E*) has enclosed lower portion of carpel. J. Nearly mature female flower.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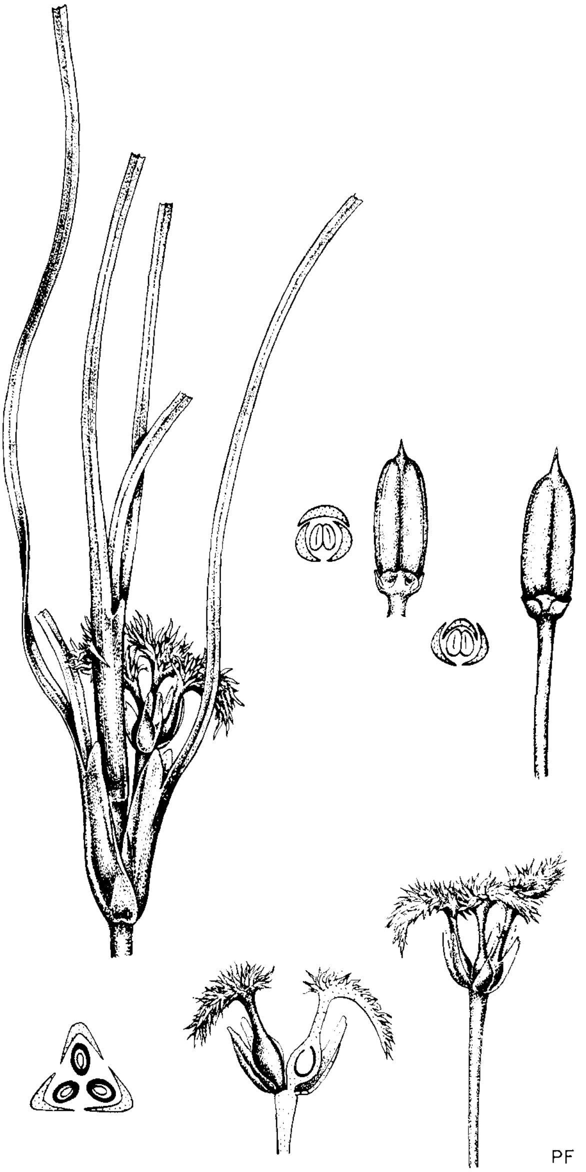

Figure 4. *Lepilaena bilocularis.* Terminal portion of flowering shoot with various views of male and female flowers.

Plate 3. *Althenia filiformis.* Side views of sympodial flowering branches. All magnifications x140. A. Vegetative apex (*V*) prior to floral induction. B. A floral shoot which has terminated in a single stamen male flower (*A*). Below, in the axil of the last formed leaf (*L*), is a still younger floral apex (*F*). C. Three floral apices at different stages of development. Two have already differentiated as male flowers (*A*). The oldest male flower is initiating its bract primordia (*B*). D. Same stage as C, but as seen from the other side. E. A sympodial floral shoot which is terminated by a tricarpellate (*G*) female flower. F. A stamen (*A*) of the terminal male flower with distinct microsporangial differentiation (darkly stained portions). G. A similar stage to F, but positioned to show prominent connective (*Ac*) and scale-like bracts (*B*). H. A young female flower below the terminal male, at a stage just prior to the initiation of the ovary walls on the three carpel primordia (*G*). Bract primordia (*B*) are already present. I. Nearly mature male flower.

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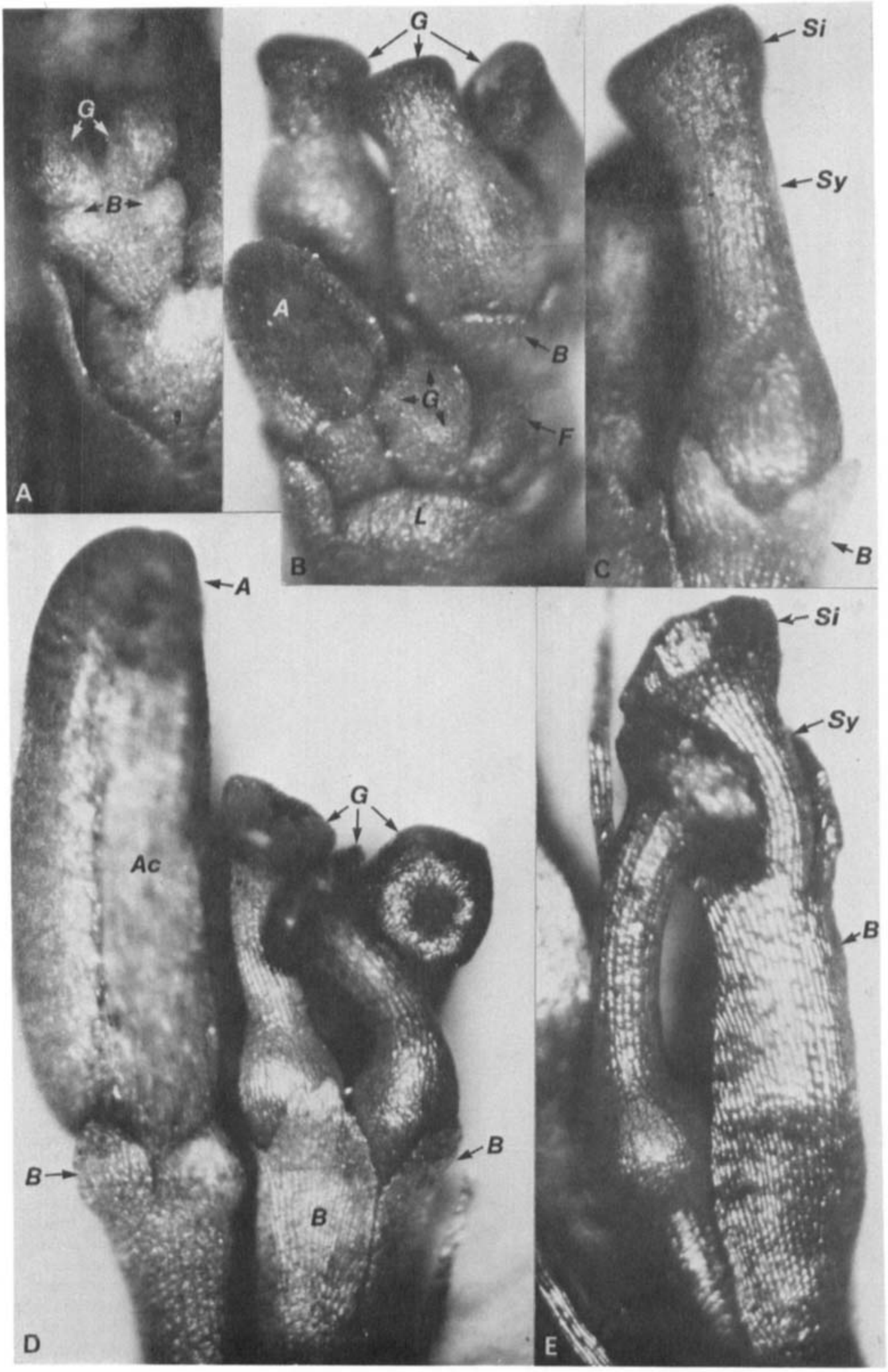
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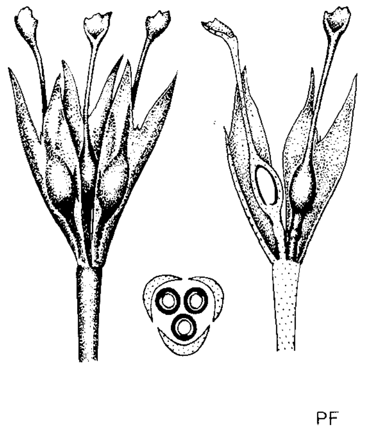


Figure 5. *Lepilaena cylindrocarpa.* Various views of mature female flower.

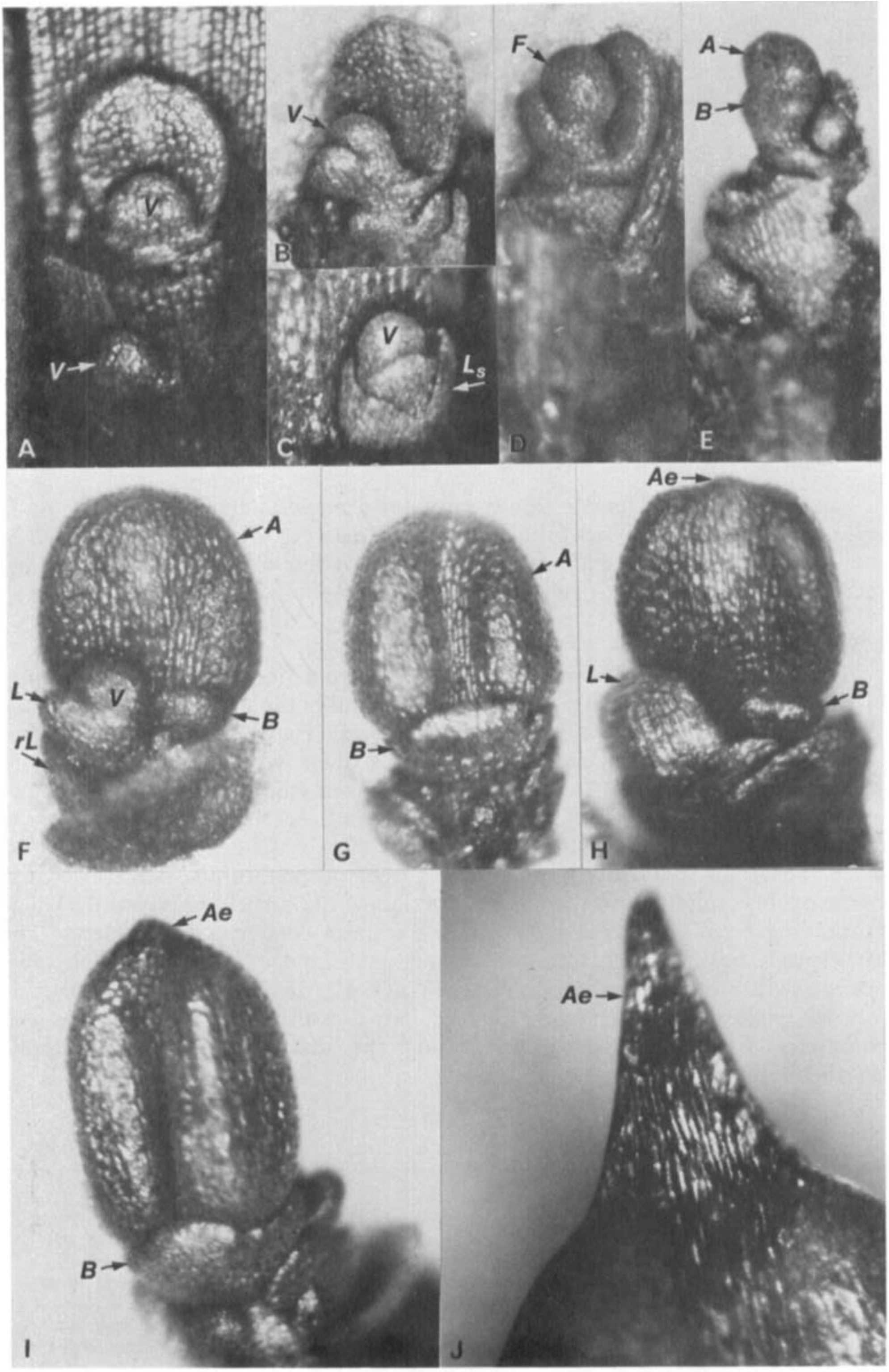
Each carpel of the female flower contains a single bitegmic ovule. The short style terminates in a broad funnel-shaped stigma (Fig. 5). At maturity each of the carpels is partially enclosed by its more or less three-lobed, subtending, membranous tepal (Fig. 5; Plate 7H).

Organogenesis

As the vegetative meristem is transformed into a floral one, it first becomes broadly dome-shaped (Plate 7A). Lateral renewal-branches continue vegetative growth in the axils of existing foliage leaves (Plate 7B, C, E). The floral meristem becomes triangular, as a result of outgrowths which are incipient carpels: simultaneously, the three primordia of the subtending tepals are initiated (Plate 7D). With further growth and development of the floral meristem the three carpel primordia become clearly delineated (Plate 7G). By more rapid marginal growth, the carpels become peltate and soon after, the single ovule is initiated on the adaxial portion of the carpel wall (Plate 7F). The subtending tepal primordia by now have a clear dorsiventral symmetry. The carpel wall begins to enclose the young ovule and at this stage each tepal becomes distinctly three-lobed (Plates 7C, G, H). Differentiation of the style follows enclosure of the ovule by the carpel wall; the style elongates and completes its development by forming the distal, funnel-shaped stigma (Plate 7G, H).

Plate 4. *Althenia filiformis.* Side views of late stages in primarily female flowering shoots. All magnifications x140.

A. Two young carpels subtended by bract primordia (*B*). B, C. Carpels after enclosing ovules, developing their styles (*Sy*) and stigmas (*Si*). Subtending bract (*B*) has become bi-lobed. D. Nearly mature male and female flowers. Bracts about male flower are still small and scale-like. Bracts subtending carpels are more elaborate with lobed margins. E. Mature female flowers with the bracts almost totally ensheathing carpels.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ANATOMICAL OBSERVATIONS

The anatomy of the reproductive parts, and especially the vasculature, is very reduced and does not aid in interpreting morphological features. For this reason it is only briefly reported here. In the vegetative parts, the vascular system is represented by a vascular strand, including a central protoxylem lacuna, surrounded by phloem tissue which is indistinctly segregated into discrete strands. A plexus, which includes numerous short tracheids, is established at each node. A cortical system of narrow strands of elongated cells is feebly developed. Details of the vascular system of *Zannichellia palustris* have been provided by Monoyer (1927) and Uhl (1947). Branching involves a simple bifurcation of the central vascular strand.

The vasculature of the reproductive parts is equally reduced. Prophylls and tepals, where present, have no vascular system. The foliage leaves or bracts associated with the flowering system each have a single median strand. The stamen has a single vascular strand, which is a direct continuation of the vegetative vascular system. Each carpel is supplied by a single strand, which bifurcates basally to produce an ovular trace and a strand running into the style.

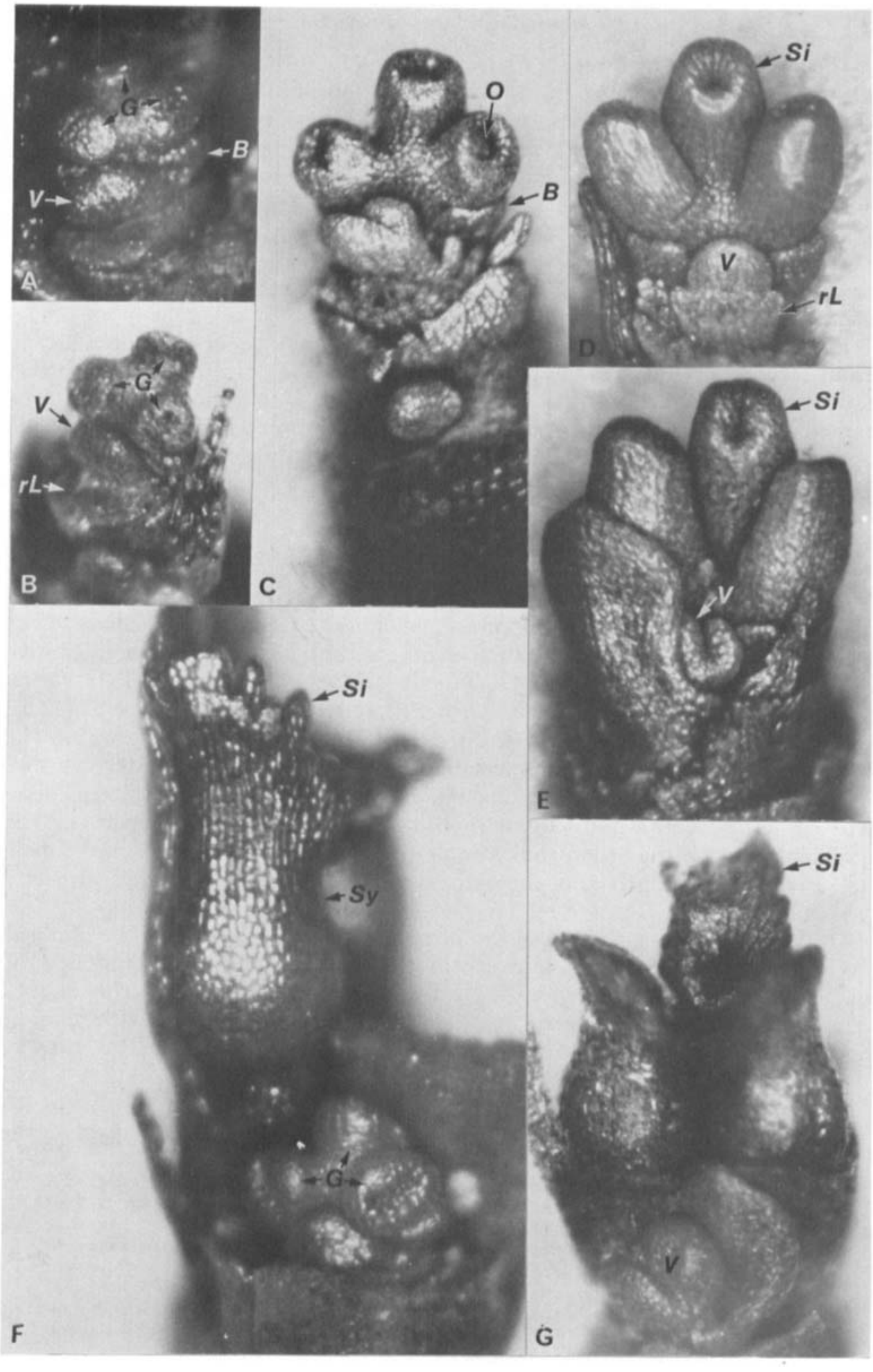
DISCUSSION

Previous descriptive work on certain members of the Zannichelliaceae has dealt with the nature of reproductive organs, but has made no comparative developmental observations (e.g. *Althenia filiformis*, Prillieux, 1864; *Vleisia aschersoniana*, Reinecke, 1964; *Zannichellia palustris*, Uhl, 1947; Singh, 1965). As pointed out by Posluszny & Sattler (1976a), an understanding of the flowering structures of the Zannichelliaceae is important in the interpretation of morphological trends in certain Alismatiales since they are intermediate between *Potamogeton* and *Ruppia*, with their spike-like inflorescences, on the one hand, and on the other, the extreme simplicity of *Najas*, in which the male unit is a single stamen and the female a single ovule within an acarpellate gynoecium.

The basic branching pattern of the flowering shoot in all the plants examined was similar, with some notable exceptions in *Vleisia*, which has a tendency for continued monopodial growth (Table 1; Fig. 7). Otherwise, flowering shoots were sympodial and terminated in either a male or female flower, with renewal shoots developing from the axils of leaves immediately below this flower.

Plate 5. *Lepilaena bilocularis.* Side views of vegetative and developing sympodial male flowering shoots. All magnifications x140.

A, B. Two views of vegetative apices (*V*) initiating distichously arranged foliage leaves. C. A vegetative lateral branch apex almost entirely girdled by sheathing prophyll (L_S). D. First indication of floral induction as the apex becomes broad and dome-like. E. A very young stage in the development of the male flower. Bract primordia (*B*) are just originating as are the areas (*A*) that will later differentiate into the pairs of microsporangia. F, G. Two sides of the same male flower showing extrorse development of microsporangial pairs. The cupular bracts (*B*) girdle the base of the developing stamen. The last formed foliage leaf (*rL*) was removed to show the renewal apex in F. H, I, J. Three stages in the development of the extension of the staminal tip (*Ae*), from initiation (H) to near maturity (J).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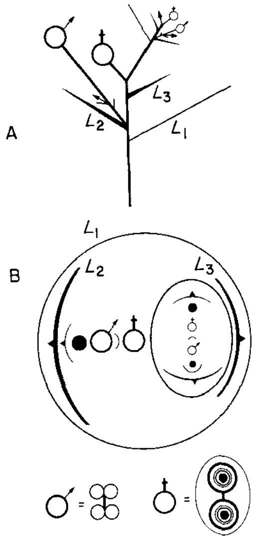


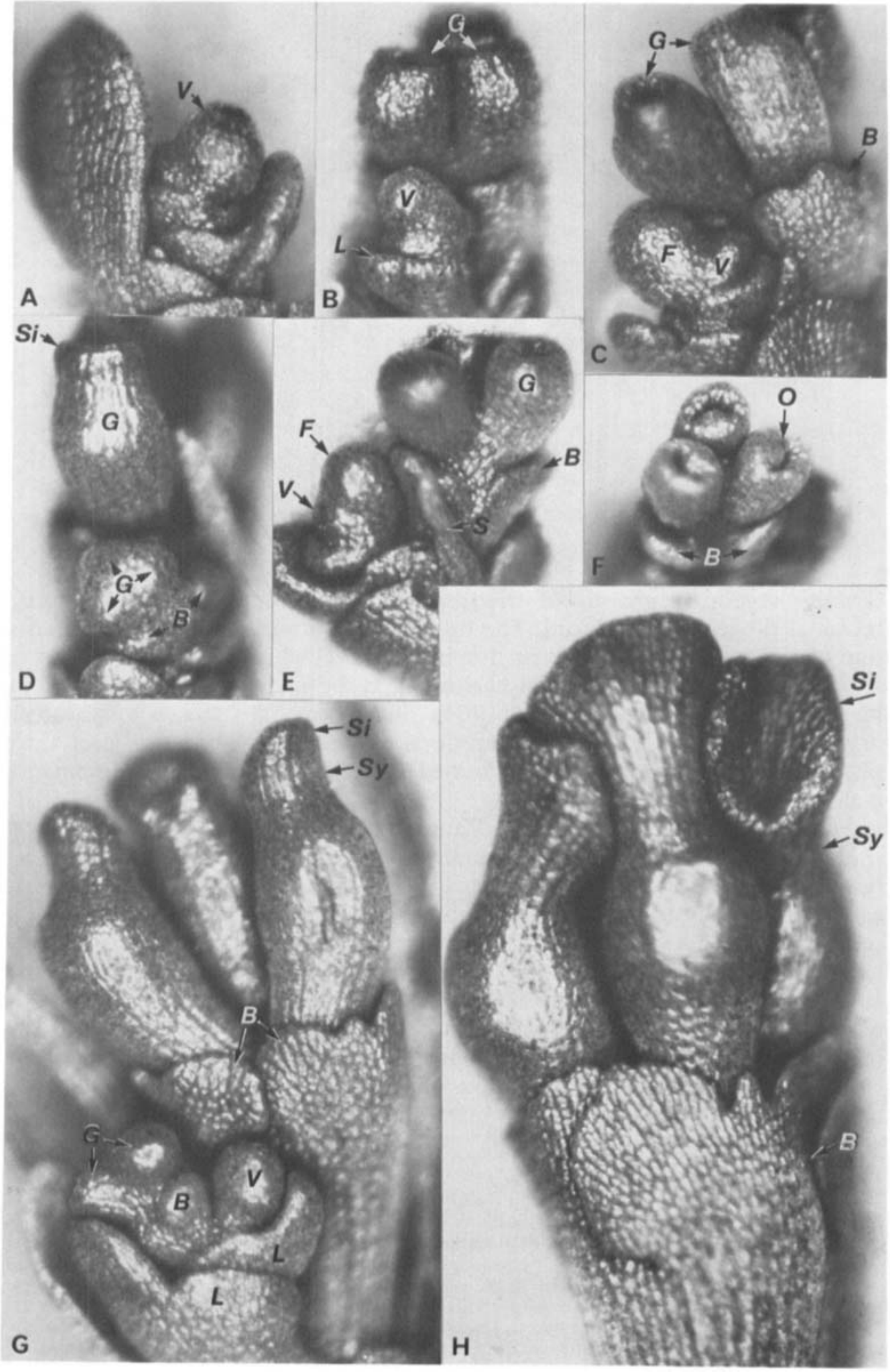
Figure 6. *Zannichellia palustris.* Diagrammatic representation of flowering shoot, showing both side (A) and top (B) views.

Usually, vegetative growth of this renewal shoot was very limited before a terminal flower was produced. The exceptional pattern of branching found as one of the two types of construction in *Vleisia,* in which the monopodial main axis develops lateral sympodial clusters, may be related to the habit of the plant, as described by Reinecke (1964). She noted that it grows in seasonally dry river basins (vleis). A single fruit develops only on the proximal part of the plant, which may be most easily buried. Distally, flowers are predominantly male.

Shoot construction within the Zannichelliaceae is most regular in *Zannichellia,* once the distal pattern of branching in association with flowering has been established. Posluszny & Sattler (1976a) showed that the pattern is most easily analysed by following its development; it is clearly sympodial, each shoot terminating in a female flower with which is associated a short lateral shoot bearing a male flower (Fig. 6). Precocious development of this lateral male shoot obscures the simplicity of the pattern, which is generally comparable with *Althenia* and *Lepilaena* but most particularly with *Vleisia.* On the other hand, the monopodial tendency noted for some shoots of *Vleisia* bears comparison with the shoot system of *Najas flexilis,* which forms its flowering branches in the axils of leaves (Posluszny & Sattler, 1976b).

Plate 6. *Lepilaena bilocularis.* Stages in the development of tri-carpellate female flowers, from initiation to near maturity. All magnifications x140.

A. Oblique top view showing the initiation of the three carpel primordia (*G*), concurrent with the initiation of the subtending bract primordia (*B*). B. Carpel primordia (*G*) becoming peltate. Renewal apex can be seen in the axil of the last formed foliage leaf (*rL*). C. Ovule primordium (*O*) initiating on adaxial portion of peltate carpel wall. D, E. Two successive stages after ovules have been enclosed by carpel walls. First indications of developing stigma (*Si*) can be seen. F, G. Side views of carpels nearing maturity with broad peltate stigmas (*Si*) beginning to become lobed at their margins.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Another consistent feature of the flowering units within the Zannichelliaceae is carpel development. This in turn is comparable with that found in *Potamogeton* and *Ruppia* (Posluszny & Sattler, 1973, 1974a, b). All originate as peltate primordia which initiate their single ovule on the abaxial portion of the carpel wall (*Querzone*). Only *Najas* is exceptional in this respect in the group of families considered here (Posluszny & Sattler, 1976b).

Male flowers in this assemblage also exhibit comparable features. In the stamens of both *Vleisia* and *Lepilaena bilocularis,* small outgrowths of the connective may be compared to the larger ones noted in *Ruppia maritima* (Posluszny & Sattler, 1974b). Cupule-like tepals surrounding the base of male flowers in *Althenia* and *Lepilaena* can be compared to the single envelope of *Najas flexilis* (Posluszny & Sattler, 1976b).

Both *Zannichellia* and *Vleisia* form a membranous envelope enclosing one or more carpels. This envelope develops late, at about the stage of ovule inception, and rapidly encircles the carpel or carpels, forming a tight enclosure at maturity (Plate 2J). In contrast, appendages in *Althenia* and *Lepilaena* appear contemporaneously with, or possibly prior to the carpel primordia and become lobed or dentate distally.

The question remains as to whether these organs are flowers or inflorescences. Much controversy has been generated by attempts to recognize (in *Zannichellia* in particular) parts of standard flowers. For example, Magnus (1870), Ascherson & Graebner (1907) and Wettstein (1935) regarded the envelope about the group of carpels in *Zannichellia* as a 'perianth' surrounding a 'flower', while Campbell (1897) and Uhl (1947) termed the same structure an 'involucral bract' about an 'inflorescence'. Study of floral development offers no solution to this problem and suggests that such clear-cut distinctions need not be made, since the categories of flower and inflorescence here merge (cf. Emberger, 1950; Sculthorpe, 1967; Posluszny & Sattler, 1976a). Developmentally we have shown that the tepal-like appendages associated with the carpels in *Althenia* and *Lepilaena* are different from the carpel envelope in *Vleisia* and *Zannichellia.* Only the former are appreciably phyllomic.

Similarly the development of the male floral units does not resolve their homologies. Singh (1965) claimed that the evidence of a double vascular supply and variation in the number of microsporangia supported an interpretation of the male 'flower' of *Zannichellia* as two fused stamens. However, in no member of the Zannichelliaceae is there developmental evidence to support this interpretation. All stamens, whether they eventually form 2, 4, 6 or 8 microsporangia are initiated as single primorida. The various cupular or

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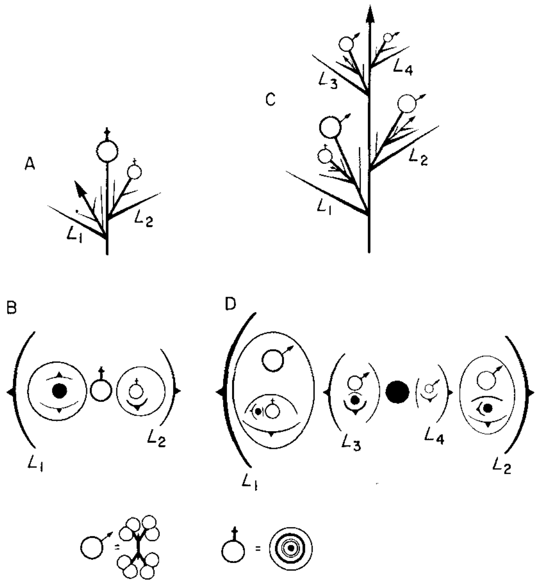
Plate 7. *Lepilaena cylindrocarpa.* Stages in the development of female sympodial flowering branches. All magnifications x140.

A. Vegetative apex (*V*) prior to floral induction. B, C. Two flowering branches terminating as female flowers (*G*) with renewal branches developing below, vegetative at first (B) before also terminating as a floral apex (C). D. The carpel of the uppermost flower is already forming its stigma (*Si*) while the lower flower is just initiating its carpels (*G*) and bracts (*B*). E. Side view of a renewal branch below a female flower which is just becoming a floral apex (*F*) while initiating its own renewal apex (*V*). F. Slightly oblique top view of three carpels of a female flower, initiating their ovules (*O*) on the adaxial portion of the carpel wall. G, H. Side views of two successive late stages in carpel development. Note the characteristic tri-lobed development of the subtending bracts (*B*).

Table 1. Comparison of morphological and developmental features of the flowers of certain Alismatales. Morphological data do not take into account minor quantitative variations

	Ruppia	*Zannichellia*	*Vleisia*	*Althenia*	*Lepilaena*	*Najas*
Habit	Basally monopodial Distally sympodial	Creeping rhizome giving rise to complex sympodial floral shoots	Rhizome sympodial, erect shoots either sympodial (proximal ♀) or monopodial (distal ♂)	Dimorphic shoot system, with a distinction between creeping sympodially-branched rooted rhizomes with scale leaves and erect foliage-bearing flowering shoots		
Development of fertile shoots	Sympodial—2 flowers per inf. spike, which terminate main and lateral shoots	Sympodial (possibly intermediate between sym. & monopodial with regard to ♀ flower)	Monopodial main shoot, sympodial flowering shoots in axils of each leaf	Sympodial	Sympodial	Monopodial
Leaves between successive flowers (inflorescences)	4	3	2	1	1	2
Sheathing prophyll	+	+	+	only on non-flowering shoots	only on non-flowering shoots	—
Perianth members (tepals)	—	♂ Naked, envelope about ♀		3 scale-like structures at base of ♂ 3 membranous tepals about ♀, one subtending each carpel		2 membranous envelopes about ♂ Naked ♀

Androecium	2 stamens per flower each initiated as elongate primordia 4 microsporangia in 2 pairs per stamen	1 terminal stamen formed from the transformed staminate floral apex				Microsporangia differentiated directly from the terminal portion of ♂ floral apex 1 microsporangium
		Initiated as elongate primordia, microsporangia form distally 4(-8) microsporangia in 2(-4) pairs per stamen	Initiated as 'square' primordium Pair of microsporangia initiated at each 'corner' 8 microsporangia in 4 pairs per stamen	Asymmetric stamen primordium, 1 pair of microsporangia initiated on only one side of connective 2 microsporangia in 1 pair per stamen	'Flattened' stamen primordium 4 microsporangia in 2 pairs per stamen	
Outgrowth or extension of stamen connective	1 small dorsiventral outgrowth slightly abaxial between the 2 pair of microsporangia Initiated after microsporangial differentiation	Slight vertical extension of stamen connective	2 small vestigial outgrowths initiated after microsporangial differentiation has begun	–	1 small cuspidate outgrowth in *L. bilocularis* Initiated after microsporangial differentiation has begun	–
Gynoecium	Each carpel is formed as a radial promordium, which becomes peltate and initiates a single ovule at the cross-zone (Querzone)					Acarpellate gynoecium wall initiated as a girdling primordium which encloses the ♀ floral apex, which transforms into the ovule
	(2-) 4 or more carpels per flower	2+ carpels per flower	1 carpel per flower	3 carpels per flower	3 carpels per flower	



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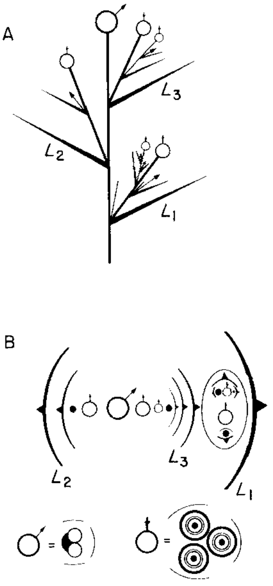

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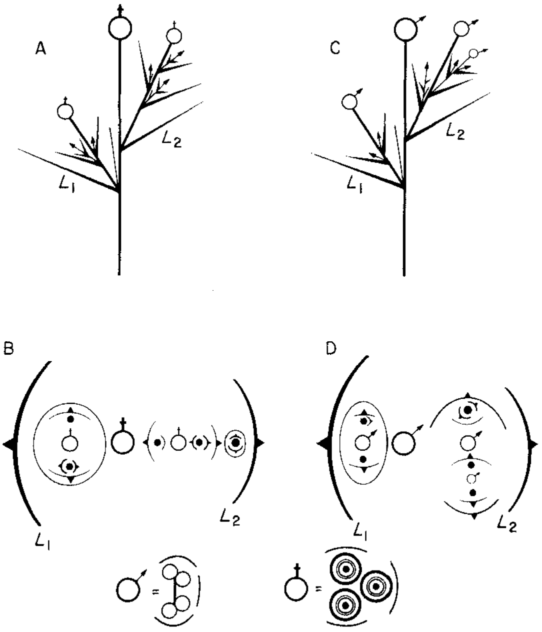

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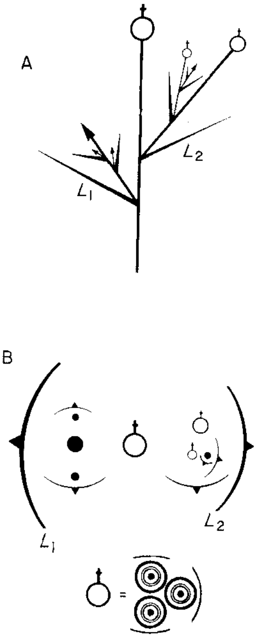

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Figures 7 to 10. Diagrammatic representations of the flowering shoots of four species showing both side (A, C) and top (B, D) views.

7, *Vleisia aschersoniana*; 8, *Althenia filiformis*; 9, *Lepilaena bilocularis*; 10, *L. cylindrocarpa*.

tepal-like appendages of the stamens usually have been interpreted as perianth members (Prillieux, 1864; Ascherson & Graebner, 1907; Miki, 1937; Eckardt, 1965). Developmental study lends support to this idea at least in *Althenia* and *Lepilaena,* since the appendages are initiated prior to microsporangial differentiation. In this they are comparable to the envelope about the male flower of *Najas flexilis* (Posluszny & Sattler, 1976b). Some developmental comparison is possible between the minute connective outgrowth in *Vleisia,* initiated after microsporangial development, and the outgrowth of *Ruppia* in a similar position.

Clearly these plants are so reduced that further search for precise homologies on the basis of morphology and anatomy are not likely to be productive unless new taxa are discovered. Future studies might be directed towards morphogenetic phenomena and floral biology.

This investigation has provided further evidence for the close affinity of the genera *Zannichellia, Vleisia, Althenia* and *Lepilaena* which is best expressed by their separation as the family Zannichelliaceae (Tomlinson & Posluszny, 1976). All these genera have similar carpel development, and the same basic shoot organization. *Vleisia* and *Zannichellia* on the one hand, *Althenia* and *Lepilaena* on the other resemble each other most closely in the form and development of the structures surrounding the male and female flowers.

In the broader context of a possible morphological series involving closely related families within the Alismatales (Singh, 1965; Posluszny & Sattler, 1976a, b) some significant gaps have been filled. Our present comparative understanding is set out in Table 1. Specific points worth mentioning are the similarity of the flowering shoot patterns, the link with *Najas* being from the monopodial tendency of *Vleisia*; the similar ontogeny and structure of carpels; the occurrence of outgrowths from the stamen connective in *Vleisia, Lepilaena bilocularis* and *Ruppia*; and the possible homology of tepals and cupules around the male flowers of *Althenia, Lepilaena,* and *Najas.* Despite the fact that this is a series of discrete and readily separated taxa, an appreciable morphological continuum seems to exist.

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ABBREVIATIONS USED IN PLATES AND FIGURES

A	Staminate flower or its primordium
Ac	Stamen connective
Aco	Outgrowth of stamen connective
Ae	Extension of stamen tip
B	Bract or its primordium
E	Membranous envelope around pistillate flower
F	Floral apex
G	Carpel or its primordium
L	Foliage leaf or its primordium
L_S	Sheathing prophyll
O	Ovule or its primordium
r	Removed, e.g. *rL*, vegetative appendage removed
S	Squamula intravaginalis or its primordium
Si	Stigma or developing stigma
Sy	Style or developing style
V	Vegetative or renewal apex