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Morphology, anatomy and cytology of the genus *Lithachne* (Poaceae: Bambusoideae)

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Abstract: *Lithachne pauciflora* and *L. humilis* were studied anatomically, morphologically and cytologically. They are typical herbaceous bambusoid grasses of the tribe Olyreae, occurring in forested tropical habitats in Central America. Both species are monoecious, with both sexes in the same axillary inflorescence in *L. pauciflora* or in separate lateral pistillate inflorescences and terminal staminate panicles in *L. humilis*. Staminate spikelets lack glumes and have three truncate lodicules. Pistillate spikelets have subequal long glumes and a single bony truncate fruit case. Leaf anatomy is typically bambusoid with a papillate epidermis bearing acute bicellular microhairs of two equal cells, siliceous cells, and rhombic stomata. In transection, blades have fusoid cells and chlorenchyma with arm cells. Chromosome number in *L. pauciflora* is $n = 11$.

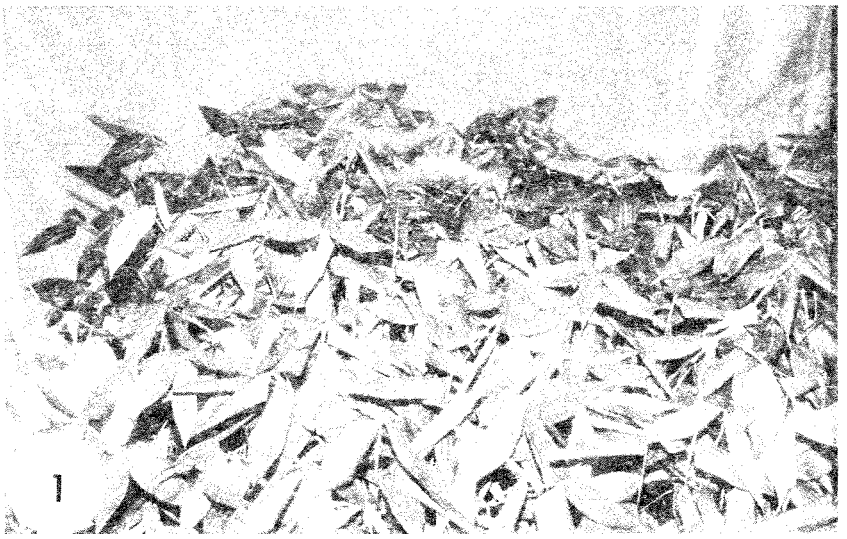
Key words: *Lithachne*, anatomy, morphology, cytology.

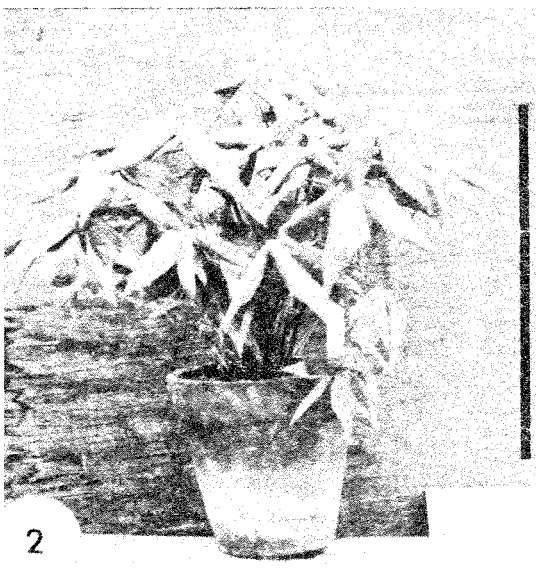
Lithachne is a small genus of herbaceous bambusoid grasses of the American tropics, presently with four known species. This study is based upon the two Central American species. *Lithachne pauciflora* (Sw.)Beauv. is a widespread species found in forests from sea level to about 1000 m elevation, from Mexico to northern Argentina. *L. humilis* Soderstrom (1980) is a recently described species restricted to brushy riverbanks in northern Honduras. Both species are cultivated in the greenhouse of the Botany Department of Iowa State University from Costa Rican and Honduran material. Living material for this study was obtained from this collection.

Both species are small caespitose grasses with flat narrowly ovate blades with conspicuously oblique bases. The blades are borne on short pubescent pseudopetioles. The blades deflex during the night. Small inflorescences are borne at middle and upper culm nodes. In *L. pauciflora*, the lateral inflorescences bear both pistillate and staminate spikelets, but in *L. humilis*, only pistillate spikelets occur in the lateral infloresences, and staminate spikelets are borne in a small terminal panicle. An outstanding feature of this genus is the obtriangular truncate laterally compressed bony fruits (lemma and palea), which give the genus its name, signifying "stone chaff".

MATERIAL AND METHODS

Fresh living material was used for all studies except measurements of herbarium specimens in ISC. Anatomical material was fixed in FAA and processed for paraffin sections. Leaf material was desilicified in 10% hydrofluoric acid to facilitate sectioning. Anthers for cytological studies were fixed in 3:1 absolute alcohol: glacial acetic acid and stained in aceto-carmine. Embryos were studied from caryopses fixed in chrome-acetic fluid 30 days after pollination. Material was dehydrated and infiltrated with paraffin for sectioning.





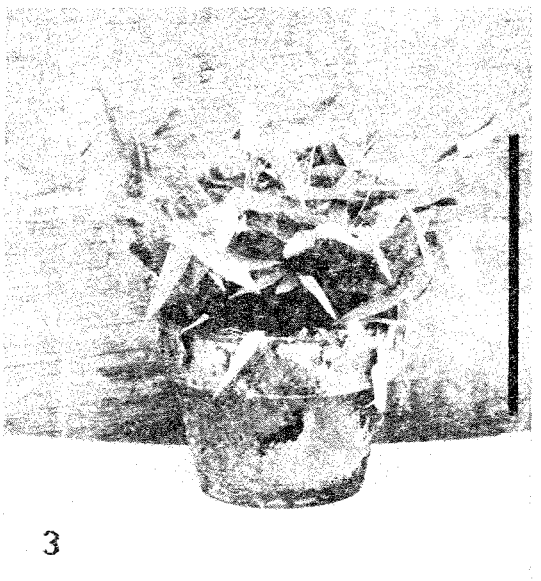


Fig. 1. *L. pauciflora* in a greenhouse chamber.
Fig. 2. *L. pauciflora*. Line scale represents 30 cm.

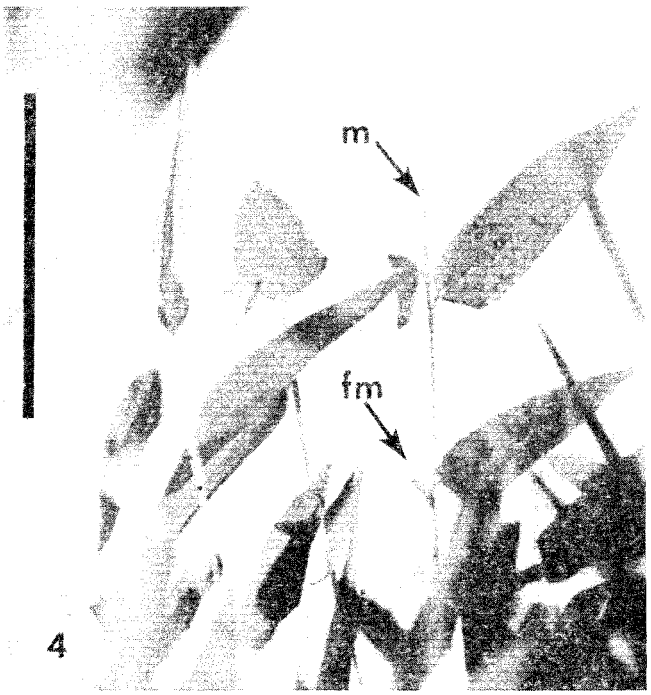
Fig. 3. *L. humilis*. Line: 15 cm.

RESULTS

Inflorescence and spikelet morphology: All species of *Lithachne* are monoecious. Pistillate spikelets are always axillary. Staminate spikelets of *L. pauciflora* are borne just below the pistillate spikelet on the same peduncle (Figs.5, 17), while those of *L. humilis* are borne in small terminal panicles (Figs.4, 19A and B).

Pistillate inflorescences of *L. pauciflora* (Figs.6, 7, 8) are axillary, arising intravaginally at culm nodes. The first partial inflorescence originates from the first short internode of a lateral branch. The later partial inflorescences are borne successively on higher nodes of this branch. Each is enclosed basally by a prophyllum. There may be from one to five borne on a single lateral branch. This arrangement is similar to that in *Olyra latifolia* L., as illustrated by Calderón and Soderstrom (1973).

Pistillate inflorescences of *L. humilis* are very simple, composed of a partial inflorescence originating on a node of a side branch. The inflorescence is intravaginal and its base is enclosed in a prophyllum (Fig. 9). In both species the prophyllum is less than half the length of the



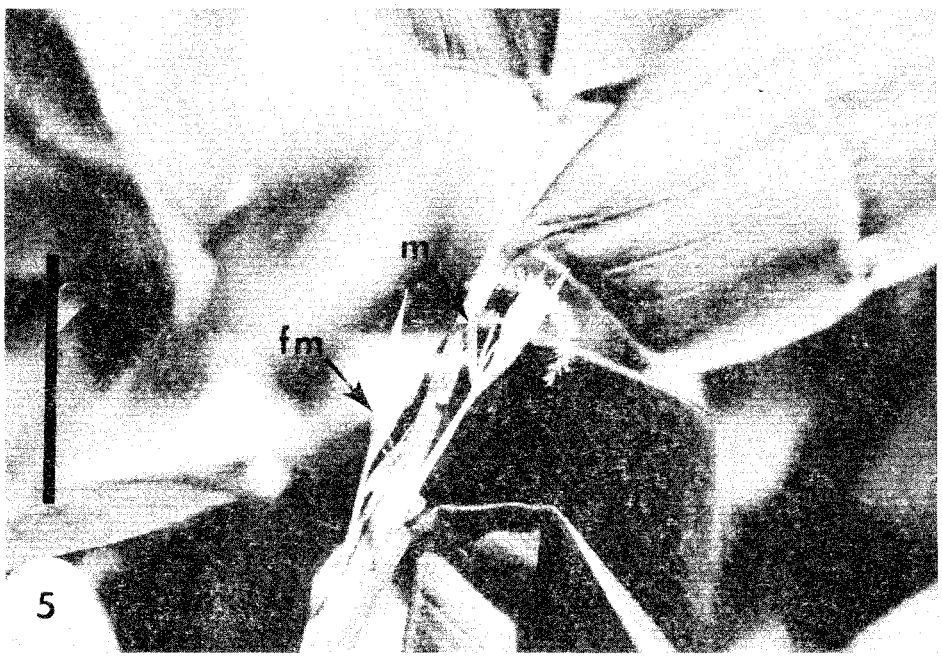
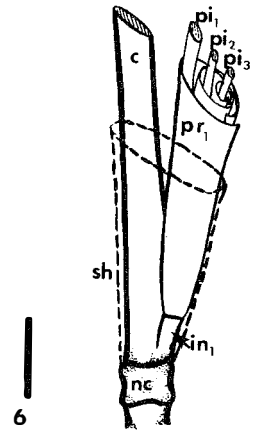
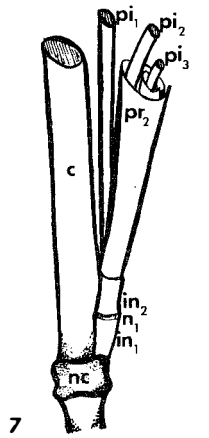
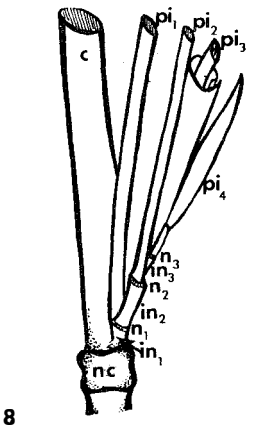


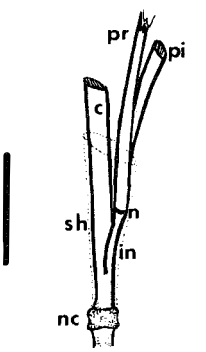
Fig. 4. *L. humilis*, inflorescences.Line: 5cm.
Fig. 5. *L. pauciflora*, inflorescences. Line: 5cm.
Abbreviations: fm = female inflorescence, m = male inflorescence.

peduncle and is never exserted. Pistillate spikelets of *L. pauciflora* have a 9-veined lower glume and a 7-veined upper glume, plus the fertile floret that is strongly laterally compressed, with a cucullate truncate bony lemma that encloses a narrow palea. The gynoecium has a single style and two stigmas (Figs.10, 14, 15). Lodicules are absent. The pistillate spikelet of *L. humilis*

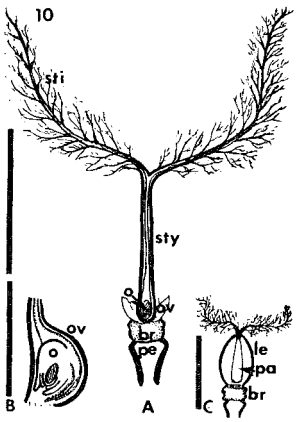


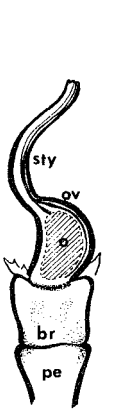


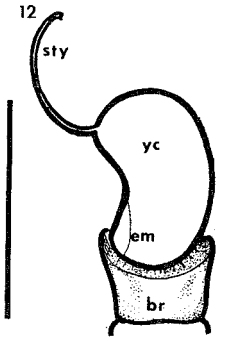


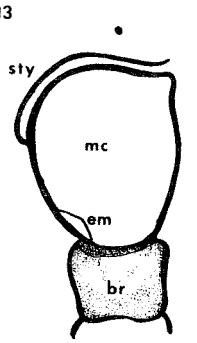


Figs. 6-8. *L. pauciflora*, female inforescences.
Fig.6. Complete inflorescence.
Fig. 7. Inflorescence without leaf sheath and first prophyllum.
Fig. 8. Inflorescence without leaf sheath, first and second prophylla.
Line: 0.5cm.
Fig. 9. *L. humilis*, has a single partial inflorescence.Line: 0.5 cm.
Abbreviations: c = main culm, in = internode, n = node, nc = node of main culm, pi = partial inflorescence, pr = prophyllum









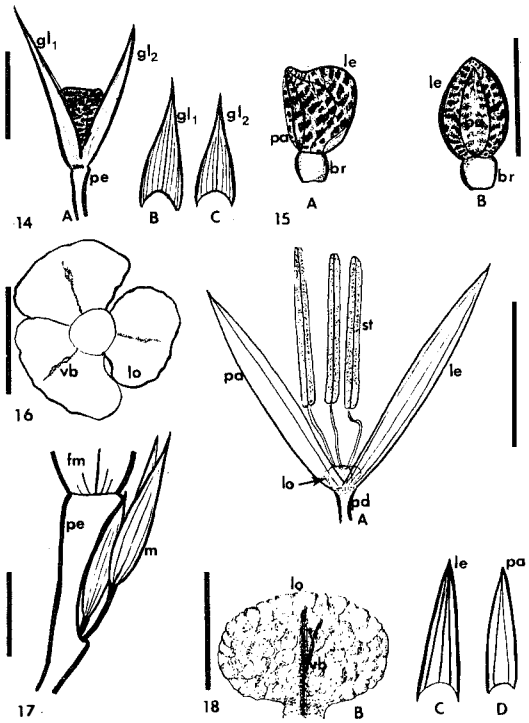
Figs. 10-13. *L. pauciflora*, gynoecium and caryopsis.
Fig. 10. A. Whole gynoeciun with style and stigmas. Line: 3 mm.
B. Semianatropous ovule. Line:80 μm.
C. Whole gynoecium with lemma and palea. Line: 5mm.
Fig. 11. Ovary after pollination. Same scale as Fig. 10 B.
Fig. 12. Young caryopsis.Line: 4mm.
Fig. 13. Mature caryopsis. Same line scale as Fig. 12.
Abbreviations: br = bony rachilla, em = embryo, le = lemma, mc = mature caryopsis, o = ovule, ov = ovary, pa = palea, pe = peduncle, sti = stigma, sty = style, yc = young caryopsis.

is similar to the previous species but has a 7-veined lower glume and a 5-veined upper glume (Figs. 19, D&F).

Staminate spikelets of *L. pauciflora* are located immmediately below the clavate-tipped peduncle (Fig. 17). The staminate spikelets are paired, one pedicellate and one sessile. One to three pairs of staminate spikelets may be borne adjacent. Staminate spikelets of *L. humilis* are borne in an exserted terminal panicle. The spikelets are similar to those of *L. pauciflora* and are paired, one pedicellate and one subsessile. Staminate spikelets of both species lack glumes and consist only of a thin, membranous 3-veined lemma, a 2-veined palea, three truncate lodicules, and three stamens (Figs. 16, 18A-D, 19C 1-3).

The abaxial epidermis of the glumes of pistillate spikelets is similar to that of leaf blades (Fig. 20). The epidermis of the fertile lemma and palea is glabrous and consists of long, thick-walled cells with narrow lumens. The epidermis of the lemma and palea of staminate spikelets is similar to that of the glumes of pistillate spikelets.

Lodicules occur only in the staminate florets



Figs. 14-18. *L. pauciflora*, staminate and pistillate spikelets.
Fig. 14. A. Pistillate spikelet.
B. First glume.
C. Second glume. Line 5 mm.
Fig. 15. A. Pistillate floret (lateral view).
B. Pistillate floret (frontal view). Line: 5 mm.
Fig. 16. Three lodicules with one vascular bundle each Line: 50 um.
Fig. 17. Staminate spikelets, one pedicellate and one subsessile. Line: 2 mm.
Fig. 18. A. Staminate spikelet with three lodicules and three stamens. Line: scale represents 2.5 mm
B. A fleshy lodicule. Line: 40 μm.
C. Lemma of a male floret. Same line scale as Fig. 17.
D. Palea of a male floret. Same line scale as Fig. 17.
Abbreviations: br = bony rachis fm = pistillate spikelet, gl = glume, le = lemma, lo = lodicule, m = staminate spikelet, pa = palea, pe = peduncle, st = stamen, vb = vascular bundle.

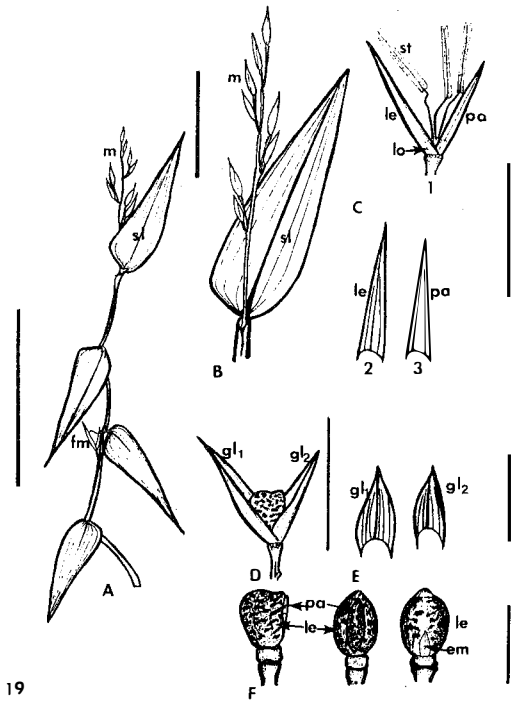
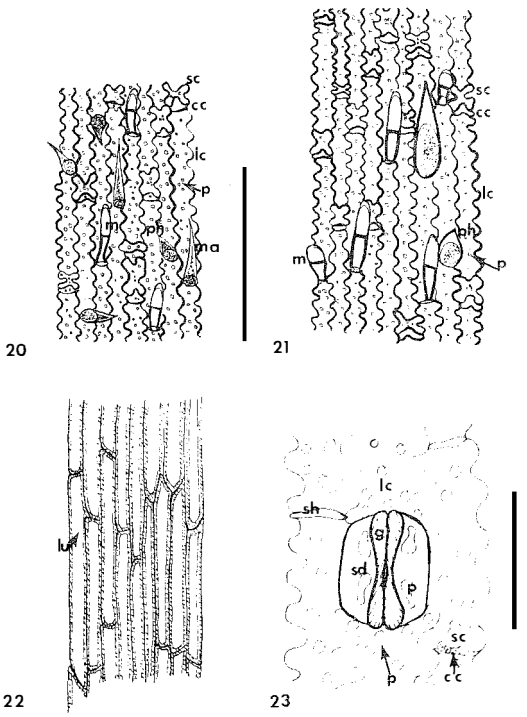


Fig. 19. *L. humilis*, inflorescences and spikelets. A. Whole culm, notice the positions of male and female inflorescences. Line: 5cm.
B. Male inflorescence with subtending leaf. Line 2 cm.
C. Staminate spikelet; 1, complete male floret; 2, lemma; 3, palea. Line: 5 mm.
D. Pistillate spikelet. Line: 8 mm.
E. First and second glumes. Line 8 mm.
F. Pistillate floret in side, front, and rear view, respectively. Line: 4mm.
Abbreviations: em = embryo fm = pistillate inflorescence gl = glume le = lemma lo = lodicule m = staminate inflorescence pa = palea sl = subtending leaf st = staminate inflorescence.

of both species examined. They are similar. As in most other Bambusoideae, there are three lodicules. In *Lithachne*, the lodicules are fleshy and have more or less truncate upper margins. Each has a single vascular bundle. Chloroplasts are lacking. The epidermis is simple, lacking stomata, siliceous cells, and bicellular microhairs. These characteristics agree with the "Olyroid" type of lodicule suggested by Calderón and Soderstrom (1973) as a subtype of the "Bambusoid" lodicule type (Figs. 16, 18A-B, 19C-1).

Gynoecia of *L. pauciflora* and *L. humilis* are similar. In early stages, the gynoecium is bottle-shaped with an ovoid or nearly spherical ovary. The ovary contains a single semianatropous ovule (Fig. 10B). Three vascular bundles arise from a single main trace which enters the ovary at its base. The lateral vascular bundles run through the ovary wall and into the stigmas. The central bundle enters the funiculus and supplies the ovule. The style is slightly conical or cylindrical and bears two plumose spreading stigmas. After pollination the stigmas dehisce and the ovary becomes inflated laterally and the style is progressively shifted to the opposite side (Fig. 11). Ultimately, the region below the style remnant becomes greatly enlar-



Figs. 20-23. *L. pauciflora* and *L. humilis*; epidermises of glumes, lemma, and palea.
Fig. 20. Epidermis of glumes of pistillate spikelet. Line 10 µm
Fig. 21. Epidermis of lemma and palea of staminate spikelet. Same line scale as Fig. 20.
Fig. 22. Epidermis of lemma and palea of pistillate floret Same line scale as Fig. 20.
Fig. 23. Stomate in the epidermis of glumes of pistillate floret and of lemma and palea of staminate floret. Line: 30µ.
Abbreviations: cc = cork cell g = guard cell lc = long cell lu = cell lumen m = bicellular microhair ma = macrohair p = papillae, ph = prickle hair sc = siliceous cell sd = subsidiary cell sh = short cell.

ged to form a gibbous caryopsis (Figs. 12-13). The development of the caryopsis requires 30-35 days from pollination.

Embryo and seedling: A whole caryopsis in longitudinal section (Fig. 24) shows a relatively small embryo and a large endosperm enclosed in the ovary wall. The ovary wall consists of a pericarp layer and an aleurone layer. The cross cells of the pericarp always stain bright red with the stains used. They appear as a black line surrounding the seed in Fig. 25.

Fig. 26 shows a longitudinal section of the embryo of *L. pauciflora*. The vascular tisssue is composed of a short central vascular bundle that diverges into the coleoptile and scutellum at the same level. An epiblast and a cleft between the lower limb of the scutellum and the coleorhiza are present. An embryonic leaf in transverse section (Fig. 26B) shows the overlapping margins and several veins. These characteristics accord with those reported for bamboos and *Lithachne* in having the F+PP formula (Reeder 1957, 1962).

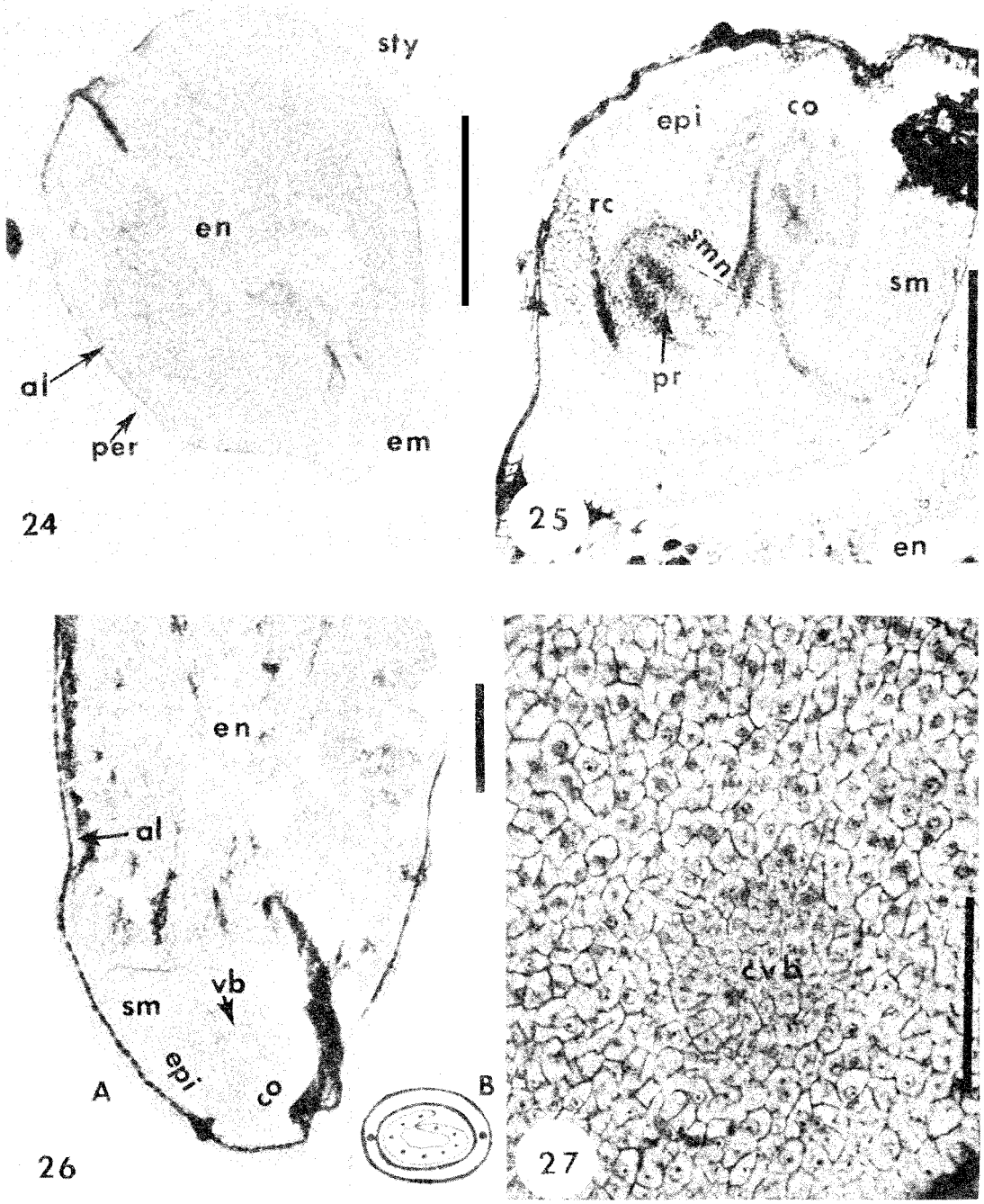
Seeds of *L. humilis* germinate profusely in the greenhouse moist chamber (Pohl 1977) and the plants carpet the granite chip floor of the chamber. James Waddick reports (pers. comm. 1990) that the seeds overwinter and germinate in the spring in Kansas City (lat.39 N, long.94:-30 W).

Fig. 28 shows developing seedlings at various ages. Seedlings do not have a visible coleoptile. The first seedling leaf is very reduced and bladeless. It appeared within 11 days. The second leaf has a reduced blade, and appeared 14 days after germination. The third and successive leaves had blades of normal size. Seedlings with three or four leaves will mature and produce flowers within 50-90 days.

Starch grains: Starch grains in the endosperm of *L. pauciflora* are simple. Figure 29 shows starch grains of *L. pauciflora* under polarized light, indicating the characteristic Maltese cross configuration that indicates radial or circumferential molecular structure. Similar starch grains also occurred in culm tips of *L. pauciflora* and *L. humilis* (Figs. 87, 88).

Chromosome counts: The meiotic chromosome number obtained for *L.pauciflora* from greenhouse-grown material was $n = 11$ (Fig. 30), which is the same as that previously reported by Pohl & Davidse (1971). Quarin (1977) also reported a tetraploid count of $n = 22$.

Leaf morphology and anatomy: Leaf blades of the two species investigated are similar, being flat, narrowly ovate, and with an inconspicuous midrib. The base of the blade is conspicuously oblique. The blades are borne on short pseudopetioles (Figs. 33-34). The ligule is thin, membranous, and inconspicuous. Culm sheaths vary from half to the full length of the internode.



Figs. 24-27. *L. pauciflora*, sections of caryopses in different planes. Fig. 24. Longisection of whole caryopsis. Line 1 mm. Fig. 25. Longisection of embryo. Line 300µm .Fig. 26. A. Oblique longisection of embryo with central vascular bundle strand extended into coleoptile. Line: 300µm B. Embryonic leaf in transverse section. Same line scale as Fig. 25. Fig. 27. Central vascular bundle. Section cut through scutellar nodal region (dashed line in Fig. 25). Line: 120µm. Abbreviations: al = aleurone layer, co = coleoptile, cvb = central vascular bundle, em = embryo, en = endosperm, epi = epiblast, per = pericarp, pr = primary root, rc = root cap, sm = scutellum, smn = scutellar node, sty = style, vb = vascular bundle.

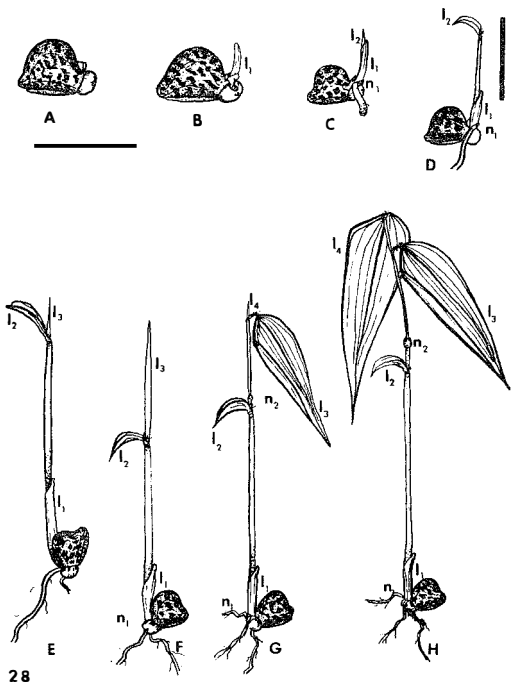


Fig. 28. *L.pauciflora*, seedling sequence of development.
A. 8 days.
B-H. 11-30 days at three day intervals.
A-B. Line 5mm.
C-H. Line 1cm.
Abbreviations: l_1 = first leaf, l_2 = second leaf, l_3 = third leaf, l_4 = fourth leaf, n_1 = first node, n_2 = second node.

Adaxial epidermis: The adaxial epidermis of the blade of both species is similar (Figs. 35, 37). The epidermis is very cutinized and with a well-differentiated costal zone above the veins and intercostal zones between them. The intercostal zones consist of three distinct bands: two stomatal bands on each side of the vein and a band of bulliform cells between.

Short cells (Figs. 35, 37) are abundant in both costal and intercostal zones of both species. The short cells are usually single but may be paired. The short cells in the intercostal zone are transversely narrow and crenate but those of the costal zone vary from transversely narrow to almost cruciform and slightly larger than those of the intercostal zone.

Siliceous cells (Figs. 35, 37, 53-55) are common in both species. Those of the intercostal zone (Fig. 53) are of the transversely narrow and crenate or Olyroid type and are always paired with cork cells; those of the costal zone are found on the midrib and lateral veins. Siliceous cells in the costal zone are usually cross-shaped (Fig. 54) with some variations (Fig. 55). The Oryzoid type of siliceous cell is occasionally found. Siliceous cells in the costal zone occur in a short row or with single cells alternating with long or short cells. Siliceous cells are completely filled with silica bodies. Granules are common in the siliceous cells of the costal zone.

Macrohairs (Figs. 35, 37, 66, 72, 73) are more common in *L. humilis* than in *L. pauciflora*. They occur mostly on veins but occasionally small macrohairs occur on the intercostal zone and at the margins of the blade. Their bases are sunken and the adjacent epidermal cells are slightly tilted.

Prickle hairs (Fig. 50) are angular in both species. They occur only on the blade margins.

Bicellular microhairs (Fig. 37) are found on the adaxial epidermis only in *L. humilis*. They are absent from the adaxial epidermis of *L. pauciflora*. They alternate with long and short cells on each side of a stomatal band and infrequently occur also on the stomatal band. They also occur on the abaxial surface.

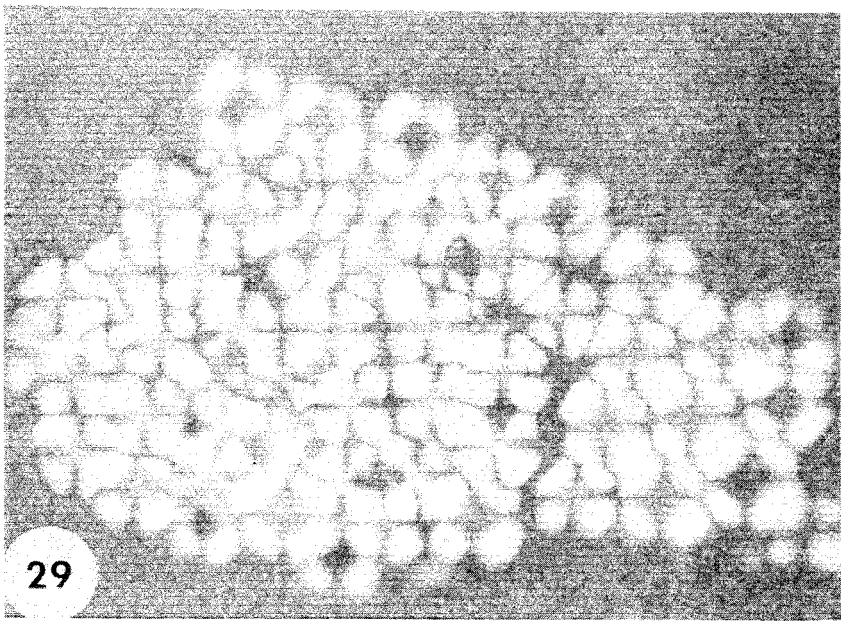
Papillae (Figs. 35, 37) are absent from the long and short cells of *L. pauciflora* but are present on the bulliform cells. In *L. humilis*, papillae are more numerous on the bulliform cells but also occur on long and short cells and interstomatal cells.

Stomata (Figs. 35, 37) are frequent in *L. humilis* and very rare in *L. pauciflora*. Each stomatal band in *L. humilis* consists of one or two rows of stomata alternating with each other on each side of a vein. The stomatal apparatus consists of two long dumbbell-shaped guard cells and two triangular subsidiary cells.

Bulliform cells (Figs. 35, 37) are arranged in two to five rows centrally in the intercostal zone. They are inflated, round to rectangular, with thick and strongly sinuous walls.

Long cells (Figs. 35, 37) are arranged in rows between the veins and the bands of bulliform cells. Their walls are also sinuous, but thinner than the walls of bulliform cells. Each long cell alternates with a transversely crenate short cell or a pair of a siliceous cell and a cork cell.

Interstomatal cells (Fig. 37) of *L. humilis* have concave end walls abutting the stomata.



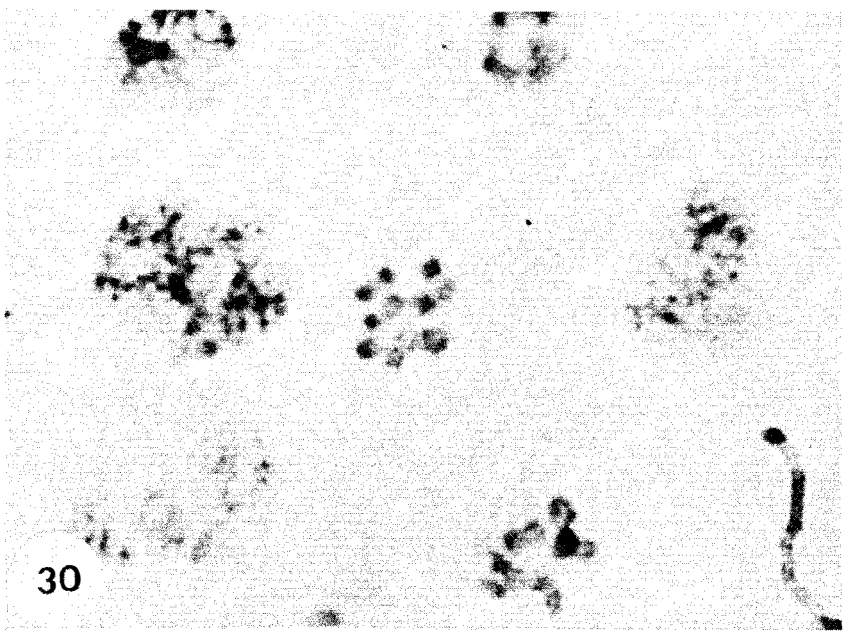
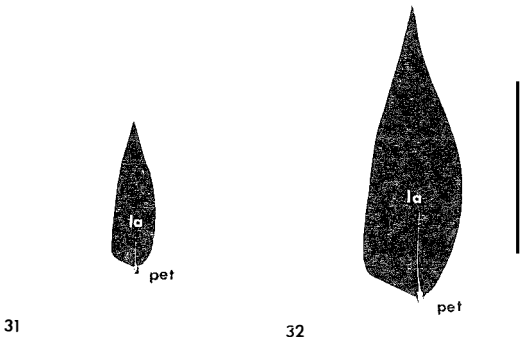


Fig. 29. *L. pauciflora*. Starch grains from the caryopsis, polarized light. 1212 X. Fig. 30. *L. pauciflora*. Chromosomes from microsporocyte. n = 11. 1212 X.

Abaxial epidermis: The abaxial epidermis of leaf blade is similar to the adaxial epidermis, having distinct costal and intercostal zones. The intercostal zone has two stomatal bands with a band of long cells between them. Bulliform cells are lacking.

Short cells (Figs. 36, 38, 40, 45) are abundant on the abaxial epidermis of both species. They are usually single, alternating with long cells, but may be associated with siliceous cells. Short cells of intercostal zones are transversely crenate but those of the costal zone



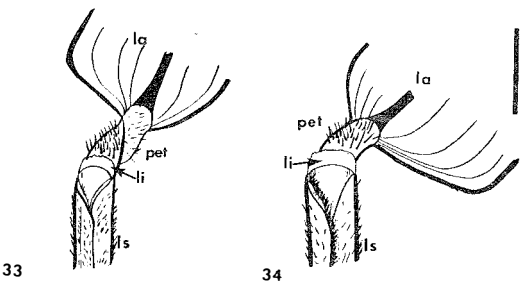


Fig. 31. *L. humilis*, leaf blade.
Fig. 32. *L. pauciflora*, leaf blade. Line: 5 cm in Figs. 31-32.
Fig. 33. *L. humilis*, pseudopetiole.
Fig. 34. *L. pauciflora*, pseudopetiole. Line: 2 mm in Figs. 33-34. Abbreviations: la = lamina, li = membranous ligule, ls = culm sheath, pet = pseudopetiole.

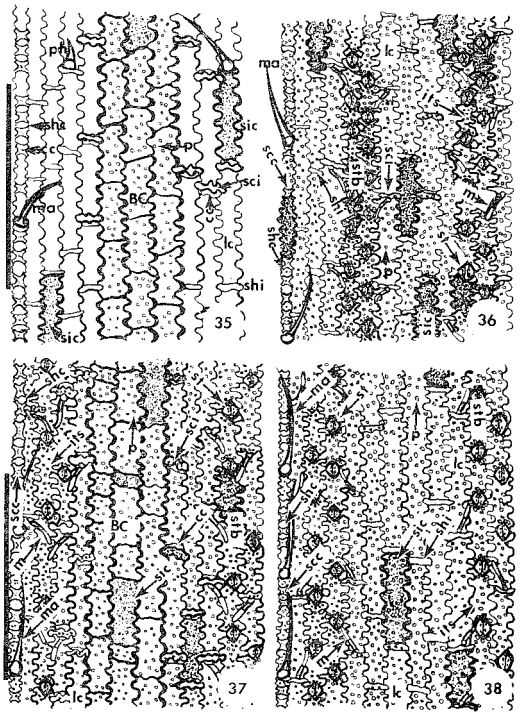


Fig. 35. *L. pauciflora*, adaxial epidermis of the leaf blade. Line: 300 µm.
Fig. 36. *L. pauciflora*, abaxial epidermis of the leaf blade. Same scale as Fig. 35. Fig. 37. *L. humilis*, adaxial epeidermis of the leaf blade. Line: 250µm.
Fig. 38. *L. humilis*, abaxial epidermis of the leaf blade. Same scale as Fig. 37. Abbreviations: bc = band of bulliform cells, cc = cork cell, its = interstomatal cell, lc = long cell, m = bicellular microhair, ma = macrohair, p = papillae, ph = prickle hair, scc = siliceous cell in the costal zone, sci = siliceous cell in intercostal zone, shc = short cell in costal zone, shi = short cell in intercostal zone, sic = silicified epidermal cell, st = stomate, stb = stomatal band.

vary from transversely crenate to cross-shaped and slightly larger.

Siliceous cells (Figs. 36, 38, 40, 45, 48, 53, 55) on the intercostal zone are less common than those of the adaxial epidermis but are of the same shape. Those of the costal zone may be cruciform, saddle-shaped, or transversely crenate (Oryzoid) type. They are usually arranged in one row on minor veins or two or more rows on major veins and the midrib. They may be single or paired or occur in short rows interrupted at intervals by a short cell.

Other epidermal cells have a tendency to be silicified, more frequently in *L. humilis* than in *L. pauciflora*. Such cells may be completely or partially filled with silica.

Cork or suberized cells are always associated with siliceous cells. They are similar to the short cells of the intercostal zone.Macrohairs are very abundant on the veins of the abaxial epidermis in both species and occasionally may occur on the intercostal zones.Prickle hairs are similar to those of the adaxial epidermis. Bicellular microhairs (Figs. 36, 38, 40, 41, 45, 46) are very common on both species. They are usually paired with short cells and restricted to both sides of a stomatal band, alternating with long cells. The two cells are about equal, but the basal cell is thicker-walled than the upper cell, which always collapses under the microtechnique process used. The upper cell has a rounded or somewhat acute tip. Microhairs of *L. pauciflora* are longer than those of *L. humilis*.

The numerous stomata (Figs. 36, 38, 42, 43, 44, 47, 49) occur in bands of one or two rows that alternate with each other. The stomatal bands are restricted to the sides of the veins. Stomata are separated by single papillate inters-

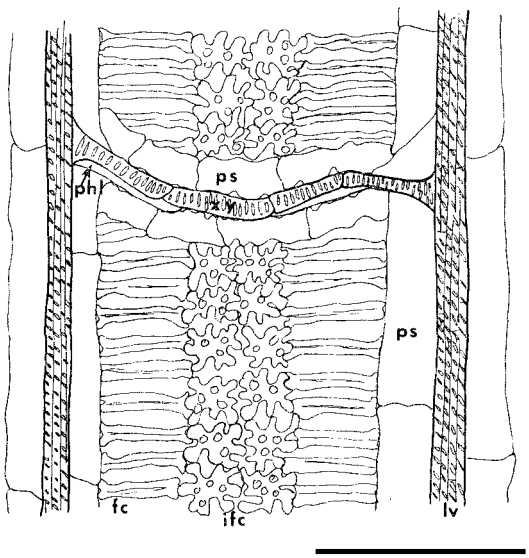


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Fig. 39. *L. pauciflora*.Cleared leaf shows the internal structure of leaf and a transverse veinlet. Line: 150µm. Abbreviations: fc = fusoid cell, ifc = interfusoidal cell, lv = longitudinal vein, phl = phloem, ps = parenchymatous sheath, xy = xylem.

tomatal cells that have concave end walls. Stomata are similar to those of the adaxial surface. Each subsidiary cell has two papillae that overarch the guard cells and obscure their outlines.

Papillae are abundant. They are small, rounded, and have a thick cuticle. Papillae of interstomatal and guard cells may overarch the stomatal openings (Figs. 40, 42-45).

Long cells (Figs. 36, 38, 40, 45) of the intercostal zones are strongly sinuous and thick-walled, with many cutinized papillae on their surfaces. Each long cell alternates with a single short cell or occasionally a pair of short cells. Long cells in a row adjacent to the stomatal band are always separated by a pair of one short cell and a bicellular microhair.

Interstomatal cells (Figs. 42, 45, 47) are strongly sinuous with concave end walls and are strongly papillate. They may be short or about as long as other long cells.

Transverse veinlets or commissural veins (Fig, 39) are very common in both species, connecting the longitudinal veins. They consist of one or two strands of xylem and phloem elements, surrounded by a parenchyma sheath of large, thin-walled cells.

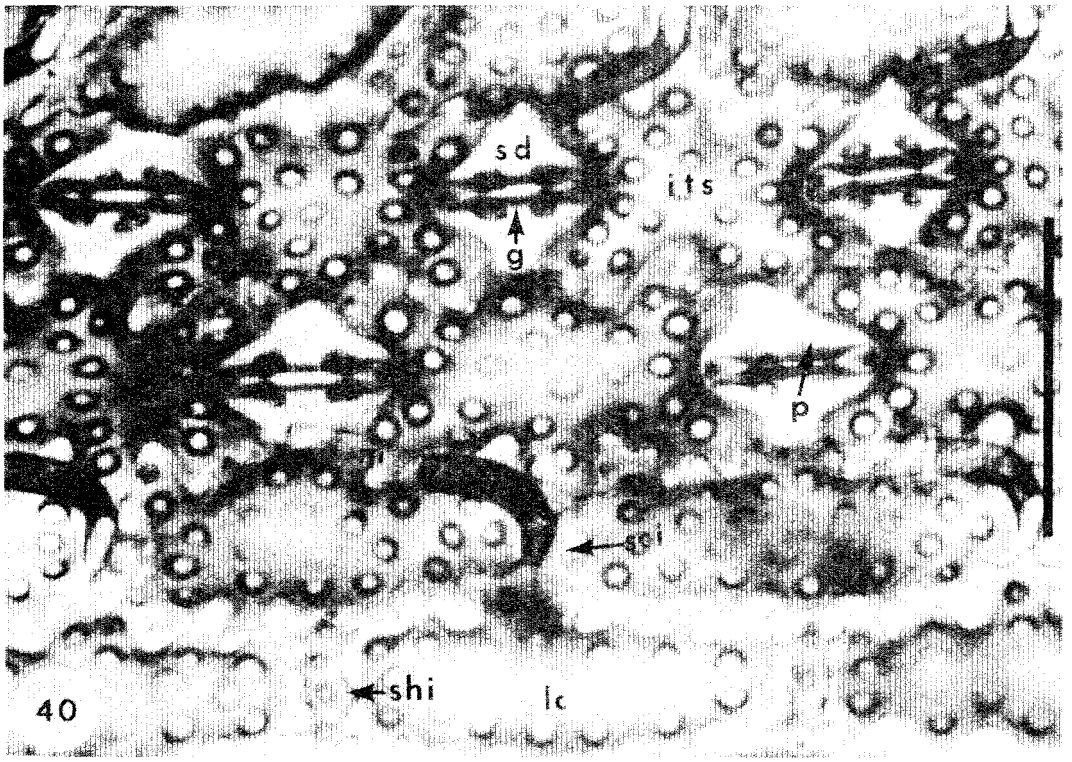
Leaf blades in transverse and longitudinal sections: Leaf blades of both species are flat and uniformly thick except at the thinner margins. The surface in both species is slightly wavy because of the bulliform cells, long cells, and veins.

Midribs of both species are inconspicuous and simple, containing only one vascular bundle in *L. pauciflora,* with an additional two small vascular bundles on either side of the midrib bundle in *L. humilis* (Figs. 60, 68, 69). Blades are slightly asymmetric, with 3-4 veins in the wider side and 2-3 in the narrower. Vascular bundles are not conspicuously angular in outline. The midrib bundle contains two or three large metaxylem vessels and a protoxylem lacuna close to the adaxial surface and the phloem close to the abaxial surface. The midrib bundle is imbedded in ground tissue close to the abaxial surface. The bundle sheath is double, with a complete mestome sheath and an abaxially interrupted outer sheath. The mestome sheath of *L . humilis* (Fig. 69) consists of several layers of sclerenchymatous cells while there is only one layer in *L. pauciflora* (Fig. 60). The outer sheath contains relatively few chloroplasts. There is a strand of sclerenchymatous fibers toward the adaxial surface and a girder of fibers toward the abaxial surface. The sclerenchyma fibers substitute for the regular epidermis and form a flat-topped keel (Fig. 60, 69).

Major veins (Figs. 61, 63, 70) of the blade are similar to the midrib bundle, differing in that the outer sheath is interrupted abaxially and adaxially by girders of sclerenchyma. The girders are hypodermal and do not interrupt the epidermal layer as in the midrib bundle.

Minor veins (Figs. 62, 71) are regularly spaced between adjacent major veins. They consist of a few xylem and phloem elements. The inner bundle sheath is not well differentiated and may be lacking. The outer bundle sheath is composed of one or two large, thin-walled cells and a few small cells with few chloroplasts.

Transverse veinlets or commissural veins (Figs. 64, 65) connect adjacent longitudinal veins. They are very simple, consisting of one or two xylem and phloem elements and thin-walled parenchyma sheath cells.



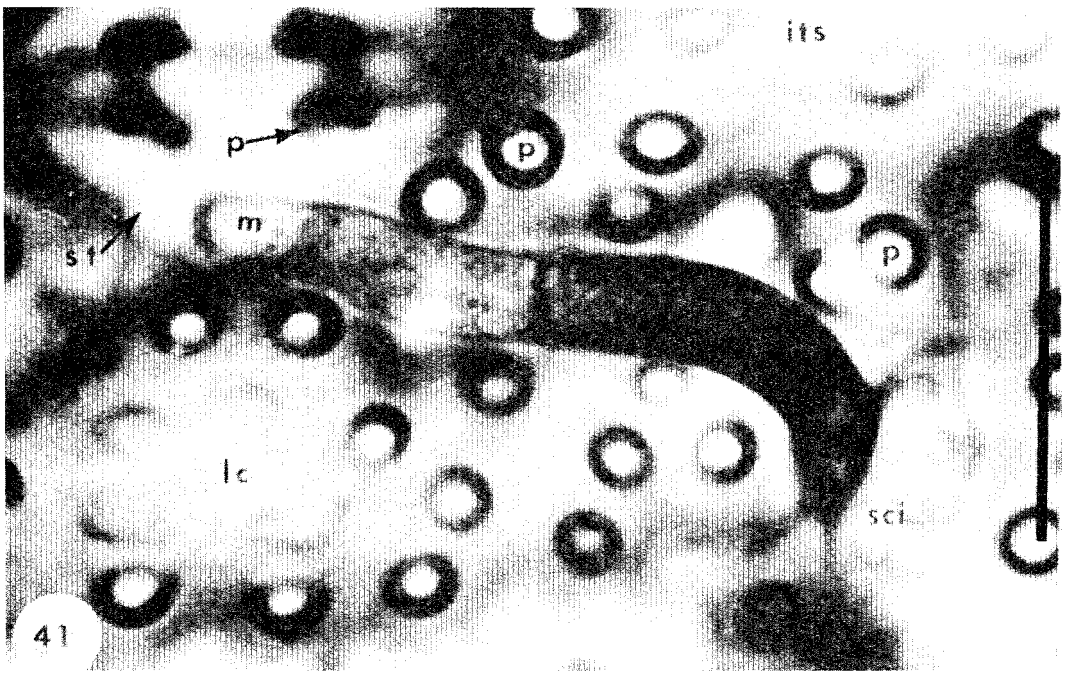


Fig. 40. *L. pauciflora,* adaxial epidermis with the stomatal band. Line: 50 µm.Fig. 41. *L. pauciflora*, adaxial epidermis, enlarged to show the bicellular microhair. Line: 25 µm.Abbreviations: g = guard cell, its = insterstomatal cell, lc = long cell, m = bicellular microhair, p = papillae, sci = siliceous cell in intercostal zone, sd = subsidiary cell, shi = short cell in intercostal zone, st = stomate.

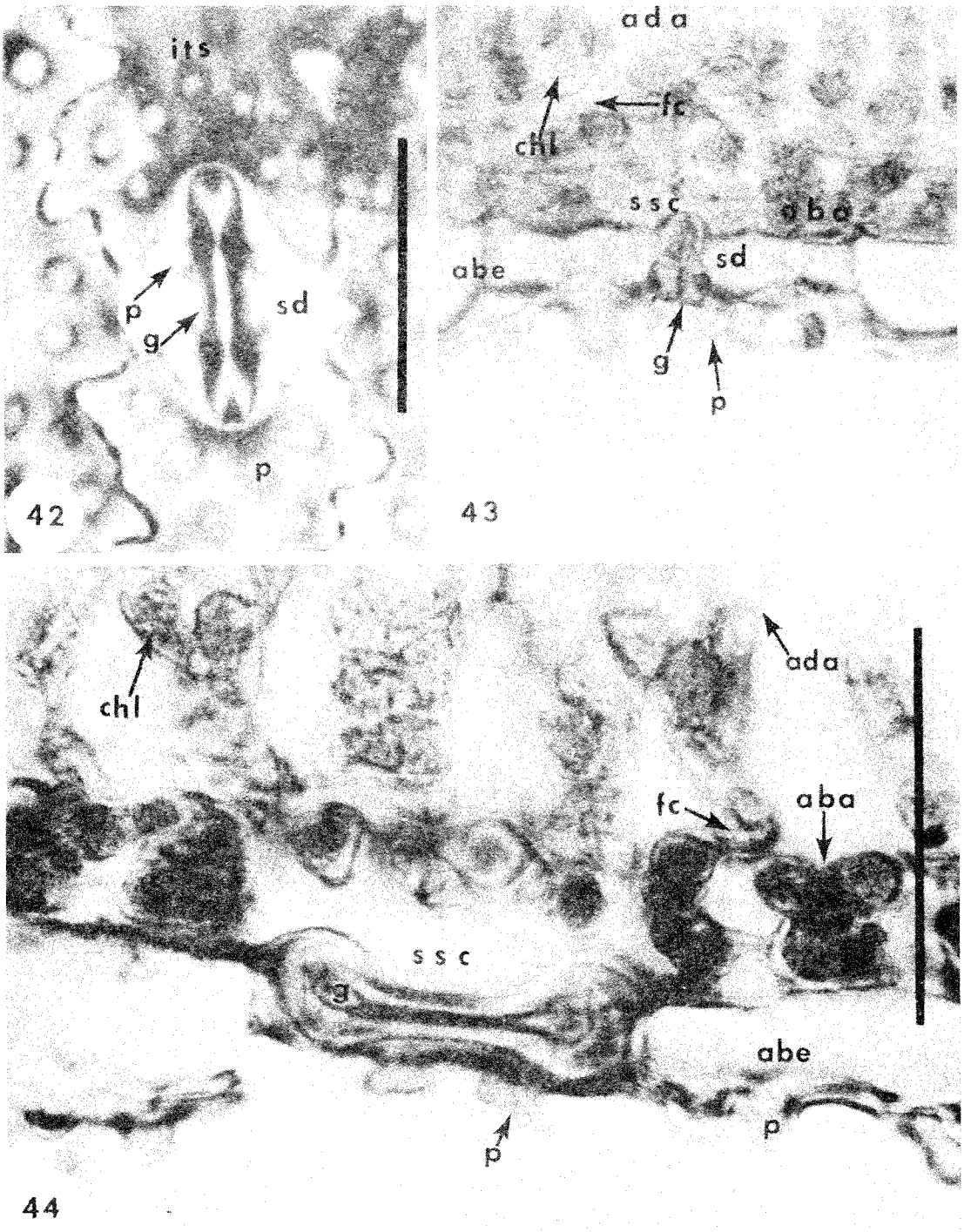


Fig. 42. *L. pauciflora*, stomate. Line: 25 µm.
Fig.43. *L. pauciflora*, stomate in transverse section. Same line scale as Fig. 42.
Fig. 44. *L. pauciflora*, stomate in longisection. Line 25 µm.
Abbreviations: aba = abaxial arm cell, abe = abaxial epidermis, ada = adaxial arm cell, chl = chloroplast, fc = fusoid cell, g = guard cell, its = interstomatal cell, p = papillae, sd = subsidiary cell, ssc = substomatal chamber.

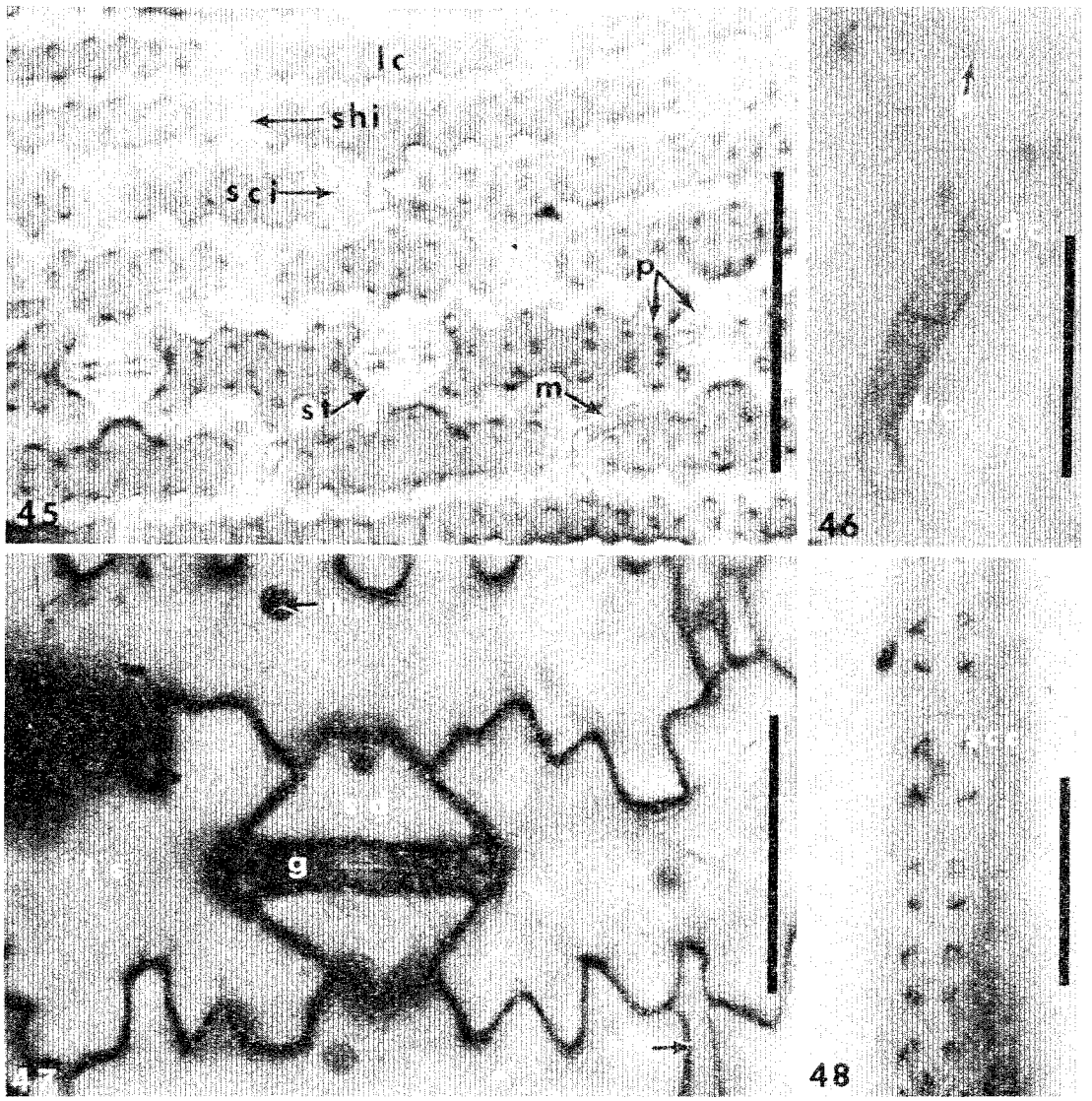


Fig. 45. *L. humilis*, abaxial epidermis with a stomatal band and bands of long cells. Line: 100 µm.
Fig. 46. *L. humilis*, abaxial epidermis with a single bicellular microhair. Line: 25µm. Fig. 47, *L. humilis*, stomate. Line: 25 µm.
Fig. 48. *L. humilis*, epidermis with a row of siliceous cells over the vein. Line: 40 µm.
Abbreviations: bc= basal cell, dc = distal cell, g = guard cell, its = interstomatal cell, lc = long cell, m = bicellular microhair, n = nucleus, p = papillae, scc = siliceous cell in intercostal zone, sd = subsidiary cell, shi = short cell in intercostal zone, st = stomate.

Mesophyll (Fig. 51) consists of arm cells, fusoid cells, and chlorenchyma and ground tissues. Fusoid cells are thin-walled and usually collapse during preparation. They lie between the adaxial and abaxial arm cell layers in a continuous transverse row (Figs. 59A-B, 67, 74). Fusoid cells appear as long translucent cavities on both sides of the vein in transverse view (Figs. 63, 70). In a surface view of a cleared leaf blade, fusoid cells are slightly wrinkle-walled, with flat or round ends (Fig. 59 C). The length of a fusoid cell is about 0.3-0.5 times the width of the intercostal zone.

Arm cells are arranged in two layers, one below the adaxial epidermis and the other below the abaxial epidermis (Figs. 52, 56, 57). A

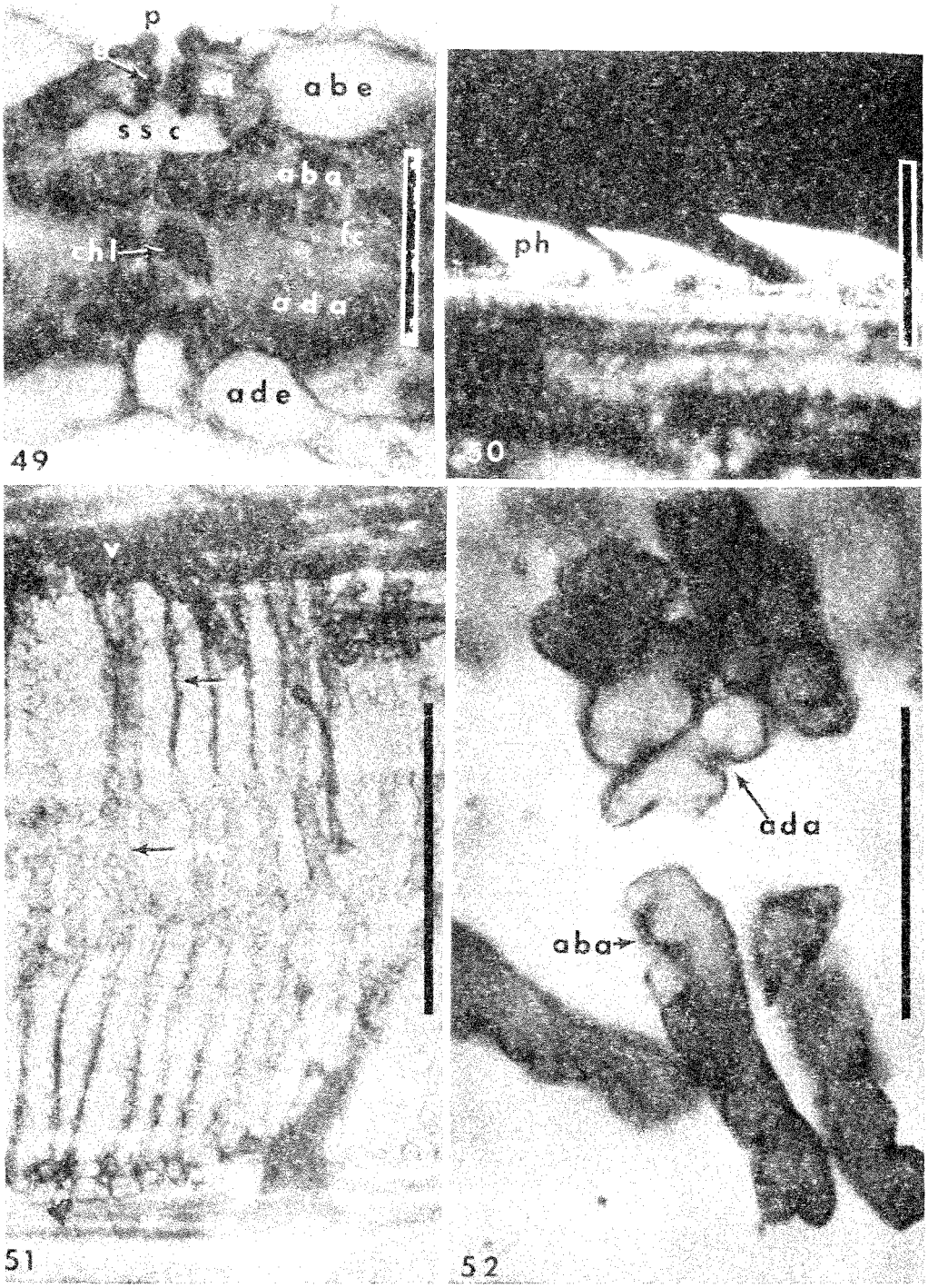


Fig. 49. *L. humilis*, leaf blade transverse section with a stomate in cross section. Line: 50 µm.Fig. 50. *L. humilis*, leaf margin with prickle hairs, polarized light. Line: 100 µm.Fig. 51. *L. humilis*, cleared leaf with mesophyll cells. Line: = 100 µm.Fig. 52. *L. humilis*, longitudinal leaf section with adaxial and abaxial arm cells. Line: 50 µm.Abbreviations: aba = abaxial arm cell, abe = abaxial epidermis, ada = adaxial arm cell, chl = chloroplast, fc = fusoid cell, g = guard cell, ifa = interfusoidal arm cell, p = papillae, pb = prickle hair, ph = prickle hair, sd = subsidiary cell, ssc = substomatal chamber, v = vein.

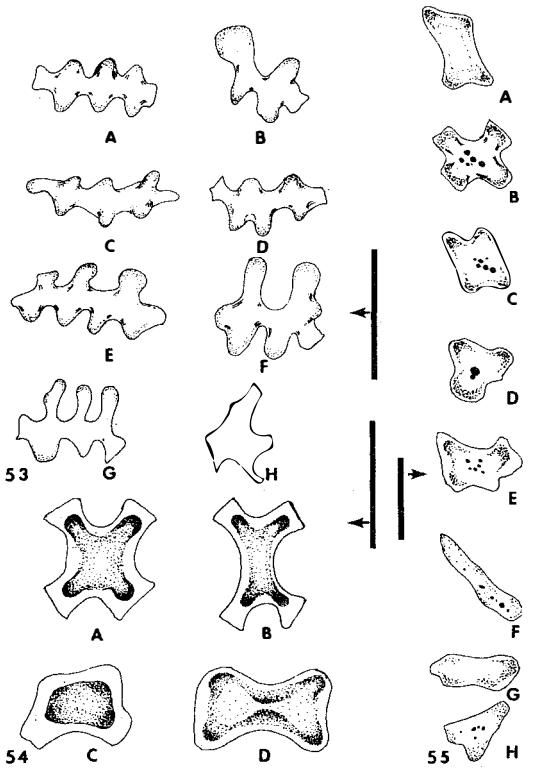


Fig. 53. A-H. Variations of siliceous cells in the intercostal zones of *L. pauciflora* and *L. humilis*. Line: 30 µm.
Fig. 54. A-D. Four main types of siliecous cells over the vein of *L. pauciflora* and *L. humilis*. Line: 15 µm.
Fig. 55. A-H. Variations of siliceous cells in the costal zones of *L. pauciflora* and *L. humilis*.

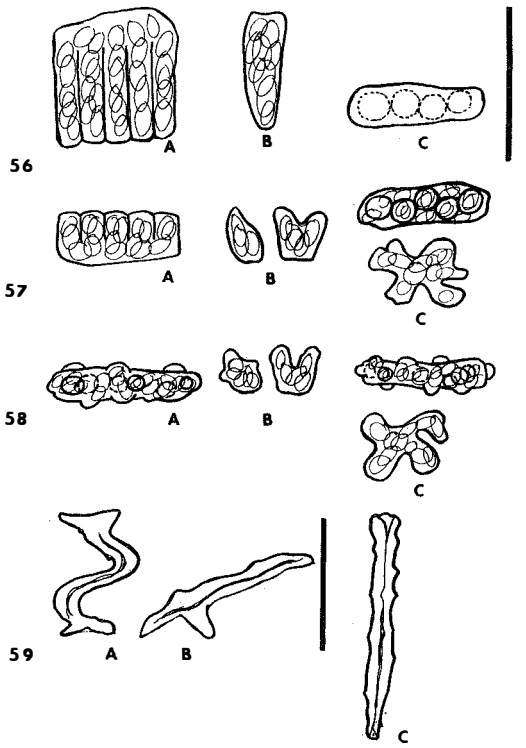


Fig. 56. Adaxial arm cells in the mesophyll of *L.pauciflora* and *L. humilis*. Line: 20 µm.
Fig. 57. Abaxial arm cells in the mesophyll of *L. pauciflora* and *L.humilis*. Same line scale as Fig. 56.
Fig. 58. Interfusoidal arm cells in the mesophyll of *L. pauciflora* and *L.humilis*. Same line scale as Fig. 56.
Figs. 56-58: A = view from transverse section of leaf blade, B = view from longisection of the leaf, C = view from adaxial or abaxial side of a cleared leaf.
Fig. 59. Fusoid cells of *L. pauciflora* and *L. humilis*.
A-B: transverse sections of fusoid cells (longisections of the leaf blade). Line: 5 µm C. view from adaxial side of a cleared leaf. Line: 50 µm.

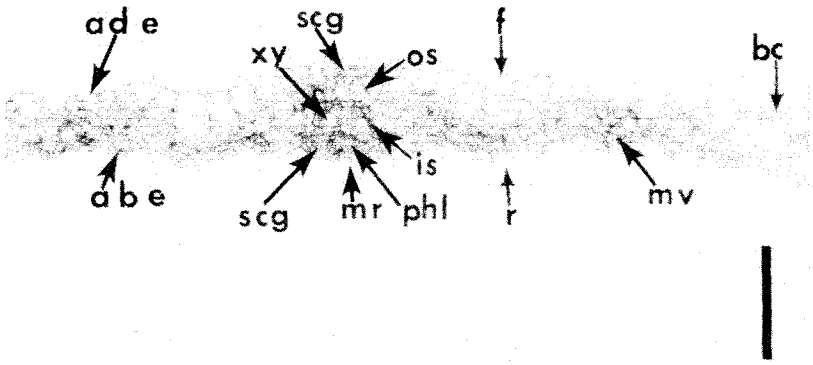
second type of arm cell occurs between the ends of two fusoid cells. These are termed "interfusoidal arm cells" (Figs. 51, 58). Arm cells are oriented with their rounded sides adjacent to the epidermis and the finger-like projections toward the fusoid cells.

Bulliform cells in transverse section (Figs. 60, 64, 68, 69) are inflated, almost round to oval in outline, and have slightly papillate outer walls. They occur in two to five files between adjacent veins.

Epidermal cells have a thick cuticle and are circular to oval in outline. Despite the cuticle, the blades are very prone to desiccation under water stress. The outer surfaces of both epidermises are similar in *L. humilis* but only the abaxial epidermis is papillate in *L. pauciflora*. The cutinized papillae in section appear as small cylindrical knobs with rounded ends on the outer walls of epidermal cells (Figs. 63, 67).

Culm anatomy: Culms of both species have solid lower internodes and hollow upper internodes. Vascular bundles are separate in the internodes and anastomose at the nodes. Wall thickenings may be scalariform, annular, or helical. The stem epidermis in longitudinal section shows long cells alternating with single short cells or a pair of a short cell and a siliceous cell. Epidermal cells are smooth or slightly papillose. The hypodermal layers, up to ten cells thick, are composed of long, thick-walled tapered cells with pointed ends (Figs. 75, 76, 77, 79, 80, 81).

The culm apices in both species (Figs. 77, 78) show two tunica layers with an inconspicuous



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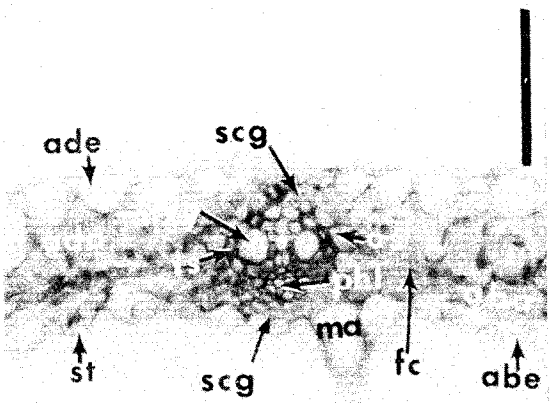
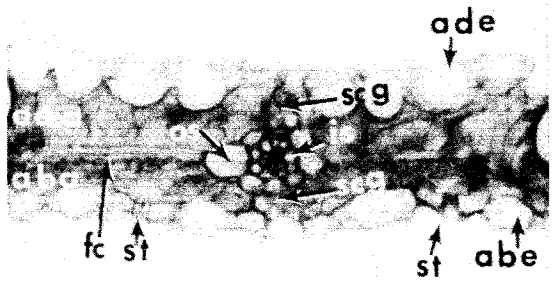


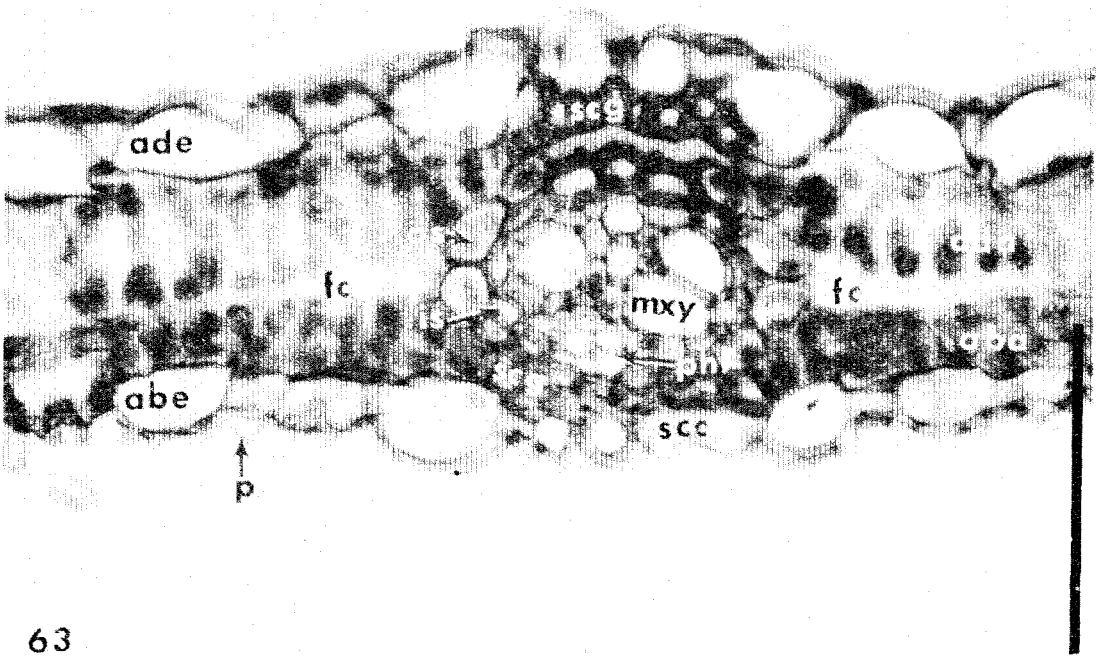


61 62

Fig. 60. *L. pauciflora* leaf; transverse section through midrib. Line: 100 µm.
Fig. 61. *L. pauciflora* leaf; blade; transverse section through major vein. Line: 100 µm.
Fig. 62. *L. pauciflora* leaf; blade, transverse section through minor vein. Same line scale as Fig. 61.
Abbreviations: aba = abaxial arm cell, abe = abaxial epidermis, ada = adaxial arm cell, ade = adaxial epidermis, bc = bulliform cell, f = furrow, fc = fusoid cell, is = inner sheath, ma = macrohair, mr = midrib, mv = minor vein, os = outer sheath, phl = phloem, r = rib, scg = sclerenchymatous girder, st = stomate, xy = xylem.

inner tunica layer and an area of corpus at the center.

Transverse sections of the culms of both species are similar. Sections of young internodes (Figs. 80, 81) show the initial stage of vascular bundle development, hypodermal layers, and the pith. The vascular bundles form two inconspicuous rings in which the outer ring is



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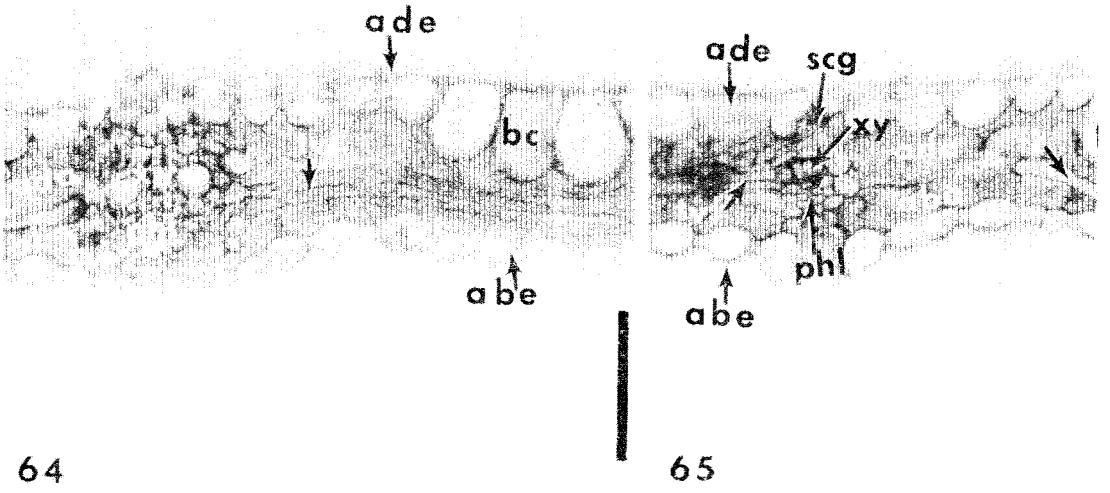

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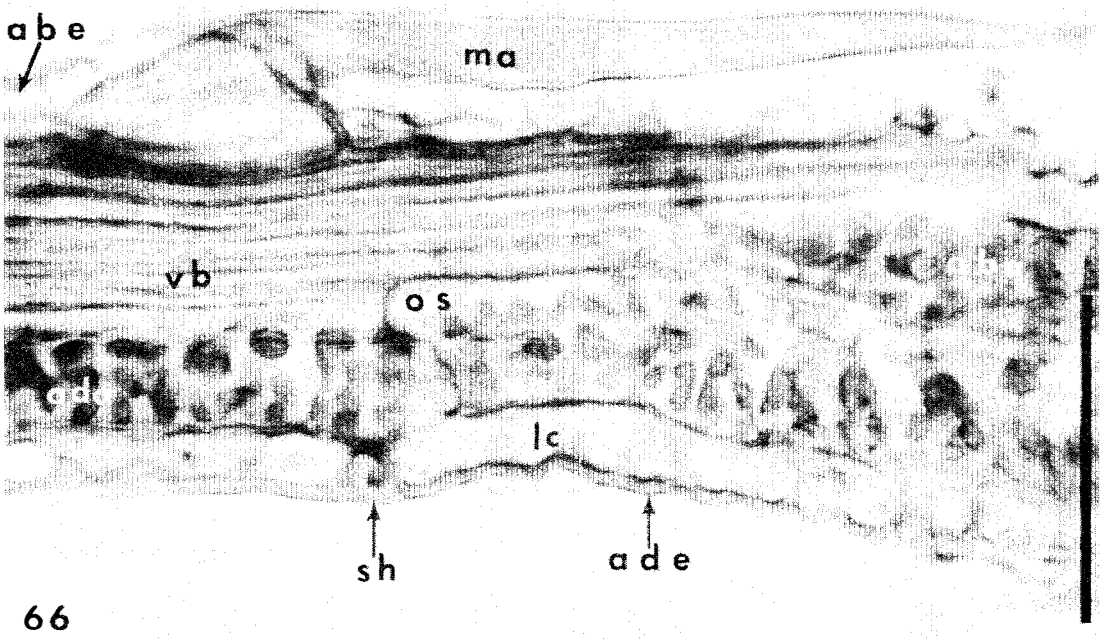
Fig. 63.Transverse section through major vein of *L. pauciflora,* with fine details. Line: 50µm.Fig. 64.Transverse section through a major vein of *L. pauciflora* with a transverse veinlet. Line: 50µm. Fig. 65. Transverse section through a minor vein of *L. pauciflora* with a transverse veinlet emerging from the minor vein. Same scale as Fig. 64. Abbreviations: aba = abaxial arm cell, abe = abaxial epidermis, ada = adaxial arm cell, ade = adaxial epidermis, bc = bulliform cell, fc = fusoid cell, is = inner sheath, mxy = metaxylem, os = outer sheath, p = papillae, phl = phloem, scc = siliceous cell in costal zone, scg = sclerenchymatous girder, tvl = transverse veinlet.



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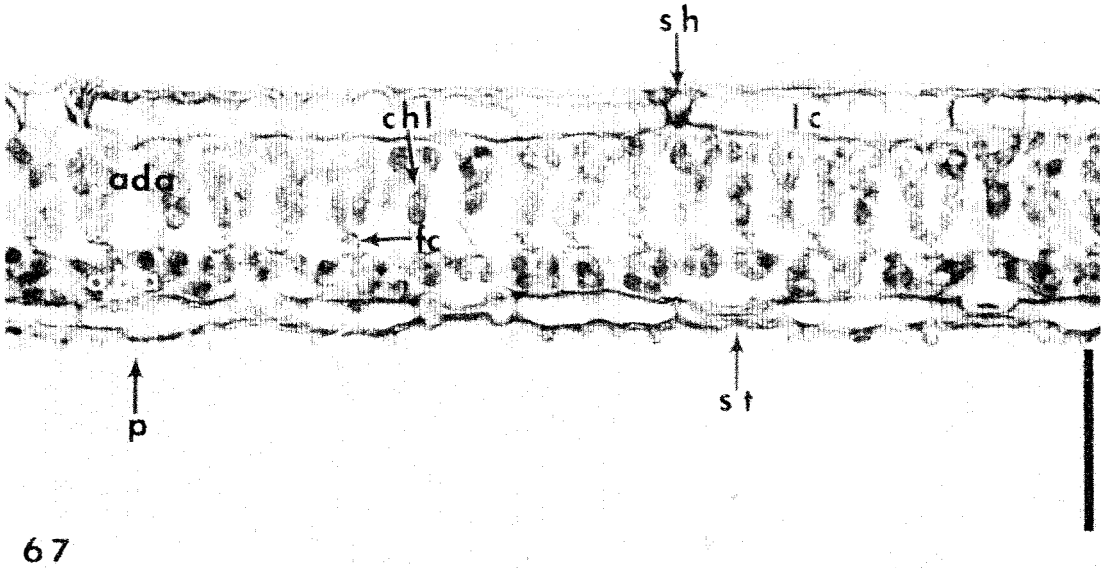

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Fig. 66. *L. pauciflora*, leaf longisection with a macrohair. Line: 50µm.
Fig. 67. *L. pauciflora*, leaf longisection with the structure.of mesophyll. Line 50µm.
Abbreviations: aba = abaxial arm cell, abe = abaxial epidermis, ada = adaxial arm cell, chl = chloroplast, lc = long cell, ma = macrohair, os = outer sheath, p = papillae, sh = short cell, st = stomate.

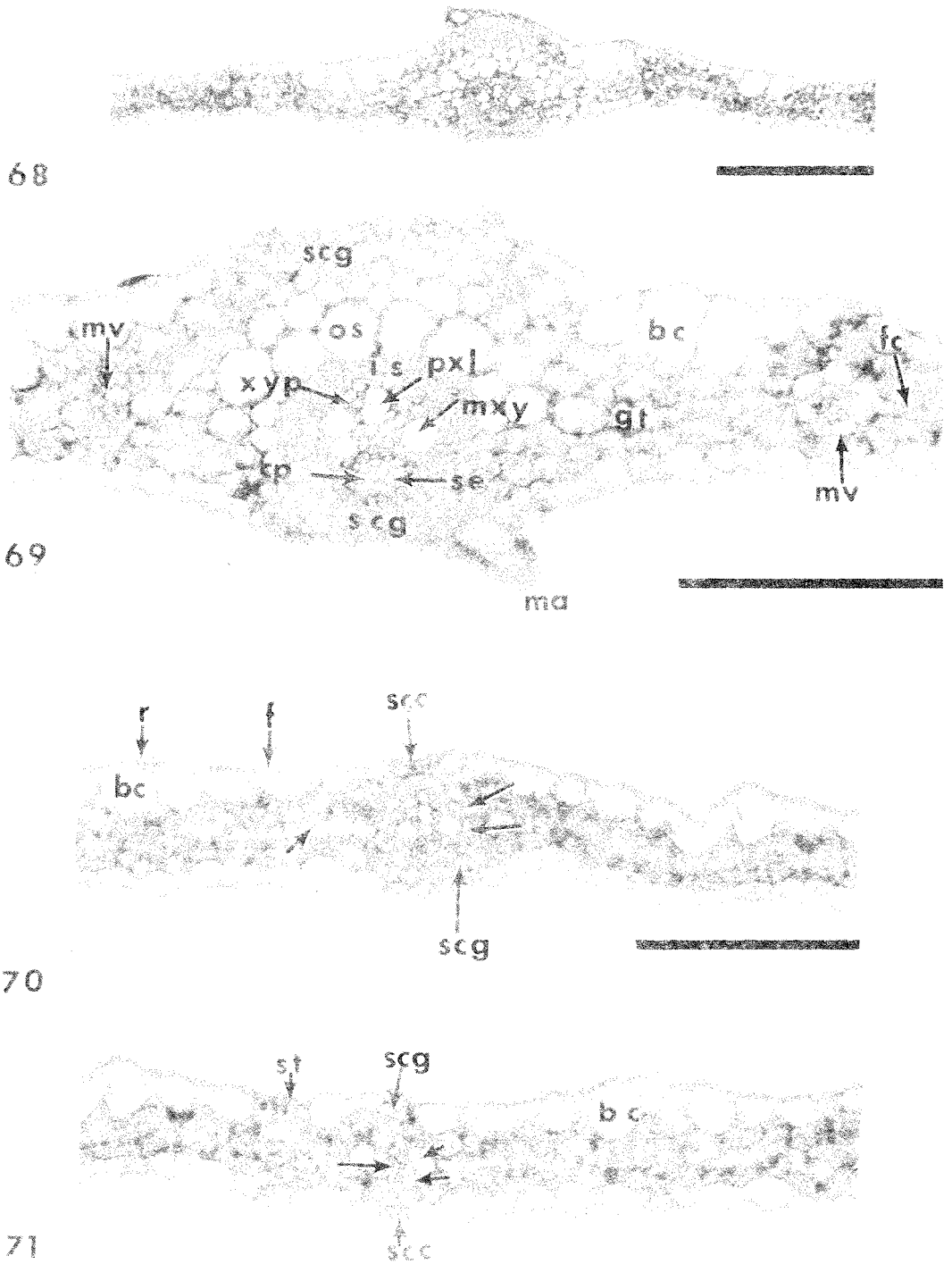
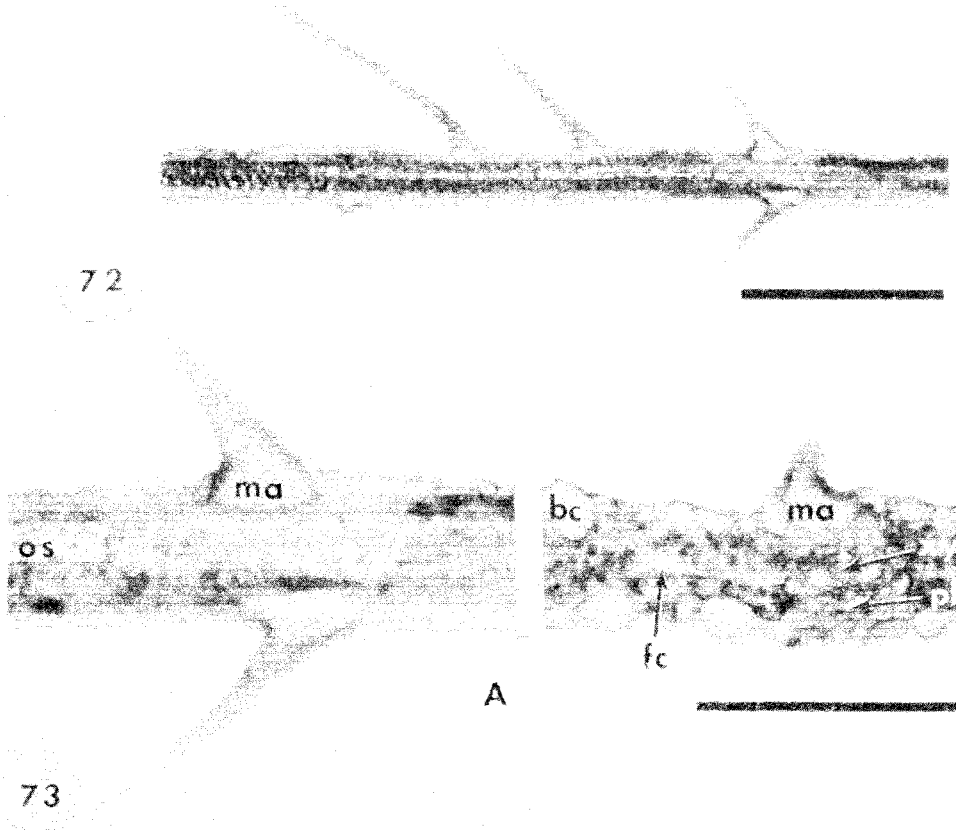


Fig. 68. Transverse section.of *L. humilis* leaf. Line: 150 µm.Fig. 69. Transverse section through midrib of *L. humilis*. Line: 100µm.Fig. 70. Transverse section of major vein. of *L. humilis*. Line 100µm.Fig. 71. Transverse section of minor vein of *L . humilis*. Same line scale as Fig. 70. Abbreviations: bc = bulliform cell, cp = companion cell, f = furrow, fc = fusoid cell, gt = ground tissue, is = inner sheath, ma = macrohair, mv = minor vein, mxy = metaxylem, os = outer sheath, pxl = protoxylem lacuna, r = rib, scc = siliceous cell in costal zone, scg = sclerenchymatous girder, se = sieve element, st = stomate, vb = vascular bundle, xyp = xylem parenchyma.



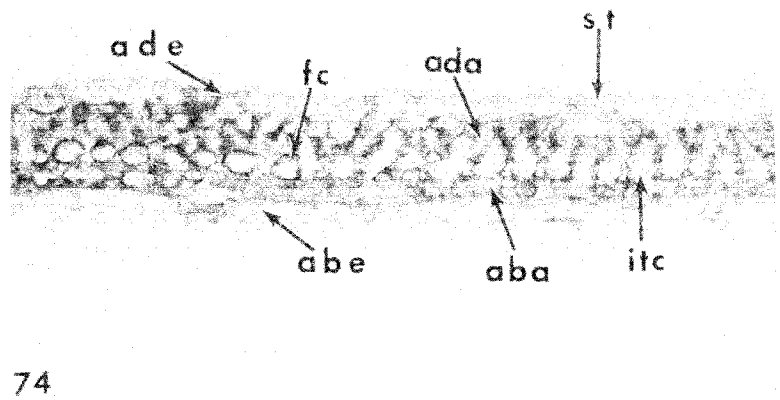


Fig. 72. *L. humilis*, leaf blade longisection with macrohairs on both surfaces. Bar = 200µ. Fig. 73. A-B. *L. humilis*, leaf blade with sunken base of macrohairs. A = longisection, B = transverse section. Bar = 100µm Fig. 74. *L. humilis*, leaf blade longisection, mesophyll structure. Same scale as Fig. 73. Abbreviations: aba = abaxial arm cell, abe = abaxial epidermis, ada = adaxial arm cell, ade = adaxial epidermis, bc = bulliform cell, fc = fusoid cell, itc = intercellular space, ma = macrohair, phl = phloem, st = stamen, xy = xylem.

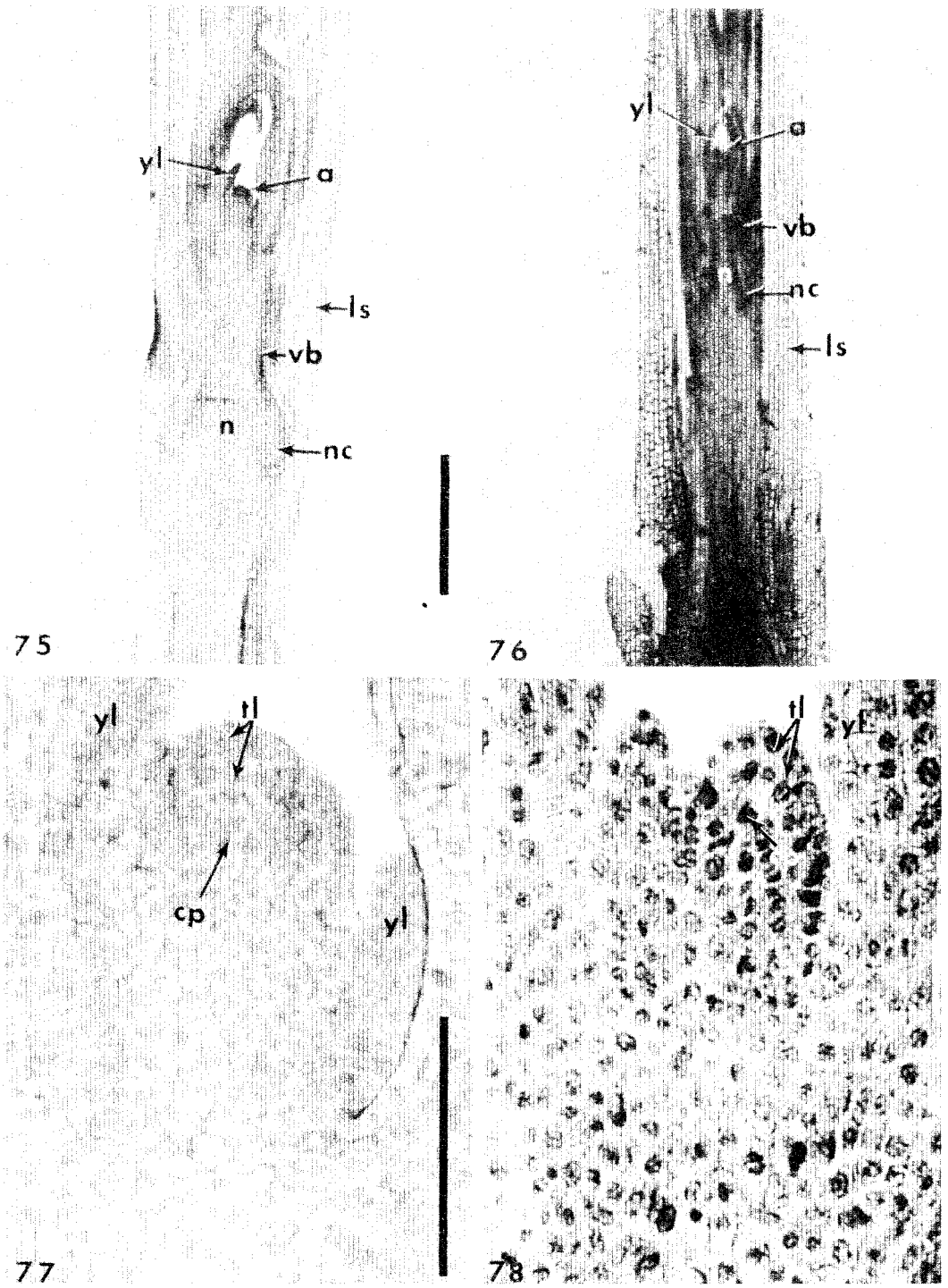


Fig. 75. Near-median longisection of shoot apex of *L. pauciflora*. Line: 500µm. Fig. 76. Median longisection of shoot apex of L. humilis. Same line scale as Fig. 75.Fig. 77. *L. pauciflora*,stem tip, near-median longisection. Line: 100µm.Fig. 78. *L. humilis*, stem tip, median longisection. Same line scale as Fig. 77. Abbreviations: a = stem apex, cp = corpus, ls = culm sheath, n= node, nc = nodal complex, tl = tunica layer, vb = vascular bundle, yl = young leaf.

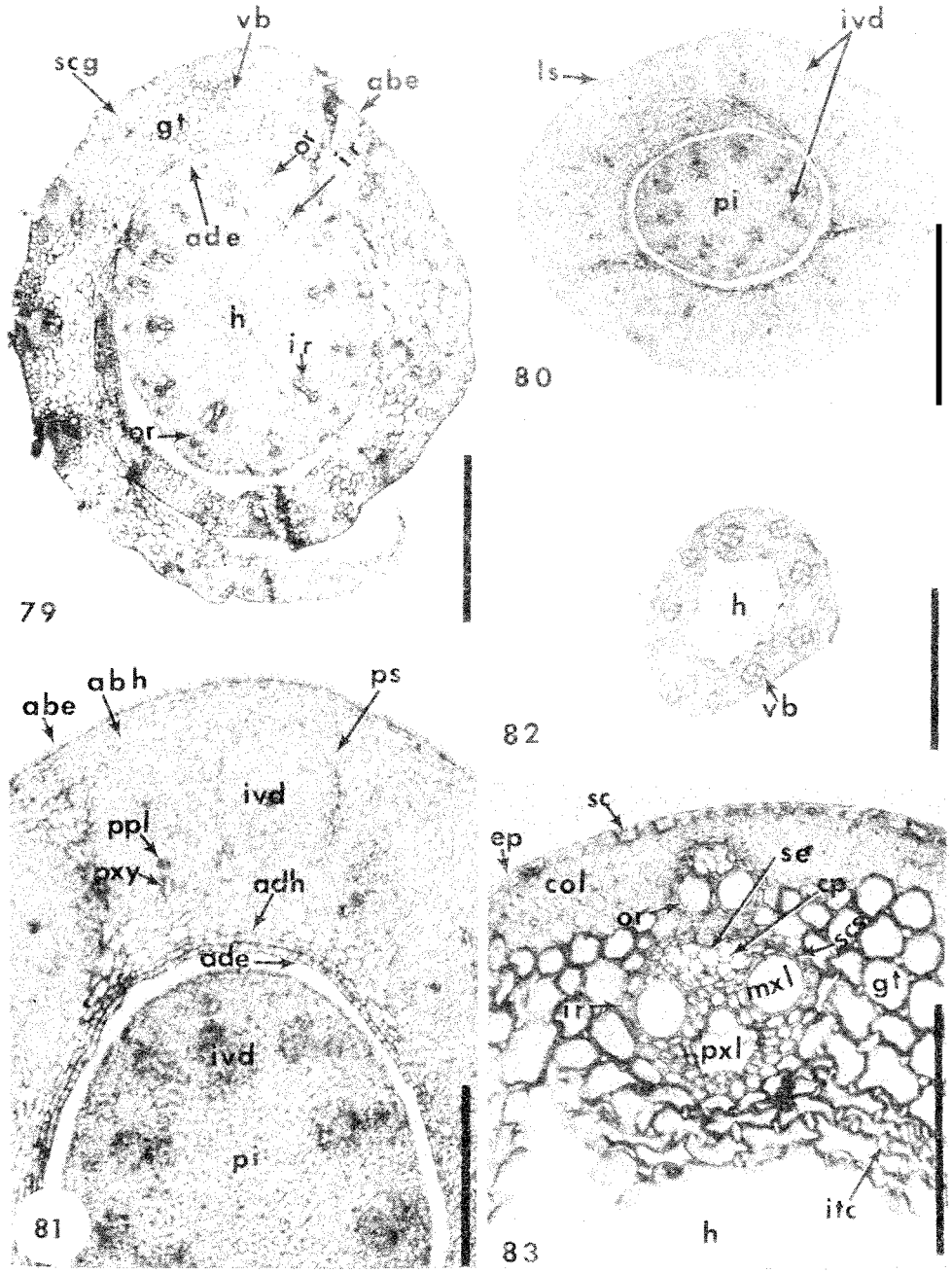


Fig. 79. Transverse section through a mature internode and the open sheath of *L. pauciflora*. Line: 500um. Fig. 80. *L. pauciflora*, transverse section through the fourth internode. Line: 500µm.Fig. 81. *L.pauciflora*, enlargement of Fig. 80. Line: 200µm.Fig. 82. *L. humilis*, transverse section of a mature internode. Line: 500µm. Fig. 83. *L. humilis*, enlargement of Fig. 82. Line: 100µm. Abbreviations: abe = abaxial epidermis, abh = abaxial hypodermis, ade = adaxial epidermis, adh = adaxial hypodermis, col = collenchymatous layer, cp = companion cell, ep = epidermis, gt = ground tissue, h = hollow in internode, ir = inner ring of vascular bundles, itc = intercellular space, ivd = inital stage of vascular bundle development, ls = culm sheath, mxl = metaxylem, or = outer ring of vascular bundles, pi = pith, ppl = protophloem, ps = parenchymatous sheath, pxy = protoxylem, sc = siliceous cell, scg = sclerenchymatous girder, scs = sclerenchymatous sheath, se = seive element.

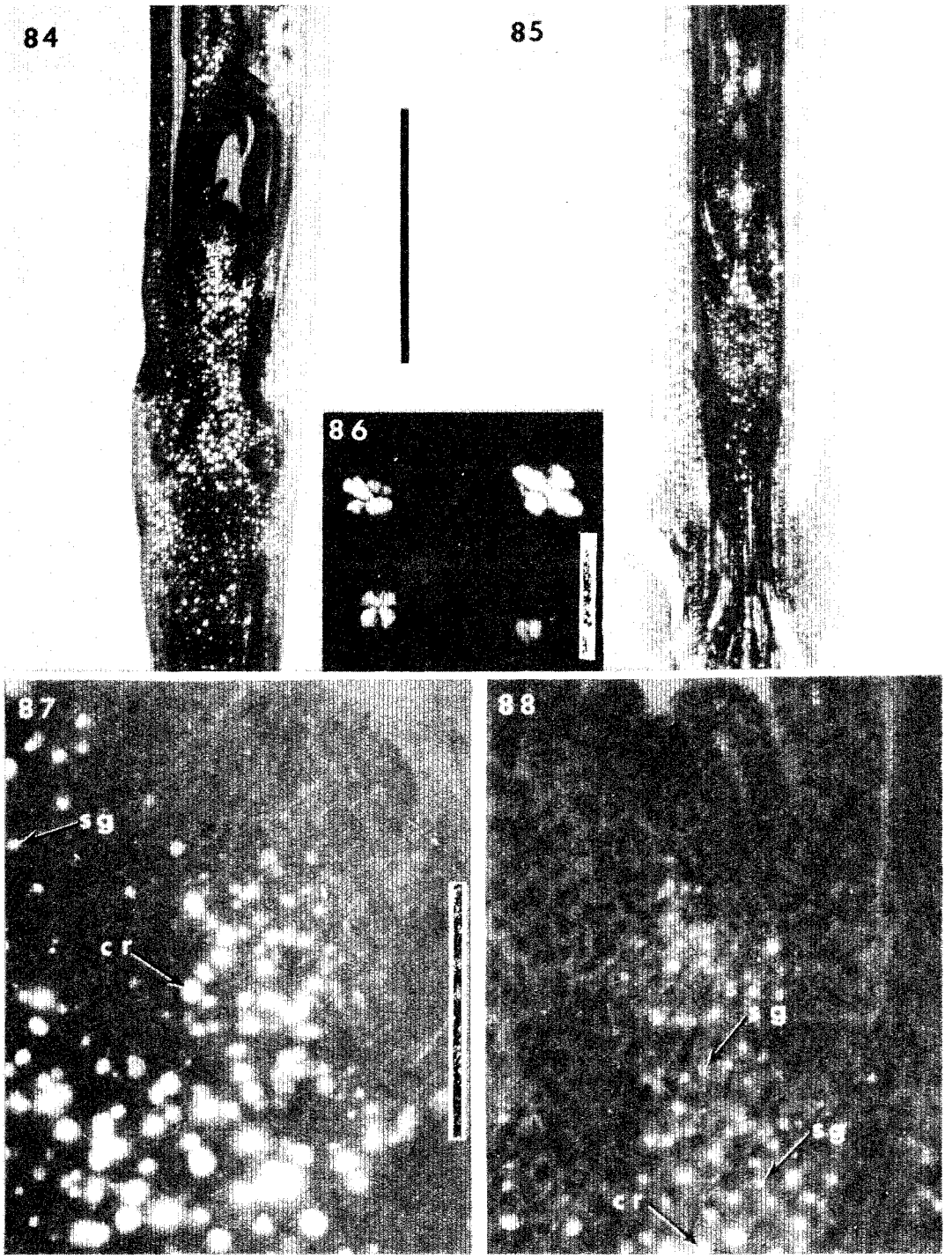


Fig. 84. *L. pauciflora*, longisection of shoot apex shows the distribution pattern of crystals, polarized light. Line: 500μm Fig. 85. *L. humilis*, longisection of shoot apex shows the distribution pattern of crystals; polarized light. Same line scale as Fig. 84. Fig. 86. Crystals from the shoot apex of *L. pauciflora*, polarized light. Line: 10μm.Fig. 87. Crystal pattern near the apex of *L. pauciflora*, polarized light. Line: 100μm. Fig. 88. Crystal pattern near the apex of *L. humilis*, polarized light. Same line scale as Fig. 87. Abbreviations: cr = crystal, sg = starch grain.

composed of smaller vascular bundles than those of the inner ring.

Vascular bundles in young stages are not well-differentiated and show one or two protoxylem and a few phloem elements. The whole vascular bundle unit is embedded in the ground tissues without a distinct bundle sheath. Ground tissues of the young culm consist of small cells at the periphery or hypodermal layer and become larger toward the center. The intercellular spaces are distinct in the pith region. The epidermis consists of uniformly sized, thin-walled cells containing relatively large nuclei. Figs. 80-81 also show a young culm sheath. This consists of distinct adaxial, abaxial, and hypodermal layers in the initial stage of vascular bundle development. The hypodermal layers are present beneath both epidermises amd consist of elongated cells. The adaxial hypodermal layer is composed of two layers of long, thin-walled cells but there are about five layers in the abaxial hypodermis. These long cells also form a single layer sheath around the young vascular bundle. The young vascular bundles consist of phloem strands and protoxylem embedded in the undifferentiated uniform-sized cells (Fig. 81). Ground tissues are composed of loosely-arranged, thin-walled cells, hexagonal in outline. The triangular intercellular spaces are distinct near the adaxial surface.

Transverse sections of the mature culms of both species (Figs. 79, 82, 83) show the hollow pith region. The epidermis of a mature culm consists of thick-walled cells. Siliceous cells are occasionally found. The thick-walled hypodermal cells form a continuous layer, associated with both outer and inner rings of vascular bundles. The vascular bundles of the mature internode are well-differentiated, with a distinct sclerenchymatous sheath. The vascular bundles are of the endarch type with the phloem at the outer pole, two metaxylem vessels at the left and right near the center, and protoxylem at the inner pole (Fig. 83).

In the mature culm sheath (Fig. 79), the vascular bundles are well separated by ground parenchyma, in contrast to the situation in the young sheath (Fig. 80), where the bundles are very near one another. Each vascular bundle has a distinct sclerenchymatous sheath and is well supported by a girder of collenchyma or a bundle cap. The abaxial epidermal cells are thickened and heavily cutinized while the adaxial hypodermal layer and the epidermis are unchanged. Siliceous cells are occasionally present in the abaxial epidermis.

Microchemical tests for crystal and cell wall components: Druse crystals (Fig. 86) occur profusely just below the shoot apex, especially in the region between the fifth node and the meristematic region of the apex. They are less abundant in the culm below the fifth node but may be found in young leaves above the apex. Figs. 87-88 show the distribution patterns of crystals in both species. However, crystals never occur in the corpus region or extend above it (Figs. 87-88). Crystals are less abundant in the shoot apex of *L. humilis*.

Druse crystals react in the cupric acetate-ferric sulfate test (Johansen 1940) to form needle-shaped crystals with cupric acetate. These crystals dissolved when ferric sulfate was added, suggesting that the druses are calcium oxalate.

Cell wall components: Tests for the chemical composition of cell walls and papillae of both species were performed. Cell walls reacted positively to the IKI-H_2SO_4 test for cellulose while papillae gave a negative reaction.

In the tests for cutin and suberin, papillae gave a positive reaction with Sudan IV but a negative reaction to the KOH-zinc chlor-iodide test, indicating that the papillae are composed of cutin.

Cell walls dissolved in 75% sulphuric acid and the papillae collapsed. Such collapsed papillae stained red with safranin, confirming the previous test for cutin. Johansen (1940) states that safranin is a specific stain for cutin.

Cell walls and papillae gave negative reactions to phloroglucin-hydrochloric acid test and the chlorine-sulfite test, indicating that the wall and papillae are composed of neither hemicellulose nor lignin.

DISCUSSION

The morphological, cytological, and anatomical characteristics of *Lithachne* indicate that it is properly placed in the Subfamily Bambusoideae of the Pooideae. Included among these characteristics are the presence of fusoid and arm cells in the leaf mesophyll, the non-radiate chlorenchyma, double bundle she-

aths, pseudopetioles, three lodicules (in the staminate flowers), and the basic chromosome number of $n = 11$. The vascular bundles are simple and similar to those of the tribe Olyreae. Bicellular microhairs are common and resemble those of other bamboos. All other leaf characteristics, such as the types of siliceous cells and the shape of the stomatal subsidiary cells, agree with bambusoid type of leaf anatomy as described by Calderón and Soderstrom (1973). Structure of the embryo provides additional evidence that *Lithachne* belongs to the Bambusoideae, having the same embryonic formula, F+PP. The inflorescence structure in *Lithachne* resembles that of *Olyra* of the tribe Olyreae of the Bambusoideae.

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RESUMEN

Se analizan la morfología, anatomía, y citología de *Lithachne pauciflora* y *L.humilis*, las dos especies centroamericanas del género, el cual pertenece a la Subfamilia Bambusoideae de las gramíneas (Poaceae). *Lithachne* es un género de bambusoideas herbáceas de América tropical. Todas las especies son monoicas. En *L. pauciflora*, las espiguillas de ambos sexos son axilares; en *L.humilis*, las espiguillas axilares son pistiladas y las espiguillas presentes en la panícula terminal son estaminadas. Las espiguillas estaminadas carecen de glumas y son compuestas de un solo flósculo con tres lodículos truncados y tres estambres. Las espiguillas pistiladas tienen dos glumas subiguales alargadas y un flósculo truncado duro. La anatomía de las hojas es típicamente bambusoide, con la epidermis papilosa, con tricomas bicelulares agudos, celulas silicificadas, y estomas romboidales. En sección transversal, las hojas exhiben las células fusoides y clorenquima compuesto de células con paredes invaginadas. El número cromosómico haploide de *L.pauciflora* es $n = 11$.

REFERENCES

Calderón, C. & T.R. Soderstrom. 1973. Morphological and anatomical considerations of the grass subfamily Bambusoideae based on the new genus *Maclurolyra*. Smithso.Contrib. Botany 11:1-55.

Clayton, W.D. & S.A. Renvoize. 1986. Genera Graminum. Kew Bull. Addit. Ser. XIII. Her Majesty's Stationery Office, London. 389 p.

Johansen, D.A. 1940. Plant microtechnique. McGraw-Hill, New York. 523 p.

Paisooksantivatana, Y. 1978. Anatomy, morphology, and taxonomy of *Lithachne pauciflora* (Sw.)Beauv. and *Lithachne humilis* Soderstrom (Gramineae: Bambusoideae). M.S. thesis, Iowa State Univ. Library.

Pohl, R.W. & G. Davidse. 1971. Chromosome numbers of Costa Rican grasses. Brittonia 23: 293-324.

Pohl, R.W. 1977. Cultivation of tropical rain forest grasses. Ann. Bot. 41: 665-666.

Quarin, C.L. 1977. Recuentos cromósomicos en gramíneas de Argentina subtropical. Hickenia 1: 73-78.

Reeder, J. R. 1957. The embryo in grass systematics. Amer. J. Bot. 44: 756-768.

Reeder, J.R. 1962. The bambusoid embryo: a reappraisal. Amer. J. Bot. 49: 639-641.

Soderstrom, T.R. 1980. A new species of *Lithachne* (Poaceae: Bambusoideae) and remarks on its sleep movements. Brittonia 32: 495-501.

Tateoka, T. 1962. Starch grains of endosperm in grass systematics. Bot. Mag. (Tokyo) 75: 377-383.