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DEVELOPMENTAL FEATURES OF THE DISCONTINUOUS STEM VASCULAR SYSTEM IN THE RATTAN PALM *CALAMUS* (ARECACEAE-CALAMOIDEAE-CALAMINEAE)[1]

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Calamus is a climbing palm marked by considerable internodal extension and limited stem-thickening growth, but with a surprisingly discontinuous axial vascular system. Stem bundles end blindly in a basipetal direction and are connected to each other only by narrow and late-developing transverse commissures. Vascular connection via leaf traces between stem and leaf is made over about nine plastochrons (P), but the dominant central system is completed by about P_7, with subsequent bundles forming the crowded fibrous peripheral system, which has reduced or no vascular tissues. The stem internode below a leaf completes its extension and maturation only by P_{10} to P_{11}. Axial stem bundles originate as procambial strands that are discontinuous apically for up to 15 plastochrons before being "captured" by a developing leaf. Their distal unconnected ends arise by dedifferentiation of ground parenchyma cells. Protoxylem is initiated as short overlapping initials that differentiate progressively during extension growth, which ruptures all but the last-formed elements. Their form, with tapered ends, means that they mature as tracheids. Metaxylem appears only late in shoot development, shortly before internodal elongation ceases (P_8) and always unconnected to the late-differentiating protoxylem. In each axial bundle protophloem differentiates as a single strand, subsequently and much later appearing as two separate metaphloem strands as the early initials, ruptured by extension growth, are replaced by fibers. It is suggested that the unique features of this stem can be ascribed to the absence of a "meristematic cap," which otherwise typifies palms of normal habit, and that discontinuity is causally related to the pronounced late stem extension growth.

Key words: Arecaceae; *Calamus*; metaxylem; procambium; protoxylem; rattan; stem histology; vascular development; vessels.

Rattans are high-climbing palms, mainly in the genus *Calamus* (about 350 spp.), with woody extended stems that produce the canes of commerce (Uhl and Dransfield, 1987). A recent investigation (Tomlinson et al., 2001) has shown that the vascular system is distinctive among monocotyledons because there is limited axial continuity of phloem and especially xylem. In the present article we describe equally distinctive features of development and show how the vascular arrangement of the *Calamus* stem is modified by the absence of features that characterize stems of palms with a normal habit. The specialization in relation to habit includes an early blocking out of the vascular pattern combined with late and extended internodal elongation without concomitant increase in diameter. The information is relevant to an understanding of long-distance transport mechanisms in woody plants. It must be emphasized that monocotyledons with a pronounced climbing habit are peculiarly vulnerable to vascular dysfunction because they cannot produce secondary vascular tissues. Furthermore, recent observation suggests that in climbing monocotyledons a close correlation exists between axial metaxylem type and degree of xylem continuity (Tomlinson and Fisher, 2000), with as yet unknown functional consequences.

The vascular system of *Calamus* is paradoxical in its apparent inefficiency for long distance transport, especially of water. Stems can reach lengths approaching 200 m (Burkill, 1966) by producing large numbers of long (up to 1 m) but narrow internodes, these supplying a single crown of leaves. However, the axial transport system consists of vascular bundles that are connected distally only to leaves, but proximally over long distances the same bundles are tapered gradually and end blindly. In the measured example (Tomlinson et al., 2001), a single bundle may extend through about 15 internodes (a total of approximately 3.5 m) with a single uninterrupted metaxylem vessel over most of this distance. Furthermore, although metaxylem vessels are wide (up to 350 μm diameter), as is to be expected in a woody liane, they are never in contact with the protoxylem tracheids that directly irrigate the leaves. The only direct connection among metaxylem vessels of different axial bundles is via short, narrow, irregular connections (transverse commissures) with narrow vessels and little phloem. This suggests that as far as water movement is concerned there is considerable internal resistance both laterally and from stem to leaf. The presence of long, wide vessels as efficient conduits seems obviated by the overall vascular architecture. There are equivalent major discontinuities in the phloem. The most likely, but still speculative, conclusion was that (a) vessels serve as much for water storage as for transport and (b) that the system minimizes xylem cavitation in an otherwise highly vulnerable system (Tomlinson et al., 2001).

In order to extend these observations the present article adds a developmental dimension by describing how the vascular system is initiated and the limited vascular continuity is maintained during late internodal extension. Associated histological features also need to be understood in the same context. Quantitative descriptions of shoot development in rattans have been provided by Fisher (1978) and Fisher and Dransfield (1977, 1979). Our observations fit well with these descriptions so our quantitative analysis of shoot form is minimal.

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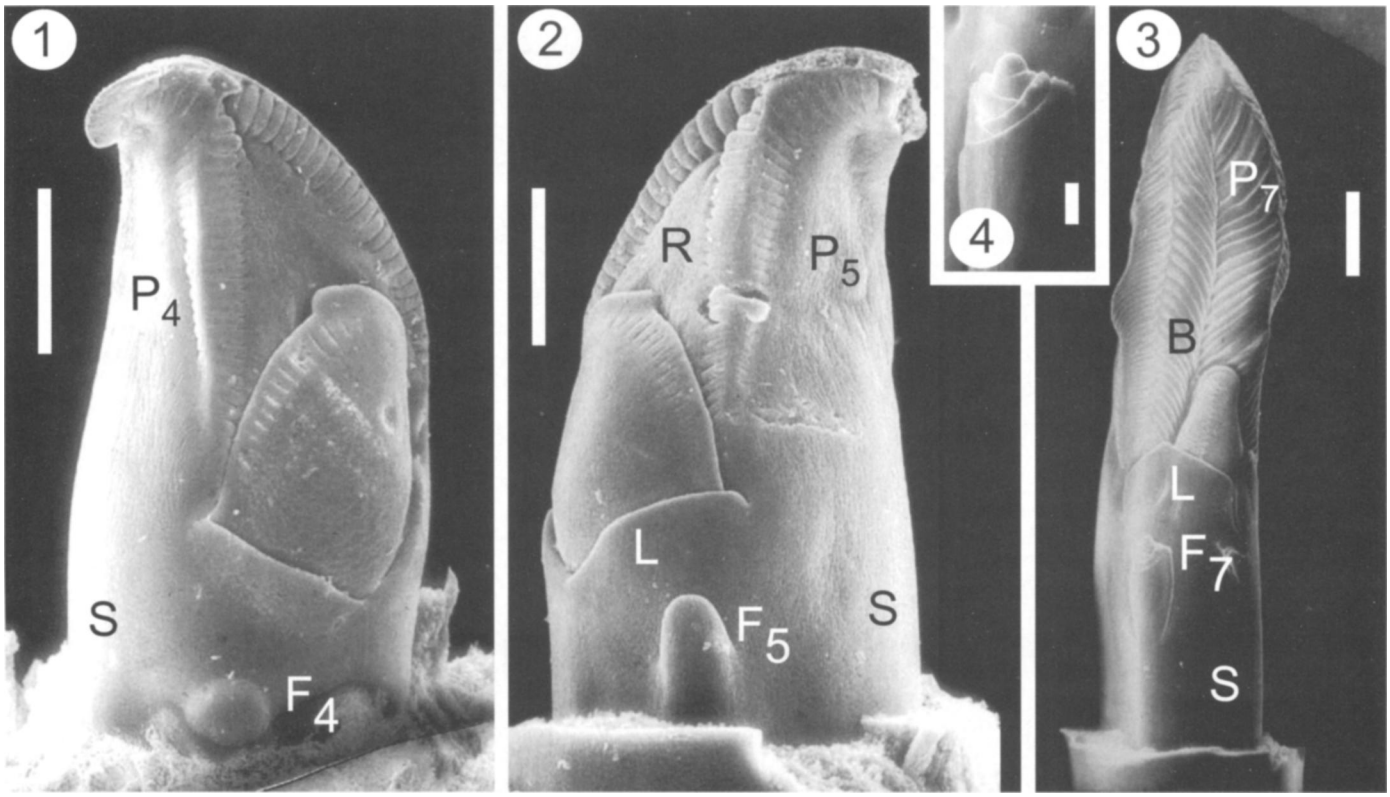


Figs. 1–4. *Calamus longipinna*. Scanning electron micrographs of developing leaves. **1.** Fourth youngest leaf (P_4) outermost on the dissected shoot, its flagellum (F_4) evident; with P_3 protruding through its sheath. **2.** Fifth youngest leaf (P_5) outermost on the dissected shoot, its flagellum (F_5) adnate to the sheath and free apically. **3.** Seventh youngest leaf (P_7), the outermost leaf on the dissected shoot, with the apex of P_6 protruding through the mouth of its sheath, the flagellum (F_7) of P_7 distally free from its sheath. **4.** Inset detail of free exposed apex of flagellum in Fig. 3 showing distichous bract primordia. Figure abbreviations: B = blade with leaflets of P_7, F = flagellum, L = ligule, P = leaf primordium, R = rachis, S = sheath. Scales = 2.5 mm for Fig. 1; 2 mm for Fig. 2; 1 mm for Fig. 3; 0.5 mm for Fig. 4.

MATERIALS AND METHODS

Canes studied in detail were derived from a single rhizomatous clump, cultivated at Fairchild Tropical Garden (FTG), and identified as *Calamus longipinna* K. Schum & Lauterb., native to Papua New Guinea. A voucher specimen is deposited at FTG. Canes were cut at widely spaced intervals in 1997, 1999, and 2000 without disturbing the overall integrity of the specimen. The method of establishing developmental events within a single shoot followed the procedures of Fisher (1978). The detached canes (up to 10 m long) were cut into shorter lengths for transport to the laboratory. Leaf dimensions from older to younger parts were measured as they were detached in order, up to the insertion of the youngest visible (but still incompletely expanded) leaf protruding from the crown, i.e., the "spear leaf," P_s, which was used as a reference level. Younger, enclosed leaves were progressively removed and measured to within a few primordia of the apex proper, but the leaves numbered basipetally, with P_1 the youngest. To provide precise dimensions of younger stages, the shoot, together with attached early leaf primordia, was fixed in formalin-acetic-alcohol (FAA). A total of 12 apices were prepared, but with some loss of material and measurements because of breakage during dissection.

For sectioning, material was embedded in "Paraplast" following routine procedures of dehydration. Serial transverse and longitudinal sections were cut on a rotary microtome at 10–15 μm, stained in safranin and alcian green, and mounted permanently in "Permount." It was found that with larger specimens there were some problems of wax penetration related to the surface texture of the stem, causing some sections to fall out of the ribbon, but less than 3% of the total number of sections were lost and axial continuity was not jeopardized. The most completely analyzed series included 570 sections and the insertion of nine developing leaves. To facilitate measurement the sections were numbered individually. Longitudinal sections produced in the same way were largely used to make measurements of early leaf stages and to complement histological observations.

For scanning electron microscopy (SEM) a number of dissected shoots were observed at different stages of leaf development (see Figs. 1–4). These were first dehydrated, critical point dried, and metal coated in the usual way and photographed with an AMR 1000 (Cambridge, Massachusetts, USA) at 10 kV. Some of these specimens were rehydrated, dissected further, and rephotographed, all were eventually rehydrated and embedded in wax for serial sectioning in order to provide as complete a series of measurements as possible for several shoots (e.g., Fig. 5B).

For vascular analysis, a video system was used in which single frame (5 s) images of successive sections were recorded. A drawing of the position of marker bundles on a transparent film mounted on the monitor was used to align each section and progressively redrawn as alignment was lost. Sequential images could then be observed in playback mode so that individual bundles could be traced unequivocally. For the most critical observation of axial bundle differentiation a series of camera-lucida drawings was used to superimpose successive sections in such a way that the earliest evidence of bundle differentiation could be established with greatest precision. Axial dimensions were unitized by section number converted to linear dimensions from section thickness (e.g., Fig. 6). In the long series studied, axial vascular bundles could be followed throughout nearly nine full plastochrons, corresponding approximately from the time of leaf inception (P_1) to the appearance of the first outwardly visible leaf termed the spear-leaf (P_s). The reconstruction of the shoot apex during development (e.g., Figs. 29, 30) depended on the knowledge of the vascular architecture of the mature system, as already established (Tomlinson et al., 2001).

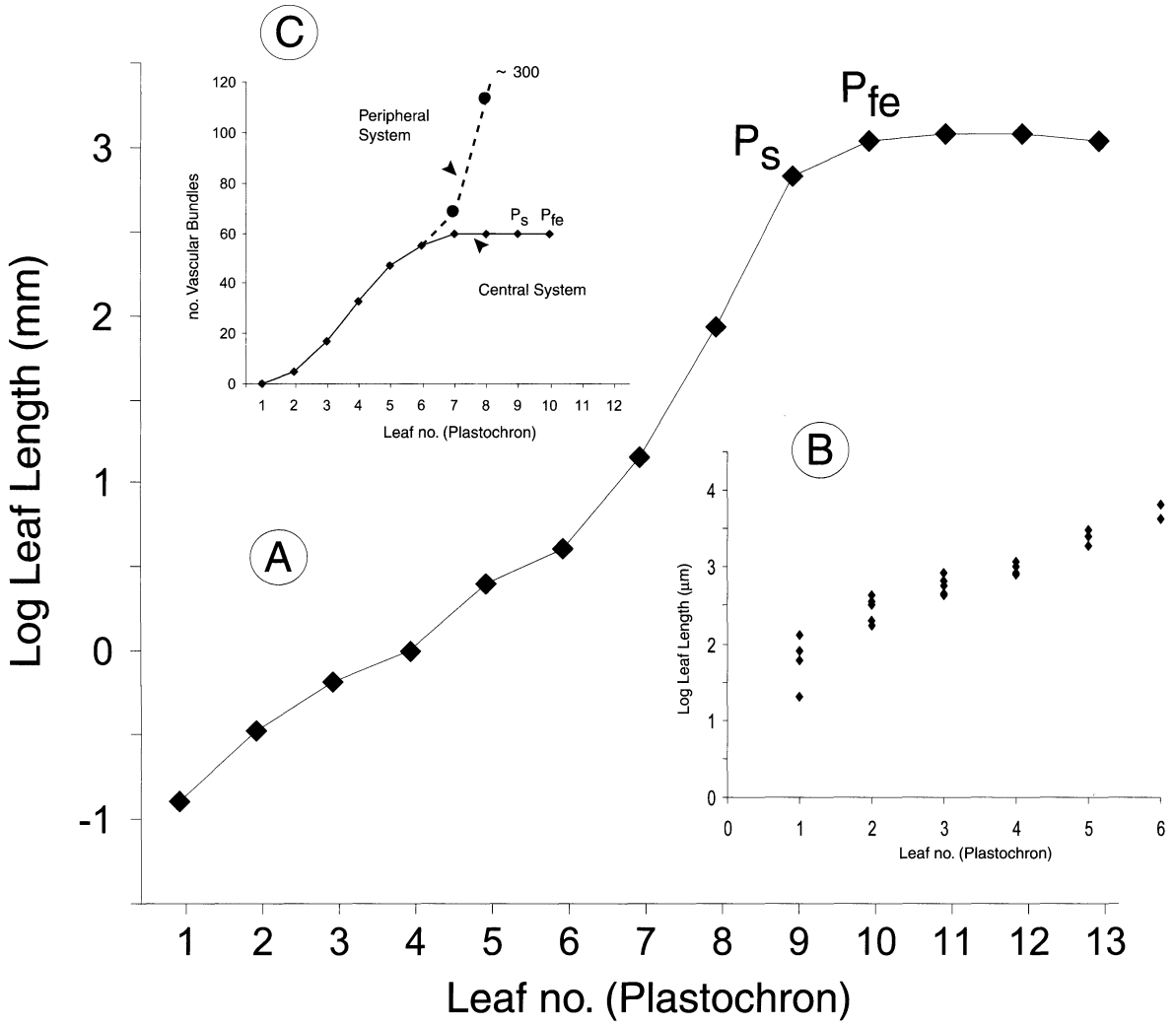


Fig. 5. *Calamus longipinna*, dimensions of developing leaves. The *x*-axis in each graph shows leaf number (P_1 youngest), i.e., plastochron number. (A) Log total leaf length for a single shoot. P_s = "spear leaf"; P_{fe} = first fully extended leaf. (B) Log total leaf length at each plastochron for the first six leaves in a number of different shoots, each diamond representing one leaf. (C) Number of vascular bundles contributed by each successive leaf at its level of insertion; the dotted line represents the approximate number of bundles in the peripheral system to a final total of about 300.

RESULTS

Shoot morphology—Canes that develop from the rhizome system show an initial phase of development that might be described as "juvenile." Juvenile leaves lack a flagellum and have a long (to 30 cm) petiole (bladeless portion of the leaf axis above the sheath). Subsequent leaves (Figs. 1–4) almost always support a flagellum, which is adnate to the stem internode and leaf sheath above its node of origin (cf. Fisher and Dransfield, 1977). The flagellar axis includes several inconspicuous, reduced tubular leaves (bracts) and is provided distally with vicious recurved hooks. We have not studied adult canes at a later stage, when the flagellum branches into a flower-bearing panicle. We studied only post-juvenile vegetative stages.

Each foliage leaf has a closed tubular, sheathing base, its mouth extended into a short ligule that is open on the inner (i.e., adaxial) side. The petiole is short (3–5 cm) and the rachis is provided with somewhat irregularly placed leaflets that project in a single plane. Leaves are spirally arranged with an average angular divergence of about 137.5°, corresponding to a phyllotactic fraction close to 3/8. Because the leaf itself has no consistently identifiable dorsal midpoint (e.g., Fig. 8) phyllotaxis was measured from the position of successive flagella (Fig. 7), since they are approximately in the ventral axillary position. Shoots may be described as right- or left-handed according to the direction of the ontogenetic helix, but chirality is most obvious from whether the flagellum lies to the right or left of the median ventral plane of the leaf (cf. Figs. 1 and 2 as left hand and right hand respectively).

Leaf and flagellum development—Dissected shoots and the developmental relation between leaf and flagellum primordia are shown in Figs. 1–4. Overall leaf dimensions (A), consistency between different shoots (B), and the progressive establishment of the stem vascular system (C) are plotted as Fig. 5, all in terms of the plastochron interval. The shoot apex remains narrowly conical throughout the different stages of each plastochron. The youngest leaf reaches a height of about 130 µm before initiation of the subsequent leaf, at which height the shoot apex is considerably overtopped. The various

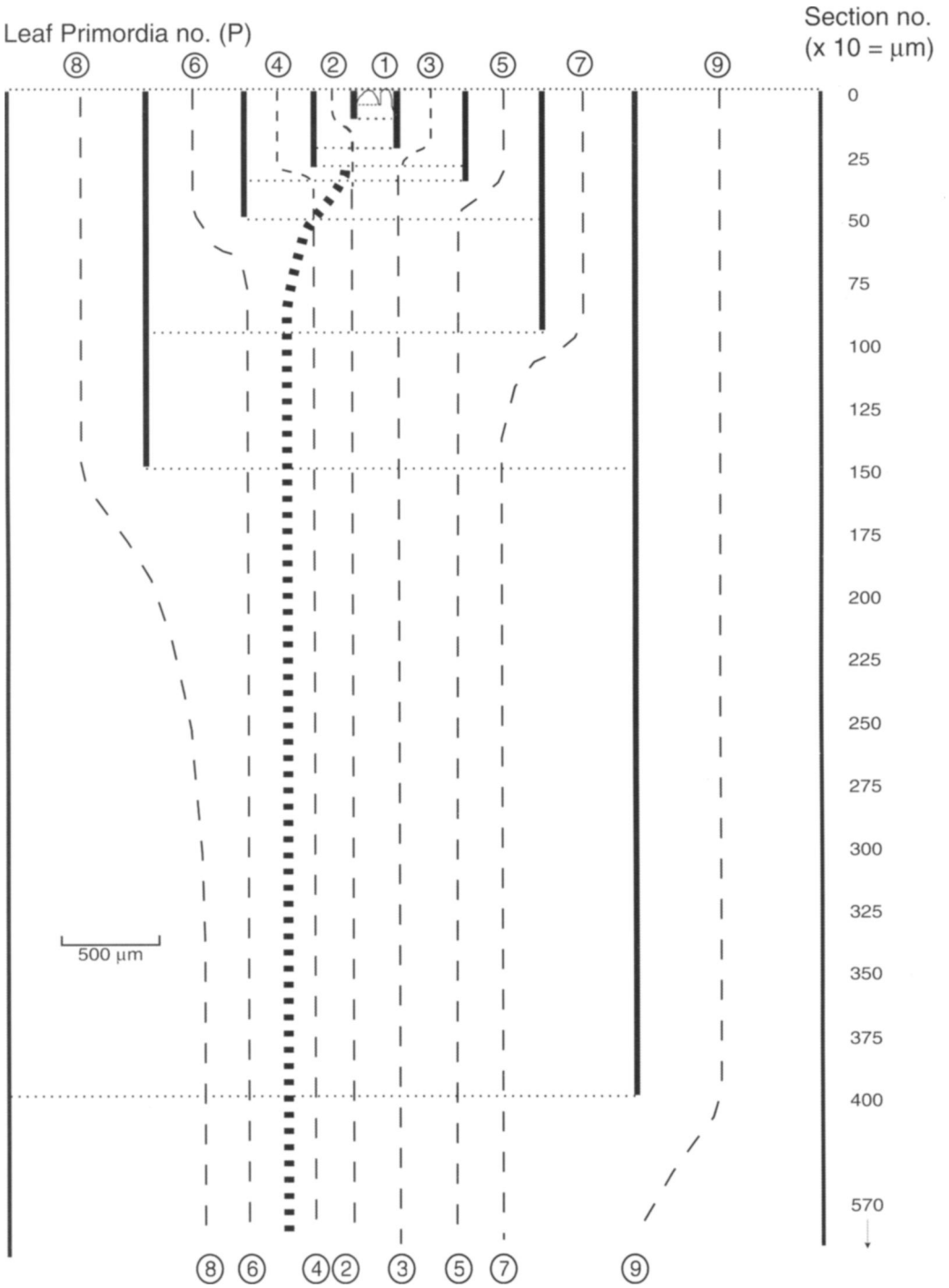
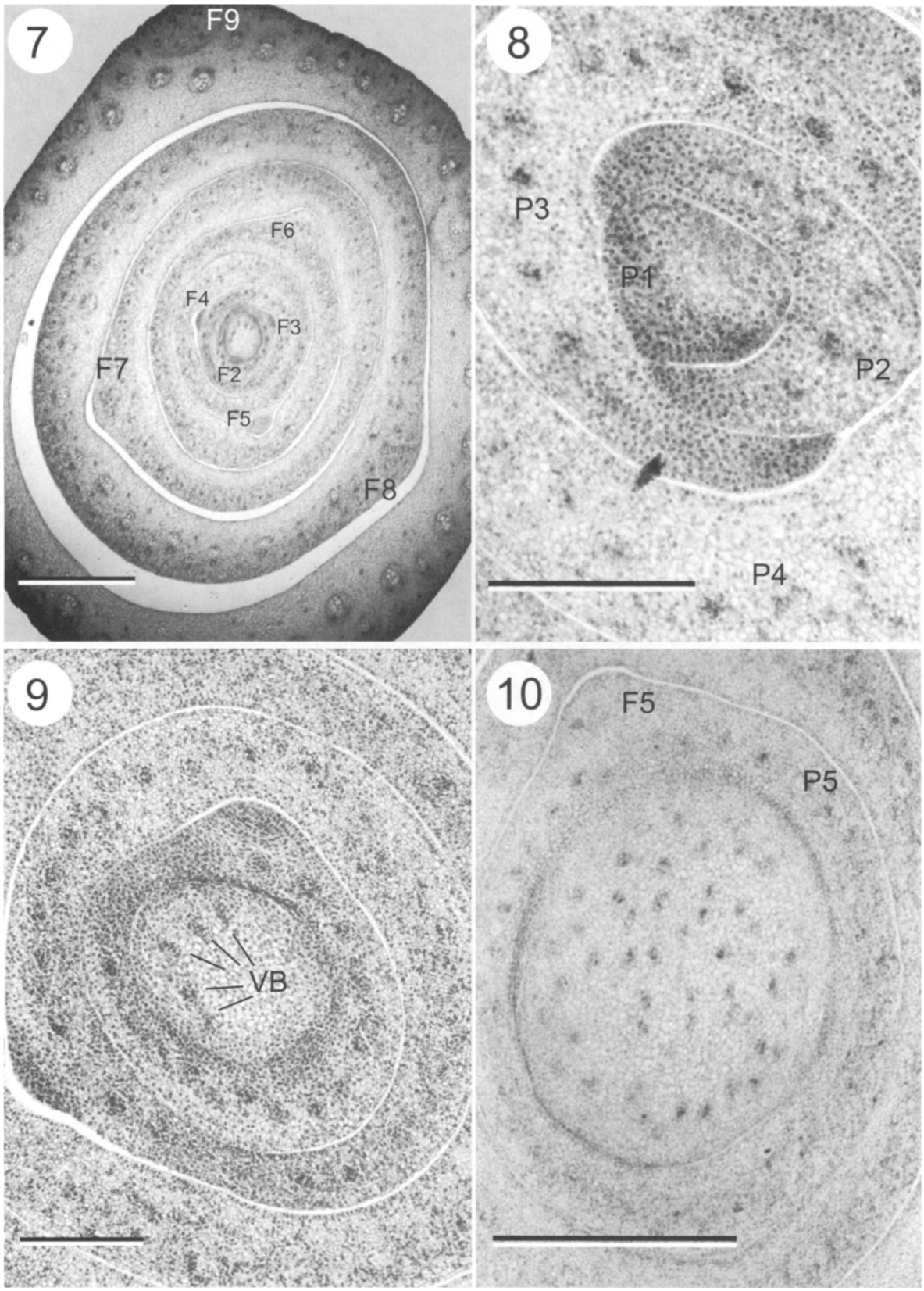


Fig. 6. Plot of shoot apex in longitudinal view from serial transverse sections cut at 10 (μm, total sections = 570; right-hand scale is section number, with 0 representing a level five section (=50 μm) above the shoot apex. Heavy vertical lines represent appressed surfaces of adjacent leaf primordial. For simplicity leaf insertions (delimited by horizontal dotted lines) are flattened into the plane, with successive insertions alternately left and right. A vascular bundle is drawn as a vertical dashed line connecting each numbered leaf with the axis. The relative position of each vascular bundle in the axis is stylized. Irregularities in the length of the youngest internodes (between the horizontal lines) represent missing sections. The barred line represents an observed axial bundle that would have connected to an unformed leaf, but could only be traced to the internode below P_4.

parts of a developing leaf are illustrated in an ontogenetic sequence (Figs. 1–3), with changes in overall dimensions of successive leaves from a single shoot (Fig. 5A). Our measurements of increase in size of each leaf part in successive plastochrons correspond closely to those in Fisher (1978) for *Calamus insignis* and are not repeated here. Notably, the flagellum shows very late and considerable internodal extension (to an overall length of 1.75 m), even though its bracts are initiated early (as on P_7 in Figs. 3 and 4).

Leaves are referred to in order of age, with P_1 the youngest,



Figs. 7–10. *Calamus longipinna*. Transverse sections of leaf bases in region of shoot apex. **7.** Low-power view with closed tubular leaf sheaths of P_3–P_9, where P_9 = spear leaf. The stem is cut at the insertion of P_2. Flagella numbered in basipetal order. **8.** Shoot apex at insertion of P_1 and above the closed portion of the sheaths of P_2 and P_3. P_1 lacks a vascular connection. **9.** Section at insertion of P_3 with its approximately 17 vascular bundles; the five vascular bundles of P_2 are evident in the stem. **10.** Section at insertion of P_5 with its approximately 47 vascular bundles. Only the central system as procambial strands is present in the stem. Figure abbreviations: P = leaf primordia, with P_1 youngest; F = flagellum with F_9 the oldest; VB = vascular bundle. Scales = 1 mm for Fig. 7; 200 μm for Figs. 8, 9; 500 μm for Fig. 10.

whereas the flagella are referred to according to the number of the leaf to which they are attached (Figs. 1–3), with F_2 the youngest. For example, F_8 is attached to P_8 (Fig. 7), although it originates in the axil of P_9. There is no F_1 because P_1 lacks an attached flagellum as a consequence of its sheath still being incompletely encircling. However, meristematic tissue that can be identified as F_1 is visible in the axil of P_2. This precocious inception of the flagellum conforms to the description by Fisher and Dransfield (1977) in other *Calamus* species. Differentiation of the various parts of the leaf are completed by P_3 (Fig. 1) in which the first evidence of leaflet primordial can be recognized, although the short petiole is little extended. The flagellum becomes protuberant and clearly adnate by P_4 (Fig. 1) and its apex is free distally by P_5 (Fig. 2). Subsequently, the free portion initiates a series of distichously arranged tubular bracts (Figs. 3 and 4), but its extensive internodal elongation is one of the last features of leaf development, as quantified by Fisher (1978). The status of the flagellum as a lateral or branch axis is confirmed by the meristematic "shell zone" visible almost from inception and forming an arc of cambial-like cells separating the flagellum primordium from differentiated stem cortical parenchyma as described by Fisher and Dransfield (1977).

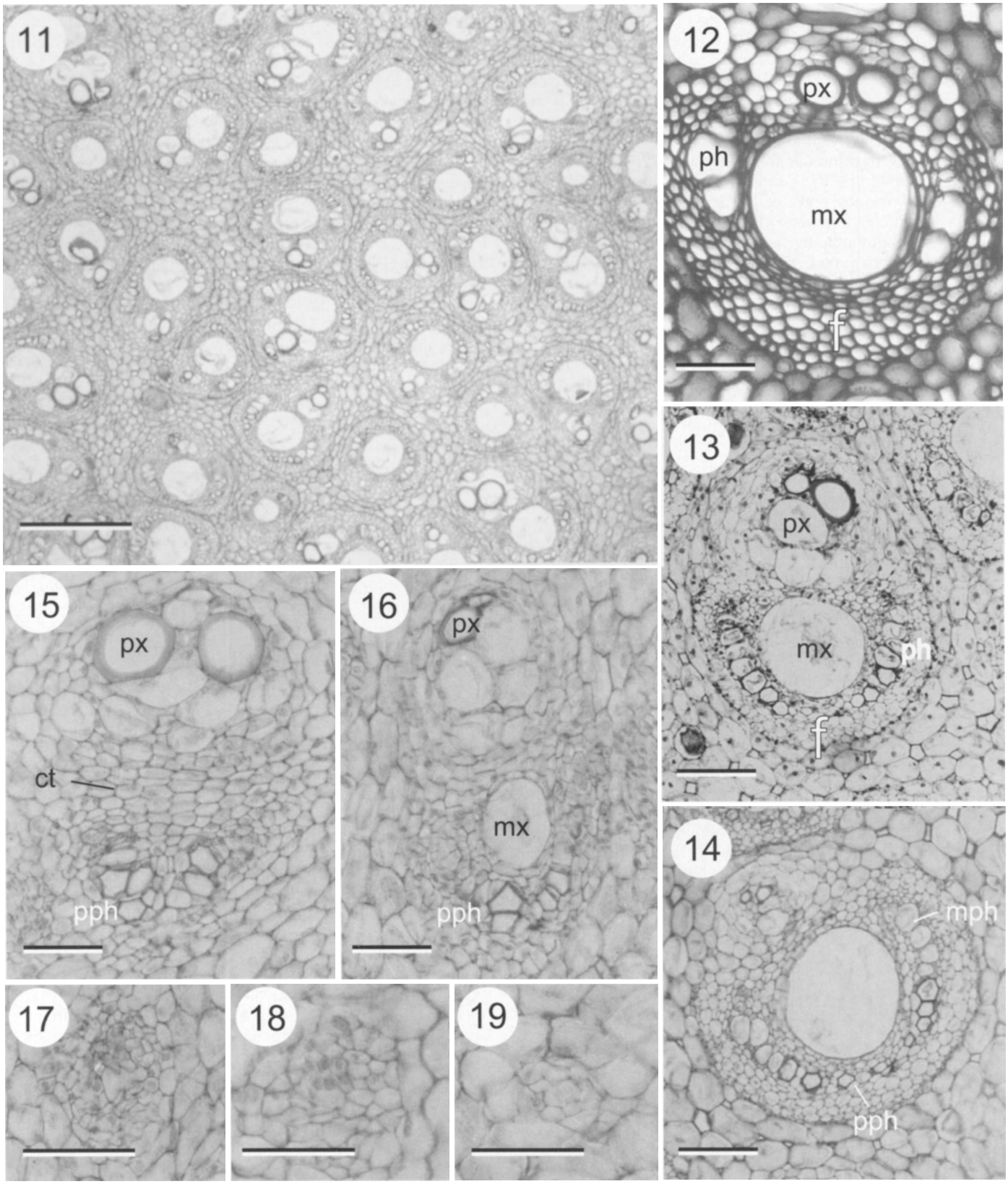
The length of the first 13 leaves on a single shoot changes through four orders of magnitude (Fig. 5A). The youngest externally visible leaf is the "spear" leaf (P_s); P_{fe} is the first fully extended leaf. Early leaf development with overall lengths for the first six leaves at the same plastochron stage but in several different shoots shows an appreciable range (Fig. 5B). There is limited overlap in leaf size from one plastochron to the next showing that leaf development is uniform in different shoots. This validates information from different samples as representing overall features of development; histological features are directly comparable from shoot to shoot. Leaf extension accelerates considerably from P_5 to P_s, the latter corresponding to P_9 in Fig. 5A. In this shoot P_{10} is the first fully extended leaf (P_{fe} in Fig. 5A) although the internode below is still incompletely extended. Consequently, a fully mature leaf–stem unit (mature in the sense of having completed extension growth), does not exist until P_{11} or P_{12} ($= P_{s+2}$), i.e., two leaves older than P_s. Therefore vascular development and maturation must proceed over a period of at least 11 internodes. Younger leaves, whose blade is exposed but whose sheath is still immature, are supported by encircling lignified sheaths of older leaves, as is usual in palms.

Origin of the vascular system—In the mature stem the vascular system includes a central core of about 200 large bundles, which occupy almost two-thirds of the total stem cross-sectional area, and a peripheral series of numerous narrow vascular and fibrous bundles. There is no discrete cortical system, as is found in palms of normal habit (Zimmermann and Tomlinson, 1965; Tomlinson, 1990). Our main focus is the origin of the central system, which becomes the main axial transport system. The vascular connection between stem and leaf in the developing shoot is made by progressive addition of leaf traces at successively older nodes of insertion as can be seen in transverse section (cf. Fig. 5C with Figs. 8–10). The youngest leaf (P_1) always lacks such a connection so that the youngest stem–leaf connection is not made until P_2 (Fig. 8). The five bundles of P_2 can be seen in a different orientation at a lower level (Fig. 9, VB). Subsequent leaves show increasing numbers of leaf traces until about P_7, which has about 60 major bundles connected to it. The insertion of P_5 adds about 47 leaf traces to the axis (Fig. 10, cf. Fig. 5C). At this node the central system includes all traces from younger leaves (P_2–P_4), i.e., 5 + 17 + 33 = 55. The internode below P_7 includes the total contribution to the central system (about 200 vascular bundles). This procambial system provides the template in which the main axial vascular tissue develops subsequently.

The peripheral system is formed as vascular bundles continue to differentiate in continuity with stem and leaf (dotted line in Fig. 5C). These are indistinctly circumscribed from ground tissue so that their number cannot be precisely counted. For the spear leaf (P_s) they number approximately 150, but their numbers are no longer additive so that there is an average of about 300 peripheral bundles in sectional view of the mature stem. Limited analysis suggests that they also end blindly below, i.e., without anastomoses, but over much shorter distances than central bundles. Except for a minority of bundles with few narrow late-differentiating xylem and phloem elements they are entirely made up of fibers and have a largely mechanical function. They are not considered further in our analysis.

Basal discontinuity—To facilitate discussion of overall shoot and vascular architecture an outline plot of the shoot is provided but with the leaves rotated into a single plane and with a single leaf trace drawn for each leaf (Fig. 6). The diagram is disarmingly simple since changes in the radial position of vascular bundles are ignored. To maintain the constant number of central bundles there must be progressive loss of vascular bundles. This is achieved by the progressive disappearance of bundles in a basal direction as demonstrated earlier (Tomlinson et al., 2001). These tapering ends are seen as narrow strands, each with few narrow procambial elements, but only in older internodes. This is exactly to be expected from our knowledge of the vascular topography of the mature stem in which basipetally blind-ending bundles have been illustrated (Tomlinson et al., 2001: fig. 43). However, traced distally these same bundles cannot connect with a leaf because we know that they may extend through as many as 15 internodes before this happens, i.e., to leaves that still have to be initiated. In fact, such bundles progressively disappear toward the younger internodes; the youngest level at which they can be recognized is about P_5. A single bundle of this type is plotted in Fig. 6 (barred line). In this sense such bundles end blindly in each direction, but must continue to grow acropetally until they are "captured" by a developing leaf. Careful search of the ground tissue reveals these incipient "uncaptured bundles" as a series of narrow cells, which become more obvious as a procambial strand when followed basipetally. Differentiation within these strands is not necessarily continuous because one may fail to recognize a strand through a short series of sections. This is particularly clear even in the youngest primordia where the distal connection has been made since a procambial strand may fail to be recognized through several successive sections before it is rediscovered at a lower or higher level.

Apical discontinuity—Vacuolation of central ground tissue begins close beneath the shoot apical meristem so that newly initiated bundles are not visibly continuous with meristematic tissue (Fig. 8). However, the discreteness of leaf traces within the vacuolated ground tissue in lower internodes is clear from Figs. 9 and 10. Axial bundles thus appear by dedifferentiation



Figs. 11–19. *Calamus longipinna*. Transverse sections of stem and developing vascular bundles. **11.** Low-power view of central system in the internode below Ps (i.e., spear leaf). Only protoxylem is prominent; the number and size of its elements in each vascular bundle reflect the distance from its departure as a leaf trace. **12.** Mature vascular bundle for comparison with Fig. 13. **13.** Vascular bundle with abundant protoxylem; the vascular tissues are blocked out and only protoxylem and some late protophloem sieve tubes are fully differentiated. Bundle sheath fibers are immature. **14.** Vascular bundle with little protoxylem; the metaphloem is blocked out but immature. **15.** Leaf trace shortly below its departure into a leaf; protoxylem and protophloem are separated by a cambium-

of ground-tissue cells and subsequent internal longitudinal division. Early stages of this process are evident only in internodes older than P_5. Each newly initiated leaf thus captures a progressive series of blind-ending vascular bundles. In order to maintain the system at progressively higher levels there must be initiated a corresponding new series of apically discontinuous axial bundles. These are not captured until a much later plastochron. This method of initiation and subsequent connection to a leaf is the most distinctive developmental feature of the rattan stem. The overall acropetal development of the vascular system thus depends on the contrasted process: on the one hand of "capture" of axial strands to form leaf traces and, on the other, generation of new apically discontinuous axial strands.

Differentiation of vascular tissue—Because of the long distance over which vascular bundles extend, a bundle connected as a procambial strand to any leaf primordium when traced basally shows a progressively differentiating complement of vascular tissues. The distance from the leaf connection for each bundle is reflected in the relative amount of differentiated and undifferentiated protoxylem. The stem center shortly below the insertion of P_9, i.e., about 5 mm below the shoot apex shows the future configuration of the vascular tissue (Fig. 11), i.e., at the base of the plotted apex in Fig. 6. Although metaxylem vessels are conspicuous, they are incompletely differentiated, whereas mature protoxylem elements are present in most bundles. A section of a single mature vascular bundle is provided for comparison with earlier stages (Fig. 12).

Initially, an axial bundle is made up of procambial cells established by dedifferentiation and division of vacuolated ground tissue cells (Figs. 9 and 10). Procambial cells develop dense cytoplasmic contents and, because of limited transverse cell division, become axially extended as overall internode length increases. Vascular bundle diameter is increased by longitudinal divisions within the first elements of the strand, which thus appear in radial files in transverse view and in longitudinal view exhibit the "tiger-tail" effect described in Zimmermann and Tomlinson (1966), i.e., where nuclei of successive divisions remain at the same level to form a banded series. The resulting radial seriation of cells in the center of the vascular bundle may remain evident late in development, especially close to the departure of an axial bundle as a leaf trace (e.g., Fig. 15, ct). This configuration is lost in the bundle at maturity, as shown for comparison in Fig. 12. Late stages in bundle maturation, i.e., in internodes below P_8 and P_9, are shown in Figs. 13–16. An axial bundle close to bundle departure from the stem as a leaf trace (Fig. 13) indicates the abundant protoxylem (px), some of it mature; the bundle in Fig. 14 is at a level more remote from its departure as a leaf trace and so has little protoxylem. At this level the original location of the protophloem (pph in Fig. 14) is being usurped by fibers (f in Fig. 13). The future metaphloem in both bundles is blocked out as lateral arcs of tissue (e.g., ph in Fig. 13), but the original continuity of phloem tissue as a single strand is most evident in Fig. 14 in the early protophloem (pph) region. The contrast in number of protoxylem elements between distal (Fig. 13) and proximal (Fig. 14) positions of the same bundle is pronounced.

The level of vascular differentiation at opposite ends of a bundle, i.e., its distal and proximal extremities, is represented in Figs. 15–19, but not from a single bundle. In Fig. 15 the protoxylem (px) and protophloem (pph) are apparent at opposite poles of the bundle. This represents a leaf trace close to its departure into a leaf, which is evident from the numerous actual and potential protoxylem elements and the absence of differentiated metaxylem. At a lower level of a comparable bundle (Fig. 16) the metaxylem vessel is present (mx) and there is more limited mature protoxylem (px). In both these bundles the pph is a single strand and there is no differentiation of phloem in the lateral parts of the bundle. This stage should be contrasted with the later condition in Figs. 13 and 14 and the mature condition in Fig. 12. The metaxylem originates in the sinus between the differentiating metaphloem strands so that it eventually appears to be flanked by their mature end products and remains unconnected to the protoxylem (cf. Fig. 16 in contrast with Fig. 12). At the basal extremity of bundles (Figs. 17–18) there is little indication of vascular tissue, until finally there remains only a strand of narrow cells that never differentiate as vascular tissue (Fig. 19). The sequence represented by these figures in the order 15–16, 13–14, and 17–18 represents features that change as one follows an entering leaf trace in a basipetal direction from its insertion into the stem. These images may be compared with the sequence shown as Figs. 10–20 in Tomlinson et al. (2001). It is important to emphasize that these changes within the developing crown are progressive apically, since a bundle present in a mature internode, with fully differentiated xylem and phloem (e.g., Fig. 12), may be continuous distally with an immature bundle (e.g., Figs. 13 or 14). The basal end of a newly initiated axial bundle (as in Fig. 19) is likely to be continuous apically with a procambial strand that will not connect to a leaf until many plastochrons later.

Contrast between protoxlem and metaxylem elements is extreme, as seen in longitudinal section, because early developing protoxylem initials form an overlapping series of fusiform cells, in contrast to the linear series of late-developing barrel-shaped metaxylem elements with transverse end walls (cf-mx in Figs. 20 and 21 with px in Fig. 21). Protoxylem elements mature progressively first as annular and then as helical elements of wider diameter with subsequent extension as internodes elongate. This produces a wide range of diameters for protoxylem elements, with the most recently formed elements still incompletely differentiated (Fig. 11). Maturation, with pitted secondary walls, of the last-formed protoxylem elements and all the metaxylem elements does not begin until internodal elongation ceases. This does not occur until after the associated leaf is fully extended (i.e., P_{fe} in Fig. 5A). The collective first appearance of the metaxylem vessel initials is surprisingly late and in the internode below P_8 (e.g., Fig. 20), with subsequent widening (Fig. 21) and then extension (Fig. 25). Metaxylem vessel differentiation refers to the extended middle part

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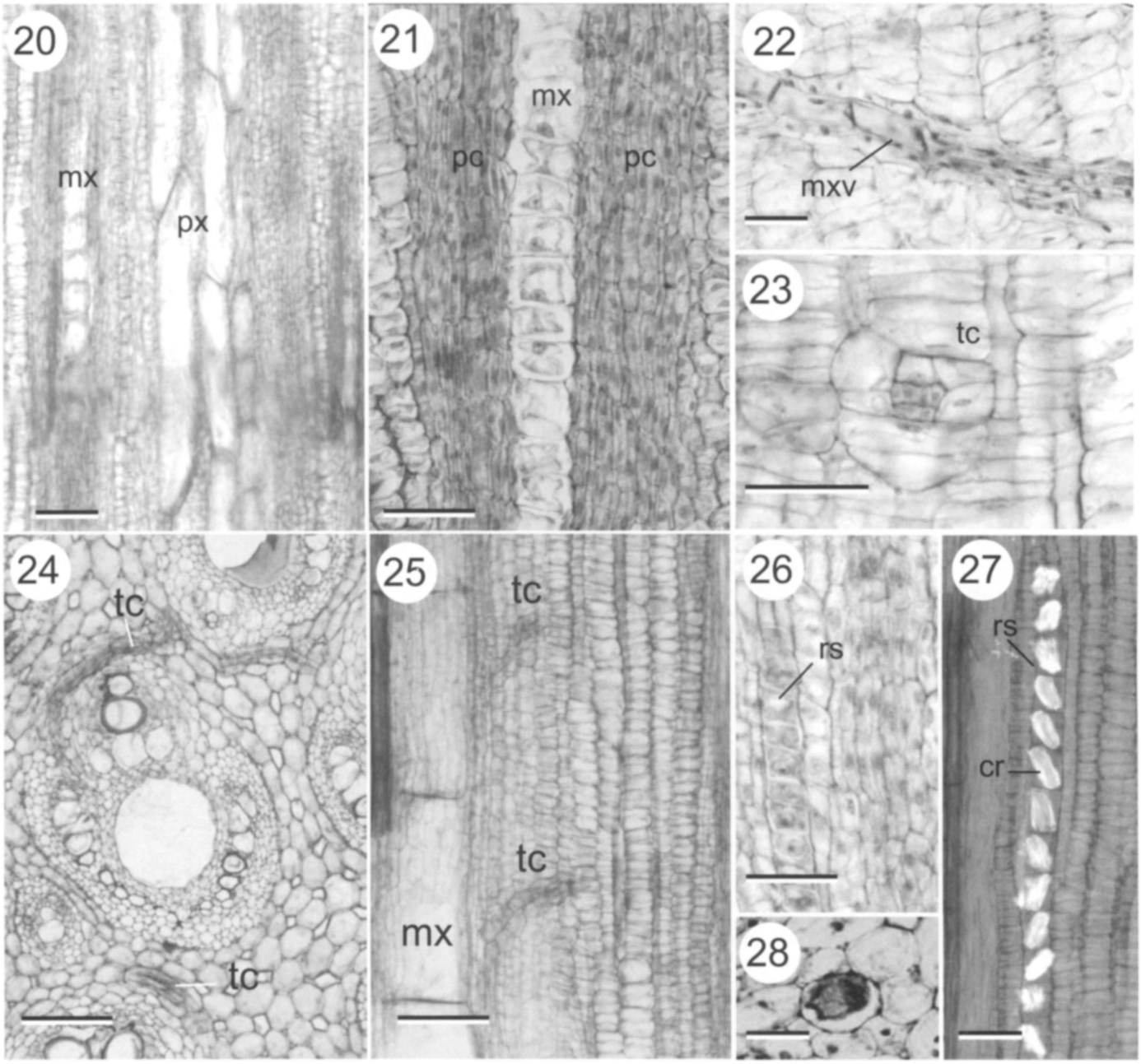
like layer, metaxylem absent. **16.** Similar bundle at a lower level, with early differentiation of metaxylem vessel. **17–19.** Sections at three successively lower levels toward the blind end of a central vascular bundle and without vascular tissue. Figure abbreviations: ct = cambium-like layer, f = fibers, mph = metaphloem, mx = metaxylem, ph = phloem, pph = protophloem, px = protoxylem. Scales = 400 μm for Fig. 11; 100 μm for Figs. 12–16; 200 μm for Figs. 17–19.



Figs. 20–28. *Calamus longipinna*. Development of various histological features in longitudinal section, except Figs. 24 and 28. **20.** Contrast in shape of young metaxylem and protoxylem elements before wall maturation. **21.** Differentiation of a metaxylem vessel as a series of wide cells contrasting with the undifferentiated procambial cells on each side. **22.** Detail of transverse commissures with early differentiation of its single metaxylem vessel. **23.** Early stage in differentiation of a transverse commissure as seen in transverse view. **24.** Vascular bundle in internode below P_8 with early development of transverse commissures as procambial strands. **25.** Transverse commissures showing beginnings of connection to the immature metaxylem vessel to the left. **26.** Early stage in formation of a raphide canal as a series of raphide sacs with part of a procambial strand to the right. **27.** Maturing raphide canal with individual cells represented by crystal bundles illuminated in polarized light. **28.** Transverse view of early raphide canal with dense plasmolyzed cytoplasmic contents. Figure abbreviations: cr = crystals, mx = metaxylem, mxv = metaxylem vessel, pc = procambium, px = protoxylem, rs = raphide sacs of raphide canal, tc = transverse commissure. Scales = 200 μm for Figs. 20, 21, 24, 25; 60 μm for Figs. 22, 23, 26; 50 μm for Fig. 25; 40 μm for Fig. 27; 20 μm for Fig. 28.

of the overall length of a vascular bundle: basally (at the blind ending of the bundle) there is a short series of overlapping narrow metaxylem elements; distally in the leaf trace the series of narrow metaxylem elements also ends blindly. The absence of mature metaxylem in the developing shoot until about P_{11} emphasizes the importance of protoxylem maturation in shoot development.

Transverse commissures—The transverse veins that connect adjacent axial bundles appear late, first evident in the internode below P_8, as dedifferentiated strands (Figs. 22 and 23) within the ground parenchyma. Here the contrast between them and the relative maturity of axial bundles is pronounced (Fig. 24). In longitudinal view they appear somewhat regularly spaced (Fig. 25). Their frequent subsequent irregular course is

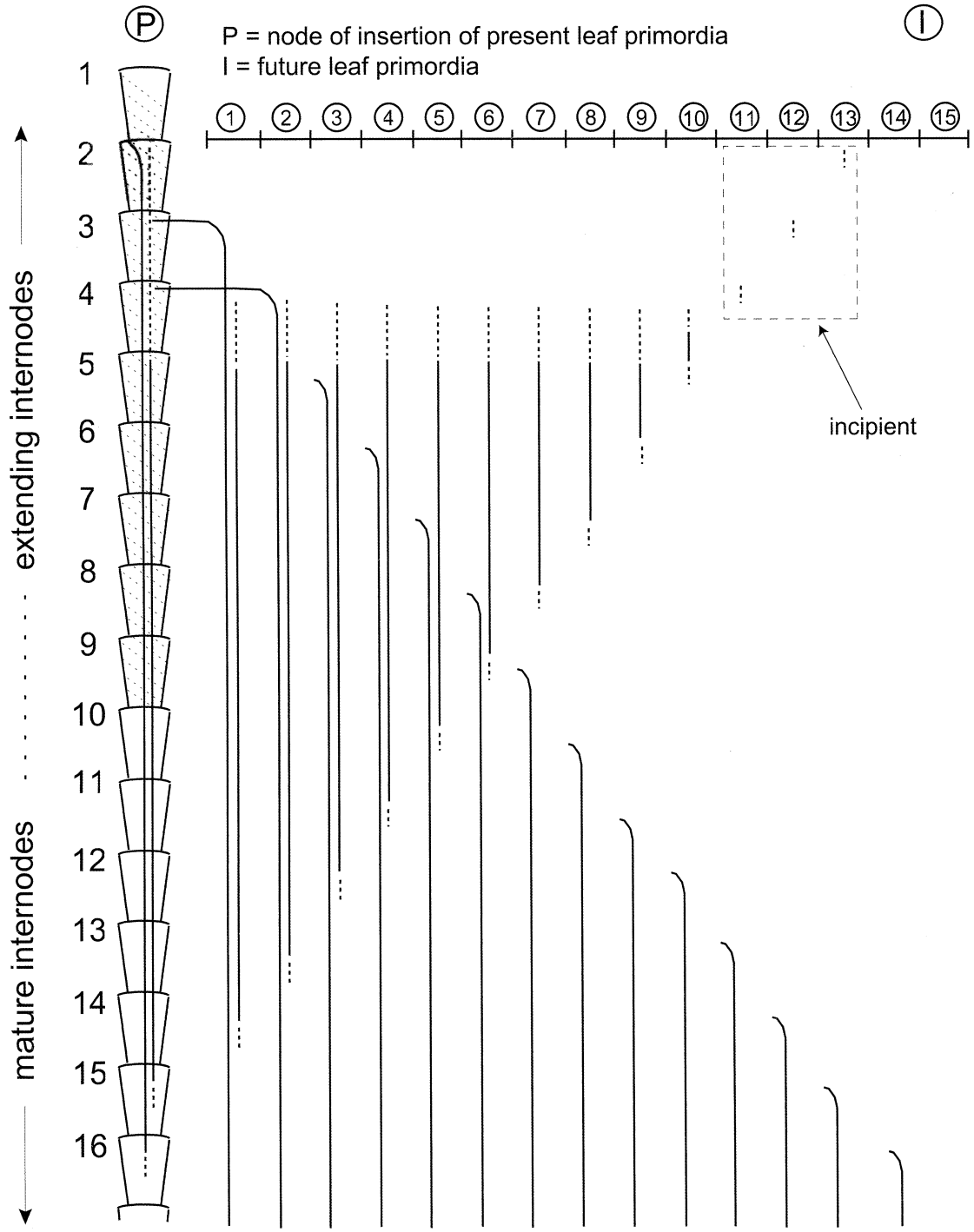
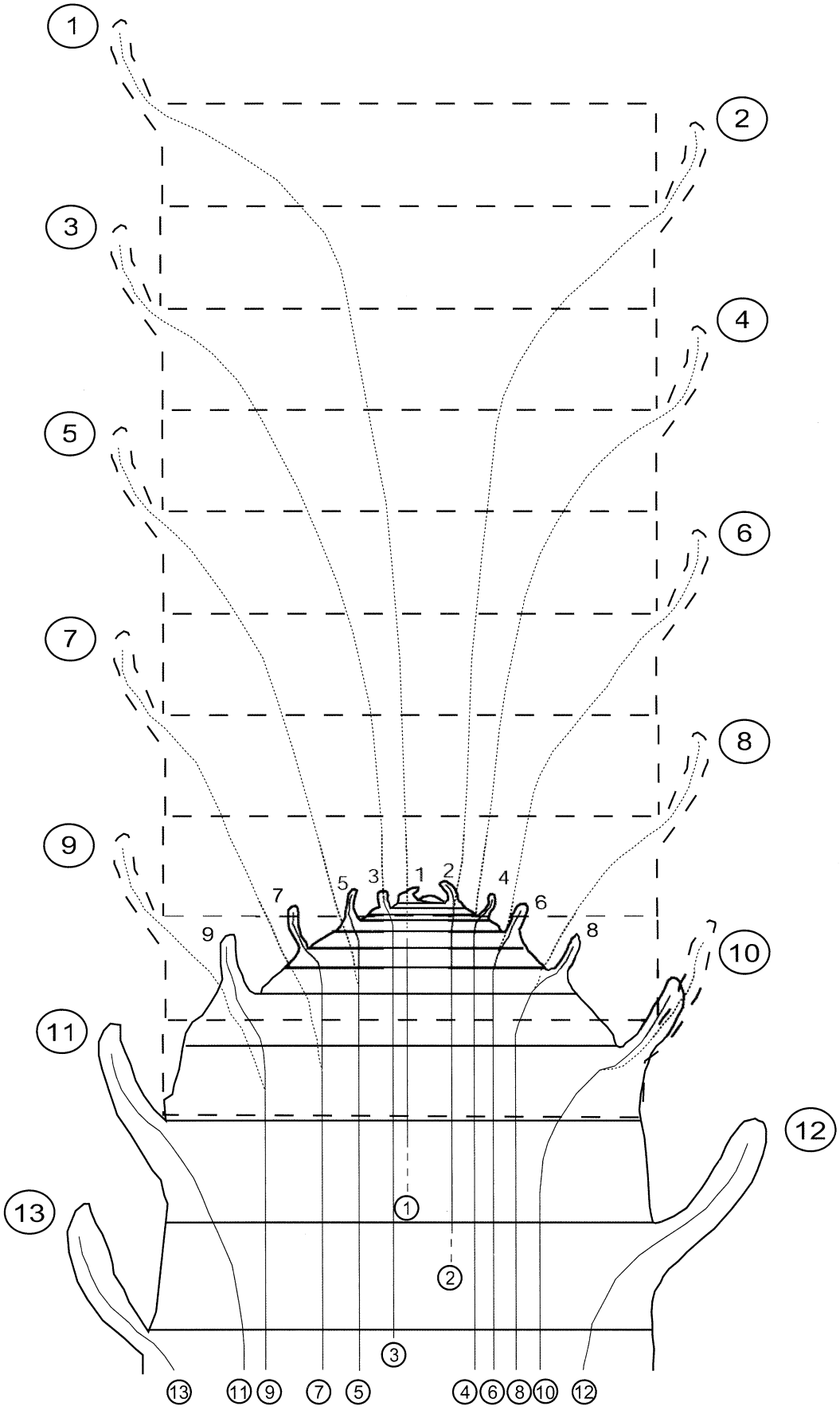


Fig. 29. Representation of actual and potential axial bundles and their leaf connections. The left-hand figure corresponds to the published plot in Tomlinson et al. (2001), with the estimated full extent of an axial strand connected distally to P_2 and ending blindly in the internode below P_{16}. The second line represents a strand extending from the internode below P_{15} and putatively connecting to the youngest leaf primordium (P_1). Its distal end (dotted) cannot be identified in the shoot apical region (hatched) of the internodes below P_1 to P_3, which are still extending. However, its basal end must exist as shown. The right-hand figure represents a series of axial bundles, one connecting to each leaf. In addition an axial bundle to each future leaf primordium (I_1–I_{10}) is shown, its basal end evident, its distal end (shown dotted) still undifferentiated. The axial bundle number remains constant because of a 1 : 1 relation among "lost" (as leaf traces) bundles and "gained" (newly differentiated) bundles. In the developing crown, the number of bundles decreases because axial bundles are still undifferentiated, although their incipient presence may be suspected, as indicated.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a consequence of their unequal levels of attachment to axial bundles (Fig. 25), which becomes much exaggerated during internodal extension. Xylem and phloem differentiate late in these elements, at about the time of overall internodal maturation, although the single metaxylem vessel is visible well in advance (Fig. 22, mxv). The process of dedifferentiation that produces these bundles can be seen in a longitudinal section that cuts transversely through a commissure (Fig. 23). It is clear from this developmental information that these commissures are not the homologue of "bridges" (strands connecting leaf traces and axial bundles in palms of normal habit) as was suggested by Weiner (1992). It is equally clear that the attachment of these strands to a metaxylem vessel will depend on the simultaneous wall maturation of xylem in the two bundle types in order to preserve a continuous pathway for transport (Fig. 25).

Raphide canals—Linear series of cells that include mucilage and a cluster of raphides are a feature of the ground tissue of the *Calamus* stem. They appear as early as the internode below P_4 as files of cells with dense cytoplasmic contents (Fig. 26) and so demonstrate histological differentiation well above the level of differentiation of vascular tissues. Surrounding cells are somewhat shorter than other ground tissue cells as a continuous layer without intercellular spaces, but do not constitute a distinct epithelium. Cell contents of the immature raphide sacs themselves stain intensely with safranin so that these initials are obvious in transverse view (Fig. 28). Once differentiated, the cells of the raphide canals do not divide so that they become considerably extended by subsequent internodal elongation. A cluster of raphide crystals appears early in each cell of the canal within one to two plastochrons of their initial differentiation (Fig. 27). Extension of the cells of the raphide canals is considerable, but the raphide crystals do not increase in number and are easily overlooked in mature tissue, although they are easily seen in mucilage exuding from cut surfaces.

Ground parenchyma—The central ground parenchyma of the axis shows vacuolation as early as the first plastochron (Figs. 8 and 9), but with continued transverse cell division so that extension growth is promoted. This results in extended files of cells within the wall of the original mother cell, as is very evident in longitudinal section (Fig. 25), but with persistence of the original intercellular space. A distinctive feature of this developmental process is the thickened, hydrated texture of the wall adjacent to the intercellular spaces, made conspicuous by the intense staining with alcian green (e.g., Figs. 13 and 24). The feature is retained in mature stem internodes even after wall thickening and lignification are completed. The significance of this structural feature is not clear. Tannin cells are not observed in these stems.

Flagellar vasculature—Even though the flagellum is recognizable as an axillary meristem as early as P_2, and is postgenitally fused to stem and leaf sheath above its node of insertion, its vascular system develops much later than that of the main axis. For the first five plastochrons it remains uniformly meristematic. Vascular bundles become recognizable as 1–2 strands in transverse sections at the level of insertion of P_5, with five bundles at a corresponding level in P_6, the number increasing to the maximum (10–13) at the insertion of F_8. The basal extent of flagellar bundles is limited to the internode below because they disappear relatively abruptly without making any connection to stem bundles. This restricted development shows that they are confined morphogenetically to the original axillary meristem. Their discreteness is further indicated by the limits of the shell zone, which in later stages becomes a zone of narrow parenchyma cells. Vascular differentiation in the flagellum is recognizable at about the level of insertion of P_8 as protoxylem and protophloem in all but the most recently formed bundles. The only displacement of axial bundles in association with the insertion of the flagellum is a slight irregularity caused by the outgoing leaf traces. Axial bundles are here seen as obliquely sectioned strands in transverse view, below the flagellar attachment.

Summary—From our knowledge of the mature vascular architecture of *Calamus* (Tomlinson et al., 2001) and the present information we can summarize our understanding of how the axial system of the shoot in *Calamus* is initiated (illustrated in Figs. 29 and 30). Figure 29 represents the described structure, based on the earlier analysis, but presented in a developmental context, with the shoot drawn in an extended form for clarity but with elongating and mature internodes at the same magnification. Within this shoot a complete leaf trace continuous into the node of insertion of P_2 is drawn, with its basal end terminating in the internode below the insertion of P_{16}, i.e., the axial bundle extends through 15 internodes, of which the upper eight are still elongating. We choose 15 internodes because this is the maximum distance estimated for the length of an axial strand (fig. 43 in Tomlinson et al., 2001). Within the same stem diagram we represent an equivalent bundle that would enter the youngest leaf primordium (P_1) by the next plastochron, this bundle having originated within the internode below the insertion of P_{15}. Its distal terminus would not easily be recognized and is represented by a dotted line. The right-hand portion of the figure represents a single bundle continuous into each successively older leaf, plus an additional bundle that would be expected to become continuous with each of the successive future leaf primordia (I_1 to I_{15}) in order of their age. Their position is shown outside the left-hand diagram to simplify the illustration. These bundles are shown existing up to I_{10}, but their distal extremities disappear (as dotted lines) in the internode below P_4. Each of them, as ex-

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Fig. 30. Diagrammatic representation of axis development in *Calamus*, based on Figs. 6 and 29. The lower figure represents the developing crown with successive leaves represented alternately in a single plane. Axial bundles (solid vertical lines) are continuous into all but P_1. It is assumed that each axial bundle is continuous basally for ten internodes, e.g., the bundle connecting to P_2 ends blindly below the insertion of P_{12}. The upper figure represents the axis after internodal extension is complete with the continuation of the existing bundles represented by dotted vertical lines. There is no branching as the diagram might imply. For simplicity, axial bundles that would exist as blind-ending bundles in younger internodes are not shown. They would exist in mature internodes and be connected to leaves younger than P_1 as in Fig. 29. The complexity of the real system comes from the large number of axial bundles and their variable length. (The figure is modeled on fig. 6 in Priestley, Scott, and Gillett 1935.)

pected, would end blindly below in order of their age. Further bundles in the "incipient" box might be expected to exist, but would not be recognizable in serial sections.

This model is based on a system with only a single bundle to each leaf and all bundles extending through 15 internodes. At any level the number of axial bundles remains constant at 15, since for each bundle lost by capture as a leaf trace, one is gained by differentiation of a new bundle from vacuolating ground parenchyma cells. The actual complexity of the stem itself can be appreciated from the fact that about 60 central bundles are lost at each leaf insertion as leaf traces and that there is no constant length for every one of them. Later-differentiating bundles are likely to be shorter.

The change in configuration of the leaf insertion and its associated internode during primary expansion of the stem that must exist in an actual shoot is illustrated (Fig. 30). The diagram is realistic because it is based on the plotted example of Fig. 6, but simplified in representing a constant distance for any axial bundle as ten internodes. The solid lines represent bundles and tissue at the initiation of the expansion of the crown, the dotted lines their further development once internodal extension and expansion is complete (i.e., ten plastochrons later). The diagram is simplified because we have not represented bundles that would be continuous into leaves younger than P_1 (cf. Fig. 29).

The only illustrated strand that is discontinuous both apically and basally is the one leading to P_1. The strands to P_2 and P_3 end blindly below within the figure, but are continuous into a leaf. All other bundles (from P_4 to P_{12}) extend basally out of the limits of the figure. The simplification of this figure is evident, but it represents a reasonable facsimile of our observations and their interpretation as a developmental process.

DISCUSSION

The unique vascular architecture of *Calamus* contrasts completely with that of nonclimbing palms, as described by Zimmermann and Tomlinson (1965, 1966), in which the cane-like palm *Rhapis excelsa* was used as a model. In *Rhapis* vascular development is determined by a "meristematic cap," i.e., a dome-shaped meristem that generates axial bundles as branches of existing leaf traces; the axial bundles eventually make contact with a leaf to become, in turn, leaf traces. As in *Calamus* axial strands are initially uncommitted because leaf traces appear well before the leaf they will supply.

Calamus differs from this system in that there is no meristematic cap. Instead, vascular bundles are generated by dedifferentiation of vacuolated ground parenchyma cells, such that their level of initiation is represented by a basally blind-ending vascular strand and their distal, also blind-ending, apical portion continues to differentiate until "captured" by a newly initiated leaf primordium. As the leaf primordium enlarges through about nine plastochrons vascular connections continue to be made up to a maximum of about 200 central bundles. These first-formed connections result in bundles of the central system, which provide the main stem-conducting system. Late-differentiating bundles are small, numerous, and mainly fibrous, but are not regarded as a cortical system, as develops in the *Rhapis* model. They have a primary mechanical function in the mature stem.

Vascular differentiation within the procambial strands follows in principle the same sequence as in *Rhapis* (Tomlinson and Vincent, 1984), with the protoxylem providing the exclusive leaf–stem hydraulic system within the developing crown. In *Calamus* protoxylem is first evident, together with protophloem, in the bundles connecting the axis with P_5. This represents the upper limit of continuously generated vascular tissue. Because such leaf traces represent the distal connection made by preexisting axial bundles, it is certain that vascular tissue already exists in older internodes. In contrast, metaxylem vessels do not begin to differentiate until the internode below P_8. They do not mature until extension growth has ceased, i.e., at about the internode below P_{10}, which is the first fully extended foliage leaf. The peculiarity of the *Calamus* system, in which axial bundles may extend unconnected for as much as 15 internodes, is that the basal end of a bundle may be fully mature, whereas its distal extremity can still be an axial strand, represented by a blind-ending procambial bundle. From our knowledge of the uniform construction of *Calamus* canes (Weiner, 1992) we can assume that these developmental details are universally applicable, although canes of larger dimensions are likely to be more complex numerically (cf. Fisher, 1978).

Although the method of vascular development is highly distinctive, some of its features may exist in other monocotyledons, albeit in examples where stems are of determinate growth. For example, in their study of *Alstroemeria*, Priestley, Scott, and Gillett (1935) demonstrate that an axial bundle diverges from a parent strand within the apical region of the stem at a level that is eight plastochrons before the appearance of the leaf it will eventually supply. Consequently, as in *Calamus*, axial strands are differentiated well before they become committed to a specific leaf primordium. On the other hand, axial bundles are interconnected in a very regular way, unlike *Calamus*. The relatively well-investigated shoot of *Zea mays* is too highly specialized to afford comparison because it is precisely determinate in organization and has pronounced nodal plexi (Sharman, 1942; Pizzolato and Sundberg, 1999). In conifers and gymnosperms, in which the vascular system is largely continuous, there is evidence that axial strands can also appear well before the leaf they will supply (Esau, 1965), but within the residual meristematic ring of the shoot apex. Here, vascular analysis is made easy by the precise association between phyllotaxis and vascular architecture, with a small number of bundles continuous over relatively short distances. The limited understanding of these developmental processes in monocotyledons makes comparative discussion difficult.

The uniqueness of the *Calamus* system is not an exclusive consequence of its pronounced late internodal elongation because a preliminary analysis of the stem of the American climbing palm *Desmoncus* shows that it is constructed according to the *Rhapis* principle. Further research should be directed to comparative analysis of the vascular architecture of climbing palms, but most obviously to those calamoid palms that show a stem anatomy contrasted with that of members of the tribe Calaminae (Uhl and Dransfield, 1987; Weiner, 1992). Furthermore, there is preliminary evidence that there are consistent correlations between vessel types and vascular anatomy of other climbing monocotyledons (Tomlinson and Fisher, 2000). The general suggestion is that more detailed comparative study of these taxa may produce informative results about the hydraulic system of plants in general. Because climbing monocotyledons lack secondary growth, they are particularly vulnerable to xylem dysfunction. The present contribution is preliminary and is certainly incomplete in details.

LITERATURE CITED

BURKILL, I. H. 1966. A dictionary of the economic products of the Malay Peninsula, 2nd printing. Ministry of Agriculture and Cooperative, Kuala Lumpur, Malaysia.

ESAU, K. 1965. Vascular differentiation in plants. Holt, Rinehart, and Winston, New York, New York, USA.

FISHER, J. B. 1978. A quantitative description of shoot development in three rattan palms. *Malaysian Forester* 41: 280–293.

FISHER, J. B., AND J. DRANSFIELD. 1977. Comparative morphology and development of inflorescence adnation in rattan palms. *Botanical Journal of the Linnean Society* 75: 119–140.

FISHER, J. B., AND J. DRANSFIELD. 1979. Development of axillary and leaf-opposed buds in rattan palms. *Annals of Botany* 44: 57–66.

PIZZOLATO, T. D., AND M. D. SUNDBERG. 1999. Initiation of the vascular system and the transition to flowering in early tassels of *Zea mays* land race chapalote (Poaceae). *International Journal of Plant Science* 160: 1111–1125.

PRIESTLEY, J. H., L. I. SCOTT, AND E. C. GILLETT. 1935. The development of the shoot in *Alstroemeria* and the unit of shoot growth in Monocotyledons. *Annals of Botany* 49: 161–179.

SHARMAN, B. C. 1942. Developmental anatomy of the shoot of *Zea mays* L. *Annals of Botany* n.s. 22: 245–282.

TOMLINSON, P. B. 1990. The structural biology of palms. Oxford University Press, Oxford, UK.

TOMLINSON, P. B., AND J. B. FISHER. 2000. Stem vasculature in climbing monocotyledons: a comparative approach. *In* K. L. Wilson and D. A. Morrison [eds.], Monocotyledons—systematics and evolution. Vol. 1. Proceedings of the Second International Conference on the Comparative Biology of the Monocotyledons, September 1998. C.S.I.R.O., Melbourne, Australia.

TOMLINSON, P. B., J. B. FISHER, R. E. SPANGLER, AND R. A. RICHER. 2001. Stem vascular architecture in the rattan *Calamus* (Arecaceae—Calamoideae—Calaminae). *American Journal of Botany* 88: 797–809.

TOMLINSON, P. B., AND J. F. VINCENT. 1984. Anatomy of the palm *Rhapis excelsa* X. Differentiation of stem conducting tissues. *Journal of the Arnold Arboretum* 65: 191–214.

UHL, N. W., AND J. DRANSFIELD. 1987. Genera palmarum. Allen Press, Lawrence, Kansas, USA.

WEINER, G. 1992. Zur Stammanatomie der Rattanpalmen. Ph.D. dissertation, University of Hamburg, Hamburg, Germany.

ZIMMERMANN, M. H., AND P. B. TOMLINSON. 1965. Anatomy of the palm *Rhapis excelsa*. I. Mature vegetative axis. *Journal of the Arnold Arboretum* 46: 160–180.

ZIMMERMANN, M. H., AND P. B. TOMLINSON. 1966. Anatomy of the palm *Rhapis excelsa*. IV. Vascular development in the apex of the vegetative aerial axis and rhizome. *Journal of the Arnold Arboretum* 48: 122–142.