INTER-PETIOLAR STIPULES OF *GALIUM MOLLUGO* L.*

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Abstract

The inter-petiolar stipules of *Galium mollugo* are separate, leaf-like in shape and size, and also in the possession of a single trace bundle. Morphogenetical studies at the shoot apex show that the initiation and vascularization of the leaves and stipules are different. Only the leaves bear axillary buds; the leaf-like stipules do not bear them. The foliar foundations by their union form a *collar* round the axis, from which it separates by the differentiation of a separation layer in its adaxial surface. The glandular hairs also arise from this layer. The axis on its separation shows a wavy contour of its surface. The crest of each initially shallow wave is occupied by a meristem which helps in the development of each ridge. From later behaviour of the apparently single leaf-trace bundle it appears that it is composed of three bundles and the laterals are concerned with the origin of the stipules and their traces.

The family Rubiaceæ, to which *Galium mollugo* belongs, have stipules as a diagnostic character. The inter-petiolar stipules of the family show a variety of forms. 'The two stipules, one from each leaf, that stand side by side, are usually *united*,' as in *Pæderia fœtida* and *Ixora parviflora*; or they may remain *separate*, as in *Galium mollugo*. There occurs another form, the *intra-petiolar*, in which the stipules unite between the petioles and the axis, 'and are frequently united to one another and to the petiole so that a *sheath* is formed round the stem,' as in *Gardenia*.

In *Galium* the stipules remain separate, in size and shape they resemble the leaves for which they are often mistaken. This happened when Hooker (1875, p. 22) stated that in the Rubiaceæ the leaves are stipulate except in the Galieæ which are without stipules.

In *Galium mollugo* the leaves and stipules are slightly stalked, 6–8 in a whorl (Fig. 1). When the stipules are separate and leaf-like, the number of such leaves and stipules should be 6 (2 + 4), but in *Galium* the number

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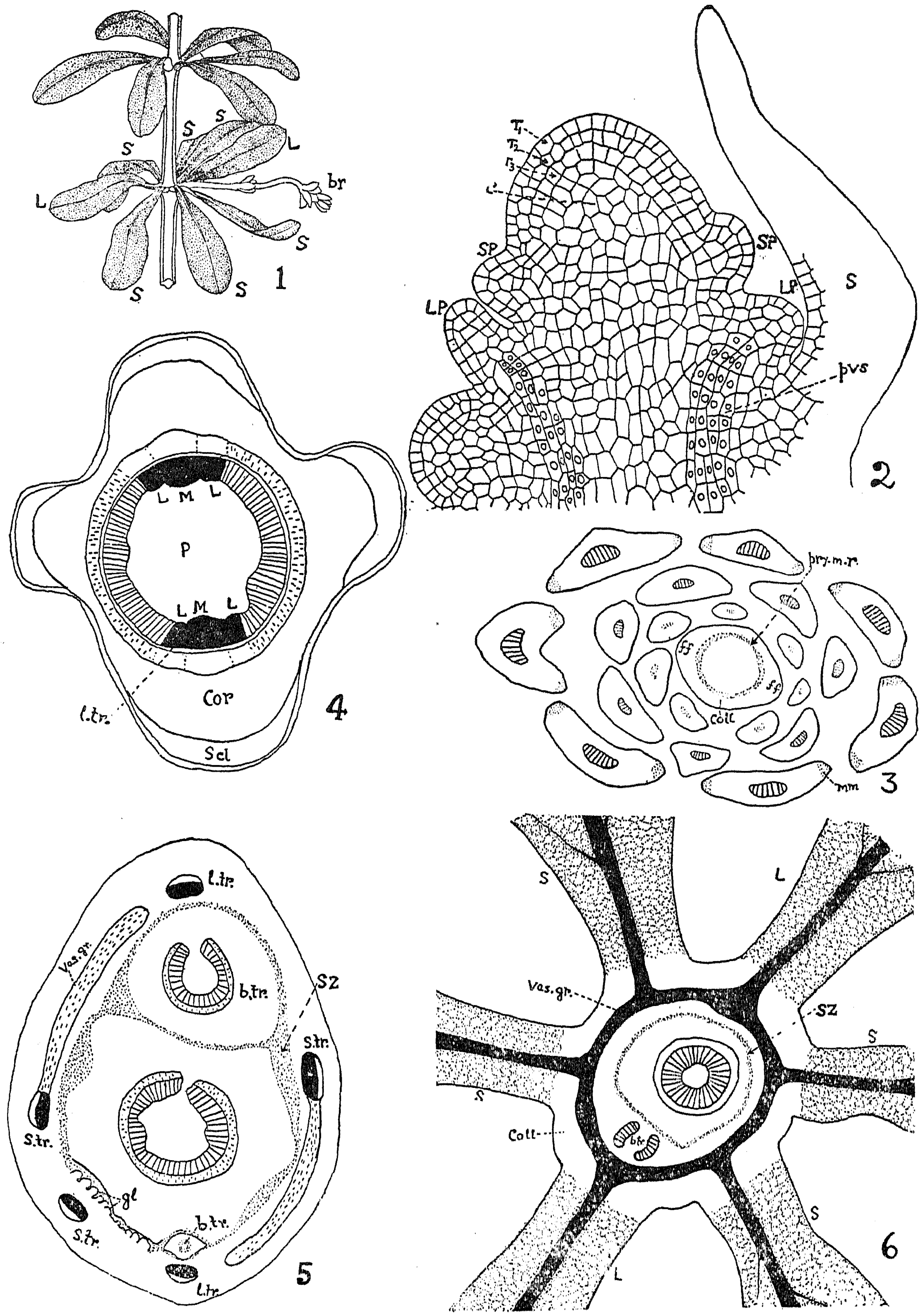
varies from 4 upwards (Strasburger, 1908; Lawrence, 1951). Willis (1951, pp. 573–74) offered an explanation for the reduced and the higher number of stipules in this species. He writes: The real leaves are those which subtend axillary buds. In the simplest case of a whorl of 6 each leaf has 2 separate stipules; if the stipules be united in pairs a whorl of 4 results; if each of the stipules be branched into 2, we get a whorl of 10; and if the centre pair of half stipules on either side be united a whorl of 8 results. In *Galium mollugo* a whorl of 7 makes the above explanation rather difficult to accept without their ontogenetical studies.

In 1914 Sinnott and Bailey while investigating on the phylogeny of the Angiosperms studied the nodal anatomy of a very large number of species under different families and suggested a clue for the determination of the morphology of the stipules. They stated that "in the majority of plants with a *trilacunar node*, stipules are present; that in almost in all with *unilacunar node*, stipules are absent, ..." (p. 442). They stated further that "each stipule obtains its vascular supply, if it has one, from branches derived from the corresponding lateral traces" (p. 447).

In the Rubiaceæ the node is generally unilacunar, and as such, the leaves should be exstipulate. Sinnott and Bailey explained this non-conformity by stating that "in the Rubiaceæ although the vascular supply causes a single gap the leaf-trace is composed of three bundles; and the stipules when present are often related to the lateral ones of this trio" (p. 449). In regard to the nature of the inter-petiolar stipules of this family they stated that these are "the products of a fusion of two formerly independent and adjacent ones; since each stipule receives its vascular supply from both leaf-traces" (p. 449).

Mitra (1948) worked on the origin, development and morphology of the inter-petiolar stipules (united) of *Pæderia fœtida* (Rubiaceæ). The leaf-trace in this species consists of two bundles placed very close to each other while being differentiated in the primary meristem ring (Helm, 1931). But before leaving for their leaf they unite, and on leaving cause *only one gap in the nodal ring*. The two fused bundles however, even before they leave the stele, show organization into three regions: a central and two laterals. Only the central one goes to the leaf as its median bundle, but the two laterals diverge around the axis through the tangentially extended foliar foundations (*collar*); the corresponding laterals from the opposite leaves meet and fuse to form a closed circuit of provascular meristem in the collar. These combined laterals then give out branches which form the traces of the stipules which are composite in nature.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FIGS. 1–6.

The free stipules of *Galium* present altogether a different problem, and the present investigation has been made with a view to study the origin and vascularization of the stipules and leaves which appear morphologically alike.

MATERIALS AND METHODS

Materials for the study were collected in Darjeeling, and fixed and preserved in Formol-acetic acid-ethyl alcohol solution. Attention of the senior author was drawn to this species, *Galium molluga* L., by his host Prof. J. Saha of Darjeeling Government College, in April 1956. Sections, both longitudinal and transverse, were cut 8–10 μ thick, and stained in safranine-fast green combination. Freehand sections through the nodal regions of adult shoot were also cut, suitably stained, and made permanent for a comparative study of the nodal structures in the young and adult shoots. To study the vascular supplies to the leaves and stipules from the axial ring *in situ* suitably thick nodal region with intact leaves and stipules was cut from the stem and treated with lactic acid at ordinary room temperature for four days to make the region perfectly clear; equally good result was obtained by heating the sections in lactic acid at 60 ° C. for 2–4 hours. The cleared material was then thoroughly washed and lightly stained with safranine solution, washed again and mounted in 50% glycerine solution, and finally sealed with paraffin wax.

The text has been illustrated with camera lucida drawings and photomicrographs; duplication has been avoided. Magnifications have been noted for each drawing in the explanations.

OBSERVATIONS

1. *Shoot Apex Organization.*—The free apex is dome-shaped measuring about 60 μ high and 75 μ wide at the level of divergence of the youngest pair of leaf primordia (*see* Table I where measurements of six apices in longitudinal sections are given).

The tunica consists of 2–3 layers of anticlinically dividing cells running over the dome, and encloses the corpus consisting of a mass of irregularly dividing cells, larger in size with contents which stain lightly. Regular zonation of the apical meristem into distinctly discernible regions, like the central mother initials, flank meristem and file meristem, is not evident (Fig. 2, 7).

2. *Initiation of the Leaf Primordia.*—About 32 μ below the extreme tip the shoot apex in transverse section shows a rounded mass of cells with two distinct regions: a central lightly stained and a peripheral deeply stained

TABLE I

Number of shoot apices	Height in μ	Width in μ
1	45*	75
2	60	75
3	60	105†
4	64	75
5	65	80
6	62	74

* This may be from a tangential l.s.

† This evidently includes the foliar foundations of the next pair of leaf primordia.

more compact cells corresponding to the corpus and tunica areas (Fig. 9). 24–30 μ further down the apex, *i.e.*, 56–62 μ below the extreme tip, the initiation of a pair of leaf primordia is indicated by the horizontal extensions of the axis giving the latter an oblong form (Fig. 10). These local extensions of the axis upon which the foliar erection is later seen to take place has been named by Grégoire (1935) and Louis (1935) *soubassements foliares,* translated *foliar buttress* by Foster, and *foliar foundation* by Majumdar. The formation of this foliar foundation at the shoot apex prior to the erection of the leaf primordium upon it has been confirmed by later workers on morphogenesis at the shoot apex of dicotyledons (Foster, Majumdar, Mitra and others).

The foliar foundations, formed in definite sectors of the apical dome and placed almost opposite to each other, then extend tangentially around the dome by divisions of the hypodermal cells until the two corresponding extending arms meet and fuse to form a *collar* congenetically united with the axis (Figs. 3, 5, 10). Meanwhile a primary meristem ring is seen to organize in the apical meristem in which the acropetally progressing trace bundles soon differentiate (Figs. 3, 11).

Further down the axis the apical bud in transverse sections shows the leaves and stipules arranged in different stages of development and separation from the axis and from one another. The primordia of the leaves and stipules also show stages in the development of the lamina from marginal meristems with very feeble secondary veins (Figs. 3, 6, 15–17). It will be seen from Figs. 17 and 18 that the stipules and leaves with their single midrib bundle cannot be distinguished one from the other except for the fact that the leaves bear vegetative buds in their axils and the stipules are without them. Only one of the two axillary buds at a node grows into a full-grown branch (Figs. 1, 15–17).

In median longitudinal sections of the apical bud the leaves and the stipules could also be distinguished. The leaves get their vascular supply directly from the axial ring, but the stipules get their supply secondarily from the girdle formed by the fusion of the laterals (?) of the leaf traces (*see* below. Figs. 6, 8, 14).

3. *The Leaf-trace.*—In *Galium* apparently only one provascular strand for each leaf primordium differentiates in the nodal ring. At a later stage of differentiation each such strand is seen to organize into three recognisable regions, namely, a central flanked by two lateral ones (Figs. 4, 12, 13). The central one later moves out towards the base of the petiole and form the single median bundle of the leaf. The laterals instead of following suit turn round and diverge in the collar along the sides of the axis, meet the corresponding ones coming from the other leaf, and coalesce to form a *complete girdle* or circuit of vascular meristem running from one primordium to the other. The two median strands stand out facing each other as two prominent knots in the girdle (Figs. 6, 14).

4. *The Stipular Trace and the Origin of the Stipules.*—In the portion of the girdle between the two median strands the trace of each stipule differentiates at equal intervals. If the two leaves are placed at the node just opposite to each other the number of stipule traces is equal on each side (*i.e.*, 2, 2 or 3, 3); but if their positions are not quite opposite then the number of such traces are unequal, *i.e.*, 2 and 3, on the two sides (Figs. 5, 18). This explains the number of leaves and stipules, 6, 7 and 8, found in a whorl in *Galium mollugo*. Increase in the number of stipules by branching or splitting has not been observed in this species. The traces show equal development in both the structures. Each stipule primordium is organized by the active division of the cells surrounding its trace.

5. *Separation of the Collar from the Axis.*—A zone of actively dividing cells appears at the junction of the collar and the axis still confluent with each other. The origin of this zone takes place in the cells of the adaxial surface of the collar (Figs. 5, 13, 14).

Separation takes place in this zone. One very interesting fact is noticed: this layer also gives rise to the appendages, the glandular hairs. These are found borne on the adaxial surface of the collar and stipules and of the leaves, but in the case of the latter they are borne near the margins, the region opposite the axillary bud which separates from the axillant leaf very late, is devoid of these glandular hairs. Separation of the collar and the development of the glandular hairs appear to have some intimate connection as they occur almost simultaneously (Figs. 5, 15).

The separation layer does not pass round the axis in a circular way. It is rather wavy in outline passing internal to the axillary buds and below the collar, so that when the axis separates it shows four or five irregular shallow ridges on its surface. A part of the separation layer also passes external to the axillary bud and later causes separation of the latter from the former (Figs. 5, 6, 13).

6. *Separation of the Leaves and Stipules from one another.*—The beginning of their separation from one another is almost simultaneous with the separation of the collar from the axis. Upgrowth of the leaf and stipule primordia is maintained by active divisions of cells immediately surrounding each trace bundle, and then by intercalary growth of each primordium. The beginning of isolation of each primordium, as a result of this local growth, is indicated by the appearance of notches or furrows on the outer surface of the collar demarcating the area of individual pieces. The furrows then proceed inwards ultimately dividing the collar into as many parts as there are leaves and stipules in a whorl (Figs. 15, 16, 17).

At separation the base of the petiole of each primordium is convex on the abaxial surface and almost flat on the adaxial, and angular where these two surfaces meet. The petiole continues as the midrib region of the blade. Its angular portions are occupied by marginal meristems which are responsible for the development of the wings of the lamina. One or two feeble branches are given out by the midrib bundle into the developing wings (Fig. 6).

7. *Appendages of the Stipules and Leaves.*—Their origin has already been described. They fill up the space formed between the stipules and the axis during its separation from the collar. These hairs have also been described and figured by Metcalfe and Chalk (1950). According to them they are composed of resiniferous cells. Their structures have well been described by Mitra (1948) in the cases of *Pæderia* and *Ixora.*

8. *Ridges of the Internode.*—The adult internode has four or five well developed ridges and as many furrows (Fig. 19). They are without any

vascular tissue, and the summit of each is occupied by a group of cells which are thick-walled and sclerenchymatous. The epidermis is provided with a few short and stiff unicellular hairs. Both these features give the stem its rough exterior.

At the node the ridges and furrows are absent. The manner of separation of the collar from the axis gives the latter a wavy contour. The crest of each wave is occupied by a meristem which by centrifugal differentiation causes its radial extension and a high ridge is formed. The cells at the summit of each ridge are finally modified into a mechanical tissue.

DISCUSSION AND CONCLUSION

The origin and morphology of the stipules have been a subject of enquiry since perhaps the days of Henry (1846). But the three outstanding contributions to this subject came from Eichler (1861), Colomb (1887) and Sinnott and Bailey (1914). Eichler was the first to point out that the stipules arise without exception in pairs secondarily from the leaf-base by intercalary growth. Colomb then established the intimate association of the origin of the stipules and their traces with the branches of the laterals of a three-bundle leaf-trace. Sinnott and Bailey from very wide and exhaustive studies of the Angiosperms came to the conclusion that:

(1) Plants with unilacunar nodes are mostly without stipules,

(2) Plants with trilacunar nodes are mostly stipulate, and

(3) the origin of the stipules and their traces is invariably correlated with the branches of the laterals of a three-bundle leaf-trace.

Mitra in a series of papers on different types of stipules, *viz.*, free lateral, inter-petiolar, intra-petiolar, adnate, foliaceous, ochreate, etc., supported Colomb and Sinnott and Bailey in all essential details and Majumdar (1955) showed that the stipules in all cases are outgrowths of the base of the stipulate leaf and thus supported Eichler. But the best proof of "correlation between stipules and lateral leaf-traces" came from Dormer (1944) and Sensarma (1957).

In *Lotononis corymbosa* (Leguminosæ) and *Azara microphylla* (Flacourtiaceæ) in which the stipules are single and of the same size and shape as the leaves, the leaf-trace consists of two bundles, the median and a lateral, the second lateral evidently is suppressed. Dormer explains that "the bilacunar condition is the result of suppression of one stipule and of the corresponding lateral trace". The conclusion is rather indirect, but Sensarma has provided direct evidence from a study of the origin and vascularization of the stipules in *Muntingia calabura* L. (Elæocarpaceæ). The node in this plant is trilacunar, and the leaf-trace consists of three bundles—the median and two laterals. One of the laterals branches and a stipule is formed on that side with the branch as its trace, whereas the other lateral does not branch and a stipule is not formed on that side. Therefore, the absence of a stipule is definitely correlated with the absence of branching of the second lateral.

In both *Pæderia* and *Galium* from its very differentiation the leaf-trace consists apparently of one bundle, and the node is unilacunar. The subsequent behaviour of the leaf-trace, however, shows that it is composed of three parts (*cf.* Sinnott and Bailey). Sinnott (1914) postulated that the trilacunar condition of the node is the primitive condition, and the unilacunar one has been derived from it either by the suppression of the two laterals, or by their approximation with the median. Studies on the nodal structures of the above species appear to support the origin of the unilacunar condition by the approximation of the three trace bundles into one, and the stipule traces are derived from the branches of the laterals.

Separation of the collar (fused foliar foundations at the node) from the axis is effected by the origin and differentiation of a *separation zone* in the adaxial surface of the collar. This meristem also gives rise to the glandular hairs.

The primordia of the leaves and stipules organize in the tissues of the collar immediately surrounding their individual trace bundle. Their upgrowth is maintained by active divisions of cells constituting each primordium. The lamina in each case is formed by a marginal meristem. Separation of each primordium from its neighbours begins in the form of notches or furrows on the outer surface of the collar between contiguous primordia. The furrows proceed inwards until the primordia are separated from one another.

Suggestions of Willis that the extra number of stipules above the normal four originate by their branching, or by branching and union, is not supported by the present developmental and anatomical studies.

Presence of glandular hairs on the stipules appears to be a regular feature in the Rubiaceæ. Their development has intimate connection with the separation of the collar from the axis. Their function seems also to be ecological.

The formation and growth of the ridges on the internode is determined by two factors, *viz.*, (1) the nature of the origin of the separation layer in the collar and internal to the axillary buds, and (2) the differentiation of a meristem at the head of each ridge. At its separation from the collar and axillary buds, the axis shows a wavy contour of its outer surface. The meristem by cutting cells centrifugally adds to the height of each ridge. Finally the meristem is modified into a mechanical tissue surmounting each ridge.

Sinnott and Bailey have suggested that the stipules are homologous with a pair of earliest teeth, or the basal lobes of a leaf. From the nature of the vascular supply to these organs (teeth, lobes and stipules) from the trace bundles, the suggestion of these two authors merit further developmental studies which the present writers propose to undertake.

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Explanation of Text-Figures

Figs. 1–6.—Fig. 1. A portion of the adult shoot showing leaves (*l*) and stipules (*s*) in whorls at nodes; only one branch (*br*) is seen developed, ×1. Fig. 2. Median longisection of the shoot apex showing distribution of tunica (*t*) and corpus (*c*) in the apical meristem; leaf (*lp*) and stipule (*sp*) primordia; provascular strand (*pvs*) going to leaf primordia from the axial ring ×270. Fig. 3. Transverse section of the vegetative bud showing leaf and stipule primordia at different stages of growth and differentiation; in the central region the axis is seen completely surrounded by the foliar foundations (*ff* and *coll*) with a primary meristem ring (*pry.m.r.*) differentiated, ×95. Fig. 4. Transverse section of an internode just below the node showing the leaf-trace (*l, m, l*) just before leaving the axial stele for its leaf, ×20. Fig. 5. Transverse section of a very young node showing that the trace bundles have already moved out in the collar; the girdle (*vas.gr.*) is broken into discrete strands—leaf (*l.tr.*) and stipule (*s.tr.*) traces with a portion of the girdle still remaining; separation zone (*sz*) is differentiated and passes round the axis in a wavy manner; glandular hairs (*gl*) are seen to form the separation zone on the adaxiar surface of the collar; only one axillary bud develops, ×15. Fig. 6. Lactic acid treated nodal region in t.s. showing the connection of the leaf and stipule traces with the vascular girdle; separation zone, bud trace and axial stele are also shown, ×18.

br–axillary branch; *b.tr.*–bud trace; *c*–corpus; *coll*–collar; *cor*–cortex; *ff*–foliar foundations; *gl*–glandular hairs; *l*–leaf; *lp*–leaf primordium; *l.tr.*–leaf-trace (*m, l* are its median and lateral bundles); *mm*–marginal meristem; *p*–pith; *pry.m.r.*–primary meristem ring; *pvs*–provascular strand; *s*–stipule; *scl*–sclerenchyma strand; *sp*–stipule primordium; *s.tr.*–stipule trace; *sz*–separation zone; T_1, T_2 and T_3–tunica layers; *vas.gr.*–vascular girdle in the collar.

Explanation of Plate XXII

Fig. 7. The shoot apex in median longisection—it shows the origin of leaf and stipule primordia, vascular supply to the former, and origin of the glandular hairs (*cf.* Fig. 2).

Fig. 8. Vegetative bud in median longisection—shows the distribution of the leaf and stipule primordia and their vascular supplies; only one axillary bud has developed.

Fig. 9. T.S. of the shoot apex 32 μ below the extreme tip.

Fig. 10. T.S. of the shoot apex 65 μ below the tip—shows the formation of the collar by the foliar foundations still congenetically united with the axis.

Fig. 11. T.S. further down the shoot apex showing differentiation of the primary meristem ring.

Fig. 12. T.S. of the axis showing the leaf-trace organised into three regions, the laterals with a tendency to diverge from the normal course.

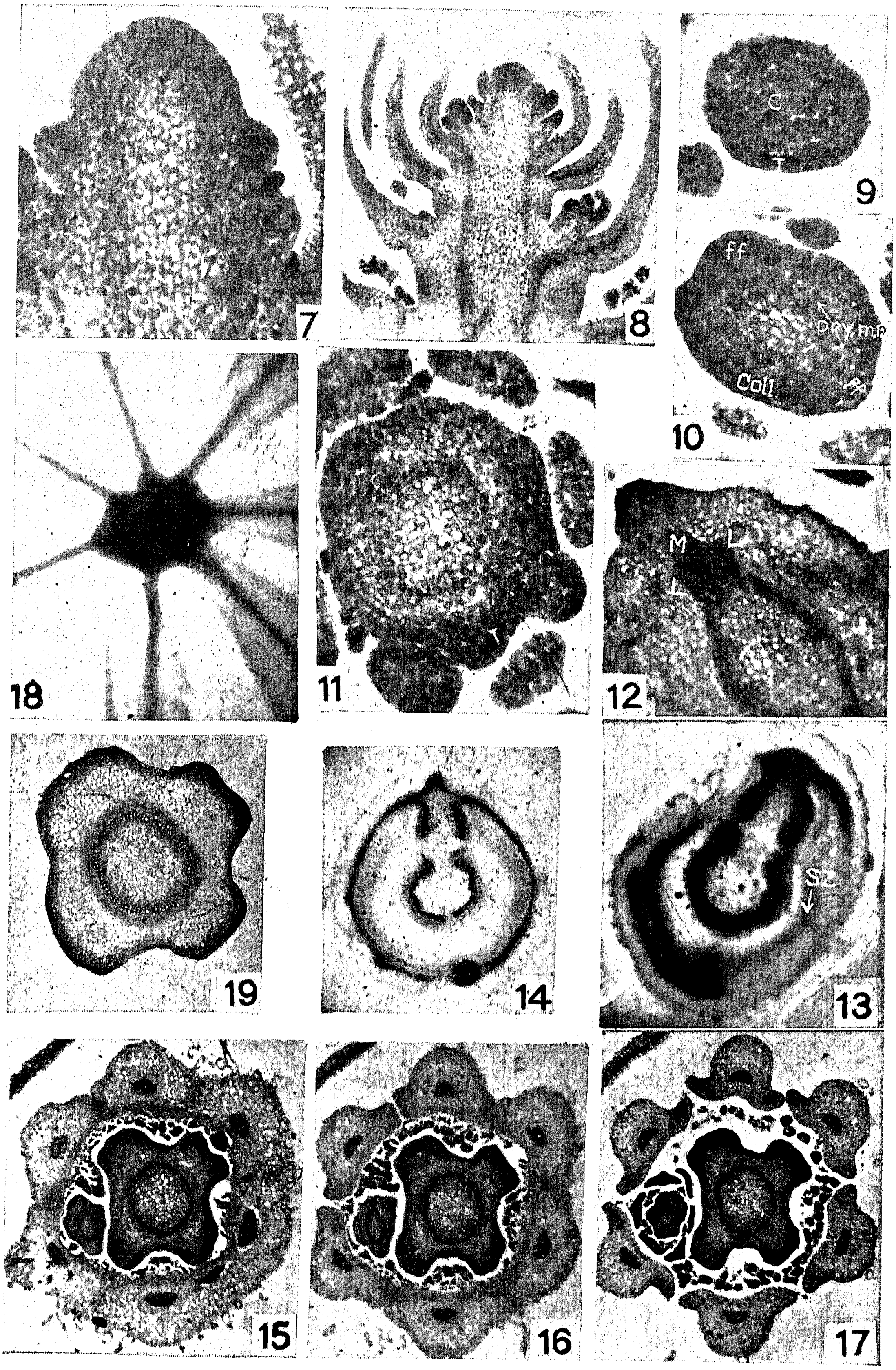
FIG. 13. T.S. of the axis with the collar showing the leaf-trace with the laterals diverged into the collar to form the vascular girdle, the bud trace and the separation zone passing round the axis.

FIG. 14. T.S. of the node showing axial stele, leaf gaps, bud-trace, leaf-traces forming a girdle around the axis with the traces of the pair of leaves and of the stipules in the girdle, and the separation zone.

FIGS. 15–17. T.S. through the node showing the separated collar with the leaf and stipule primordia organized around their individual traces; beginning and full separation of the primordia from one another; differentiation of marginal meristem in each primordium, position of the glandular hairs, formation of the lamina from the marginal meristem, development of only one axillary bud, the other atrophied (Figs. 16, 17); and the outline of the axis into broad furrows and ridge with meristems occupying apex of each ridge.

FIG. 18. Lactic acid treated nodal region showing the supply of the leaf and stipule traces from the vascular girdle of the collar (for details *see* Fig. 6).

FIG. 19. T.S. of adult internode.



FIGS. 7–19