

TAXONOMIC POSITION OF THE GENUS *NYCTANTHES*

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A B S T R A C T

The genus *Nyctanthes* with only one species *N. arbor-tristis* Linn. having flowers like that of *Jasminum* was originally included in Oleaceae. In view of its strongly quadrangular stem and its apparent resemblance to *Tectona* and other members of the family Verbenaceae, Airy Shaw (1952) placed the genus in a new subfamily under the family Verbenaceae. Stant (1952) gave some anatomical evidence for the inclusion of *Nyctanthes* in the Verbenaceae. On the basis of comparative study of *Nyctanthes*, along with some members of Oleaceae, Verbenaceae and Loganiaceae on cytology, general anatomy of stem and leaf, wood anatomy, floral anatomy and palynology and also on the preliminary data of the chemical constituents present in the plants, the authors state that *Nyctanthes* has not much affinity to the members of the Verbenaceae, although it has some similarity with several oleaceous members. After taking all points into consideration this genus is assigned to a new family Nyctanthaceae.

INTRODUCTION

The Genus *Nyctanthes* Linn. with only one species *N. arbor-tristis* Linn. was originally included in the family Oleaceae mainly on account of the structure of the flower which is somewhat like that of *Jasminum*. A second species *N. aculeata* Craib was later described from Siam (Craib, 1916). Till recently the status of the genus *Nyctanthes* as belonging to the family Oleaceae was maintained by all workers on Taxonomy.

Lindley (1846) following Robert Brown (Prodromus) split up the family Oleaceae into Oleaceae and Jasminaceae. He was the first to suggest a verbenaceous affinity of Jasminaceae in which the genus *Nyctanthes* was included. During recent years Airy Shaw (1952) has remarked that in general features and appearance *N. arbor-tristis* bears little resemblance to *Jasminum* or for that matter, to any member of the Oleaceae, but resembles the members of the family Verbenaceae, particularly *Tectona*, on account of its strongly quadrangular stem. He included the genus in a new subfamily Nyctanthoideae Airy Shaw under the family Verbenaceae mainly on the basis of morphological characters. He refers to the earlier work of the Italian botanist Bertoloni (1858) who apparently being unacquainted with the genus, described a specimen of *Nyctanthes arbor-tristis* Linn. received from Mozambique, East Africa, as a new genus and species, and assigned the new genus to the family Verbenaceae. From the anatomical study of leaf and stem of *Nyctanthes arbor-tristis* Linn., Stant (1952) supported Airy Shaw and states that there is some evidence that the structure of *Nyctan-*

thes is consistent with its being included in the Verbenaceae. On the basis of anatomical and palynological studies on *Nyctanthes arbor-tristis*, Kundu (1966) was of opinion that *Nyctanthes* belongs neither to the Oleaceae nor to the Verbenaceae. More recently, Kundu and Chakraborty (1966) studying the anatomical and also chemical constituents of *Nyctanthes arbor-tristis* in the light of modern chemotaxonomic ideas supported the placement of the genus in a separate family related to Loganiaceae.

It is felt that more detailed anatomical data, particularly on the structure of the secondary wood and of the leaves should be studied carefully before any definite conclusion could be made. Comparative anatomy alone may no doubt, solve many difficult taxonomic problems, however, many workers now feel that taxonomic relationships should be based on the study of all fields of plant sciences. The authors undertook detailed anatomical and palynological studies in order to find out whether the genus *Nyctanthes* should be retained in the family Oleaceae or included in the family Verbenaceae or should be treated separately.

A detailed comparative study of *Nyctanthes* along with some members of Oleaceae, Loganiaceae and Verbenaceae on anatomy including floral anatomy, cytology, palynology etc. has been made by the present authors in order to evaluate the correct taxonomic position of the genus.

MATERIALS AND METHODS

For the present study along with *Nyctanthes arbor-tristis* the following members of three differ-

ent families which seem to have some affinity with the genus, were investigated from various aspects.

I. Oleaceae

1. *Jasminum grandiflorum*, 2. *Olea cuspidata*,
3. *O. fragrans*, 4. *Fraxinus floribunda*.

II. Verbenaceae

1. *Clerodendrum infortunatum*, 2. *C. siphonanthus*, 3. *Tectona grandis*, 4. *Vitex negundo*, 5. *Phyla nodiflora*.

III. Loganiaceae

1. *Strychnos nux-vomica*, 2. *Buddleja davidi*,
3. *B. paniculata*.

The materials were collected from the Bose Institute garden, Indian Botanic Gardens, Shibpur and Royal Botanic Garden, Darjeeling, India.

The investigation had been attempted from a number of aspects, but it was not possible to study the above mentioned members through all aspects due to non-availability of materials.

For the study of nature of trichomes, stomata and epidermal cells, cuticular peeling was made, stained in cotton blue and mounted in 10% glycerine. The materials for general and floral anatomical studies were mostly fixed in Formal: Acetic: Alcohol (F. A. A.). After fixation, materials were dehydrated, infiltrated and embedded in paraffin following customary schedule. Sections were cut at 12-15 μ thickness and stained in Safranin and Light Green combination. Wood anatomical studies were made from the sections of wood 40-60 μ thick obtained through wood microtome. The sections were stained in Safranin and Fast Green. Macerations technique was also adopted following usual method.

Polleniferous materials were collected from dry Herbarium sheets or from freshly collected flowers to study the morphology of pollen grains. Slides were prepared following Erdtman's modified method.

To study the somatic number and morphology of chromosomes in *Nyctanthes*, young healthy root-tips from germinated seeds were pretreated with 8-Oxy-quinoline for 1½ hrs. and fixed in Acetic: Alcohol (1:3). Temporary squash preparations were made by Orcein-N HCl (9:1) staining method. Camera Lucida drawings were made at suitable magnifications.

OBSERVATIONS

I. Cytology

The somatic chromosome number of *Nyctanthes arbör-tristis* Linn. is found to be 46 (reported here for the first time). Chromosomes are of medium to

short size. Three pairs of them possess secondary constrictions. The primary constrictions of most of the chromosomes are of sub-median type, though chromosomes with median or sub-terminal primary constrictions have also been found (Fig. 1).

Regarding chromosome number it may be stated that nearly 50 genera of the family Oleaceae possess 46 somatic chromosomes in their diploid cells (Darlington & Wylie, 1955). The members of the Verbenaceae and Loganiaceae possess different diploid numbers other than 46, excepting *Clerodendrum thomsoniae* of Verbenaceae (Darlington & Wylie, 1955).

II. General Anatomy

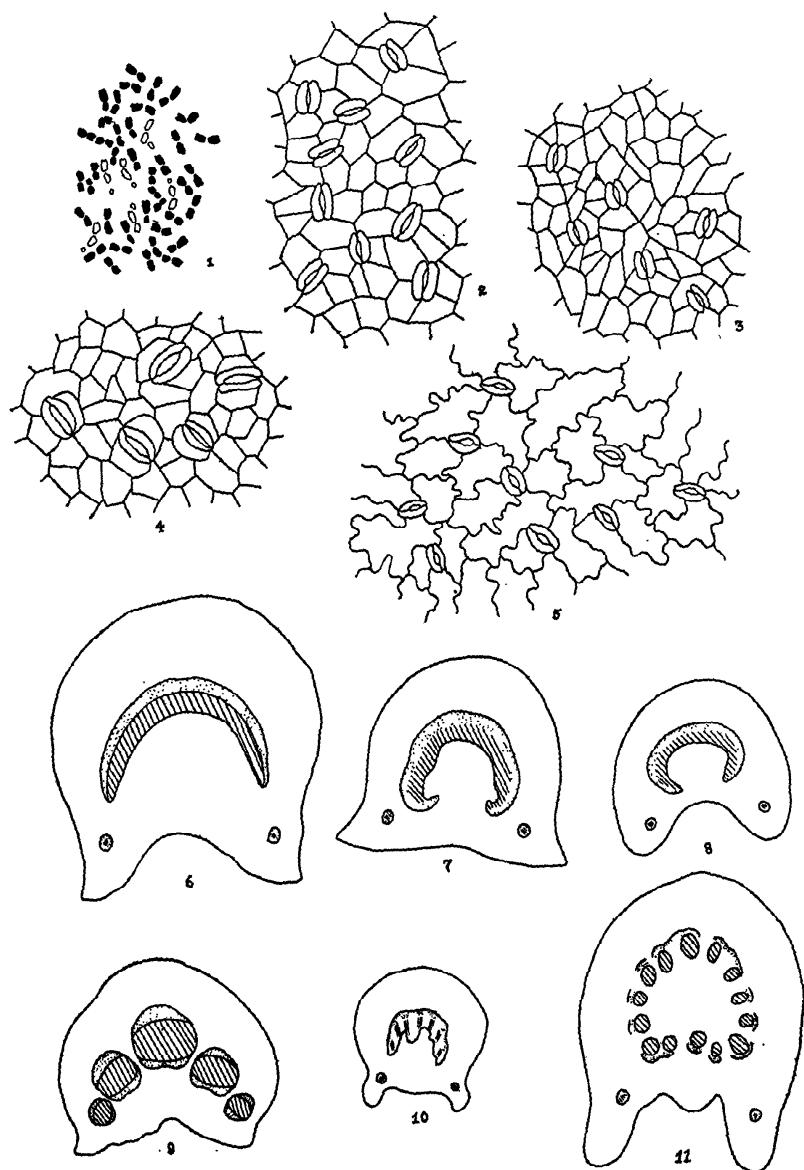
1. Shoot Apex: As the shoot apex is the seat of all primary formative activities, it may show characters which may be used for taxonomical considerations. The structure of the shoot tip of *Nyctanthes arbör-tristis* was compared to that of the shoot tips *Jasminum* spp., *Clerodendrum* spp., *Phyla nodiflora*, *Lippia geminata*, *Lantana camara*, *Tectona grandis*, etc. studied by Kundu, Mitra and Saha (1966). In the relatively shrubby and woody species of the family Verbenaceae a large and wide apical dome with massive and complex corpus consisting of clearly defined central iepithals, flank and rib meristemes has been observed. In all of them tunica layers varied from 2-4 layers. In *Tectona grandis* a distinct zone of cambial-like cells as observed in *Chrysanthemum morifolium* by Popham and Chan (1952) is observed. Such a zone is, however, absent in *Clerodendrum* and other woody species studied. In *Jasminum* sp. the tunica is 3-layered. The cells of the T₂ layers are somewhat different from those of T₁ layers, being elongated in the longitudinal direction. In the corpus a central zone is clearly demarcated from the peripheral zone. In the majority of cases the tunica in *Nyctanthes* is two layered, although a 3-layered tunica has been observed in several cases. The distinction of the central and peripheral zone of the corpus is not clear in *Nyctanthes*. Therefore, shoot apex of *Nyctanthes* can be distinguished from *Jasminum* sp. (Oleaceae) and the woody shrubby members of the family Verbenaceae, particularly from *Tectona grandis*.

2. Leaf: (a) **Petiole**—Sections through the distal end of petioles of *Nyctanthes arbör-tristis* in transverse plane show a crescent shaped vascular mass with two or three lateral bundles (Fig. 6). In *Jasminum* the vascular structure in petiole appears as a

shallow crescent-shaped mass accompanied by one or two bundles (Fig. 8). Most of the other members of Oleaceae, possess crescent shaped vascular structure, the degree of curvature varying from species to species.

In the Verbenaceae the distal end of petiole in

transverse sections show a shallow crescentic, median vascular strand in *Lantana*, *Lippia* and *Vernena*, a deeper crescentic strand with fine ends in species of *Lantana* and *Vitex* (Fig. 10). In *Clerodendrum infortunatum* the two end of the crescentic strand consisting of separate vascular bundles meet making



Figs. 1-11: 1. Somatic chromosomes, of *Nyctanthes arbor-tristis*. 2-5. Structure of epidermal cells and stomata; 2. *Nyctanthes*. 3. *Jasminum*. 4. *Strychnos nux-vomica*. 5. *Clerodendrum infortunatum*. 6-11. Structures of T. S. of the distal ends of petioles: 6. *Nyctanthes*. 7. *Buddleja paniculata*. 8. *Jasminum*. 9. *Strychnos nux-vomica*. 10. *Vitex negundo*. 11. *Clerodendrum infortunatum*.

it more or less round (Fig. 11). Metcalf and Chalk (1960) states that in the crescentic vascular strand of *Clerodendrum fargesii* the xylem is directed into separate groups in contact with a continuous arc of phloem. In *Tectona grandis* the crescentic vascular strand consists of a crescentic ring of discrete bundles with a few medullary ones.

In *Buddleja paniculata* of Loganiaceae, the petiole in transverse sections exhibit a wide U-shaped median vascular strand with inwardly curved ends accompanied by smaller accessory bundles (Fig. 7). The petiole of *Strychnos*, the other member of Loganiaceae investigated here is supplied by three collateral bundles accompanied by two more lateral ones. Each of the bundles possesses intraxylary phloem towards the adaxial side (Fig. 9).

(b) *Stomata*—In *Nyctanthes* as well as in most of the members of Oleaceae investigated ranunculaceous or anomocytic stomata are predominant (Figs. 2 & 3). Stomata in *Clerodendrum* (Fig. 5) and *Duranta* sp. and in a few other members of Verbenaceae are also anomocytic; in *Tectona* and in a few other members, they are paracytic; most of the other members of the Verbenaceae possess diacytic type of stomata. In the family Loganiaceae the type of stomata is variable, cruciferous or anisocytic in most genera, rubiaceous or paracytic in *Strychnos nux-vomica* (Fig. 4) and *Buddleja paniculata*. Ranunculaceous or anomocytic types are also observed (Metcalf & Chalk, 1950).

(c) *Trichomes*—Two types of trichomes, nonglandular and glandular type are present in *Nyctanthes arbor-tristis*. (i) Unicellular trichomes i.e. nonglandular type with pointed ends are found profusely on the upper surfaces of leaves. This type of trichomes at the base is surrounded by a group of 8 or 9 cells protruding from the surface. (ii) Glandular hairs—Each of the hairs possesses a globose head made up of four cells resting on a unicellular stalk. The base of the hair is deeply seated in epidermal layer.

Unicellular non-glandular and glandular hairs also occur in *Tectona* of Verbenaceae while *Clerodendrum infortunatum* of the same family has uniseriate type of multicellular hairs. Glandular hairs have also been recorded in *Olea*, *Fraxinus*, *Jasminum* of Oleaceae and also in *Buddleja* of Loganiaceae. Besides glandular hairs, *Buddleja* possesses stellate hairs. The presence of peltate hairs is characteristic features of the members of Oleaceae (Metcalf & Chalk, 1950) and they are absent in *Nyctanthes*.

3. *Stem*: (a) *Internode*—The internode of *Nyctanthes* is square in shape with quadrangular vascular stele and four cortical bundles with inverse orientation of their elements, one at each corner of the square stem outside the stele (Fig. 12). It has been found from the present study and past records (Metcalf & Chalk, 1950) that no oleaceous member possesses a quadrangular type of stem which is the characteristic feature of the members of Verbenaceae. In the case of the members of Loganiaceae the stele in the internodes is generally round in shape. Presence of cortical bundles has also been recorded in *Fagraea*, a member of Loganiaceae (Hasselberg, 1931). Stant (1952) reports that in this respect there is a resemblance of *Nyctanthes* to *Chloanthes*, a verbenaceous genus, in which the stem is provided with wings supplied by a system of vascular bundles. According to Fotider (1939) and Majumdar (1941) the cortical bundles that are present in *Nyctanthes* are not connected with main stele of stem, but arise from lateral leaf trace bundles.

(b) *Node*—Each leaf of *Nyctanthes arbor-tristis* altogether receives three traces, a massive trace from main stele leaving behind a unilocular node and two laterals from two cortical bundles (Figs. 12-16). In *Jasminum* as well as in other members of Oleaceae the node is unilacunar, sending off a single massive trace to each leaf (Fig. 22). No cortical bundle is observed.

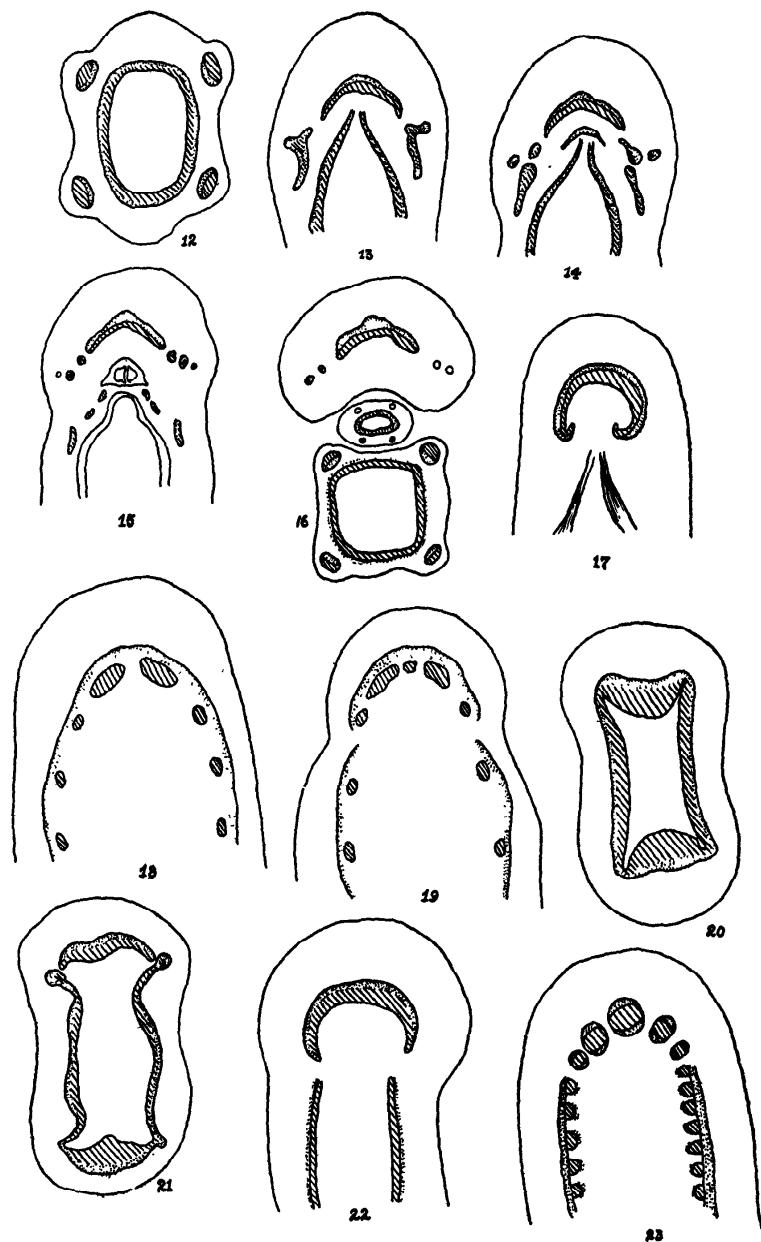
On the other hand the members of Loganiaceae though show unilacunar node the number of traces that depart for a leaf vary from genus to genus. *Strychnos nux-vomica* and *S. potatorum* both have five traces for a leaf whereas *Buddleja davidi* and *B. paniculata* have a single massive trace for each leaf (Fig. 17). The leaf of the most members of Verbenaceae receive a single massive trace or a number of trace bundles (Figs. 18-21) from a unilacunar node. In *Clerodendrum trichotomum* two discrete vascular strands subtended by a single gap, supply a leaf (Marsden & Bailey, 1955). Hasselberg (1931) reports that in *Fagraea* of Loganiaceae, apart from main stele the cortical bundles give supplies to leaf.

III. Wood anatomy

One of the most important aspect of taxonomic investigations is the comparative study of xylem, particularly secondary xylem. The many variations in the structure and arrangement of vessels, tra-

cheids, fibres, rays and vertical parenchyma in the wood have been widely used in the characterisation

of genera and families, particularly in relation to their phylogeny. Details of the anatomy of the secon-



Figs. 12-23: 12-16. *Nyctanthes arbor-tristis*: 12. T. S. of internode. 13-15. Stages in the departure of the leaf trace from the vascular cylinder of axis. 16. T. S. of internode just below leaf insertion showing a bud in the axil of the leaf. 17. Departure of leaf trace in *Buddleja paniculata*. 18-19. Stages in the departure of the leaf trace in *Clerodendrum infortunatum*. It is observed that in the single leaf trace bundle the xylem is dissected into separate groups in contact with a continuous arc of phloem. 20-21. T. S. of node and stage of departure of the single leaf trace bundle in *Vitex negundo*. 22. Departure of the single leaf trace bundle in *Jasminum* sp. 23. T. S. of node of *Strychnos nux-vomica* showing departure of the leaf trace bundles.

dary xylem of *Nyctanthes* and some members of Oleaceae, Verbenaceae and Loganiaceae are presented below.

***Nyctanthes arbor-tristis* Linn.**

Wood diffuse porous, large and small pores more or less uniformly distributed in late and early wood. Wood is composed of vessels, fibre-tracheids and ray parenchyma. Axial parenchyma is absent here. Vessels occur singly or in multiples of 2-5, sometimes 6-8 and rarely 9-13 and have simple perforations and bordered pits. Length of vessels varies from 436 to 834μ . Breadth 50 to 84μ . Rays 1-3 seriate, procumbent, homocellular. Tracheids are mostly modified into wood fibres and fibre tracheids. Fibre-tracheids are long and slender and possess degenerating bordered pits. Fibres which are non-septate are the most frequently occurring elements having the following size range: 469 - 1173μ long and 10 - 30μ wide.

***Jasminum grandiflorum* R.**

Wood diffuse porous, small to large pores gradually increase from late to early wood. Wood is composed of vessels, tracheids, fibre-tracheids, ray and axial parenchyma. Vessels with bordered pits and simple perforation occur singly or in groups. Vessel ranges from 503 - $871\mu \times 50$ - 84μ . Tracheids with bordered pits are elongated and thin-walled, 503 - $905\mu \times 24$ - 35μ . Fibre tracheids are thickwalled and possess irregularly distributed bordered pits, 687 - $2010\mu \times 18$ - 24μ . Fibres occur very infrequently with degenerated bordered pits. Axial parenchyma are paratracheal in nature. Ray parenchyma, 1-2 seriate, heterocellular.

***Olea fragrans* L.**

Wood diffuse porous, large and small pores uniformly scattered in the late and early wood. Wood composed of vessels, tracheids and its elements, ray and axial parenchyma. Tyloses present frequently in early wood. Vessels solitary or in clusters of 2 to 3 having scalariform bordered pits and simple perforation, 264 - $495\mu \times 22$ - 33μ . Tracheids, elongated, gradually tapering at both ends, with opposite bordered pits, 291 - $429\mu \times 11$ - 22μ . Fibre tracheids, lumen narrower, with opposite or alternate border pits, 352 - $1880\mu \times 16$ - 22μ . Fibres with very narrow lumen and small number of degenerating bordered pits scattered irregularly, 1692 - $2350\mu \times 11$ - 22μ . Axial parenchyma, paratracheal. Ray 1-2 seriate, heterocellular.

Fraxinus floribunda

Wood ring porous. Pores in late wood few, solitary or in small groups. Late wood occasionally without distinct rays or patches. Ground mass usually of fibre-tracheids; libriform fibres also present. Vessels, large solitary or in groups of 1-4, rarely 5, pits bordered and simple perforation, 121 - $440\mu \times 33$ - 132μ . Axial parenchyma paratracheal, often aliform and becoming confluent. Rays 1-3 seriate, homocellular.

Clerodendrum siphonanthus

Wood diffuse porous, large and small pores uniformly scattered in late and early wood. Wood composed of vessels, fibres, axial parenchyma and ray parenchyma. Vessels solitary or in groups, with alternate pits and simple perforation, 242 - $429\mu \times 33$ - 88μ . Fibres non-septate with simple pits. Axial parenchyma, vasicentric, paratracheal becoming aliform. Rays 1-3 seriate sometimes 4-seriate, heterocellular.

Tectona grandis

Wood diffuse porous, late wood with distinct radial rays. Pores in late wood few. Wood made up of vessels, fibre-tracheids, fibres and wood parenchyma. Vessels solitary or in groups of 2-3 with alternate simple pits and simple perforations, 220 - $330\mu \times 49$ - 82μ . Fibre tracheids, elongated with simple alternately arranged pits. Fibres occasionally septate. Axial parenchyma, vasicentric paratracheal becoming aliform. Rays 1-3 seriate, upright, heterocellular.

Strychnos nux-vomica

Wood ring porous. Pores in late wood small and few. Wood comprises vessels, tracheary elements and wood parenchyma. Ground mass mainly of fibre-tracheids. Vessels solitary or in groups of two to many with alternately arranged bordered pits and simple perforations, 88 - $374\mu \times 33$ - 77μ . Fibre tracheids with bordered pits alternately arranged. Axial parenchyma confluent paratracheal. Rays 1-3 seriate, rarely 4-seriate, homocellular.

Buddleja paniculata

Wood diffuse porous, large and small pores more or less uniformly distributed in late and early wood. Wood made up of vessels, tracheids, fibres, axial parenchyma and ray parenchyma.

Vessels solitary or in multiples, with alternate bordered pits and simple perforation, 363 - $561\mu \times 27$ -

55 μ Tracheids with bordered pits, alternately arranged, $374-429\mu \times 16-27\mu$. Fibres with narrow lumen and small degenerating bordered pits, $1316-2820\mu \times 11-22\mu$. Axial parenchyma paratracheal. Rays 1-3 seriate, heterocellular.

IV. Floral Anatomy

The necessity for the study of the vascular anatomy of the flowers has been in recent years stressed by modern leaders of plant morphology with regard to the interpretation of difficult taxonomical problems.

The basic vascular organisation in flowers of *Nyctanthes arbor-tristis*, *Jasminum grandiflorum* of Oleaceae, *Clerodendrum siphonanthus* of Verbenaceae, *Strychnos nux-vomica* and *Buddleja paniculata* of Loganiaceae has, therefore, been studied to find out the relation among the members of the families regarding their basic plan.

Nyctanthes arbor-tristis Linn.

Like stem, pedicel of a flower has a square shaped axial stele with four cortical bundles at four ridges. These cortical bundles disorganise near the base of receptacle with the departure of bract traces.

As the stele approaches the receptacle base, it takes the form of a pentagon and from each of the projections and their alternate furrows ten to eleven traces arise and branch out profusely to give supplies to calyx tube (Fig. 24). Immediately after the departure of calyx traces the corolla tube receives at first eight traces and later, two traces from two opposite points enter the corolla tube as stamen traces (Fig. 25). The eight traces for petals branch out irregularly immediately after their departure from the main stele, while the staminal traces remain unbranched. Simultaneously with the departure of traces to corolla tube, the traces for carpel differentiate out from the irregularly arranged vascular masses of axial stele. Each of the carpels receives a large number of trace bundles and the ovule in each carpel is supplied by fused ventrals of same carpel (Figs. 26, 27). In the mean time the two stamens from two opposite points of the corolla tube gradually separate out, each having a single trace.

Jasminum grandiflorum

The pedicel of *Jasminum* contains a number of closely placed vascular bundles arranged in a ring. With the departure of two traces for bracts, the axial stele like that of *Nyctanthes* assumes a five-angled

shape. From the angular points and the furrows ten traces gradually depart for the calyx (Fig. 28). After the departure of calyx traces, six traces further dividing form ten traces and give supplies to the corolla tube as petal traces. Immediately after the departure of petal traces, two more traces enter the corolla tube as stamen traces. After the departure of last staminal traces, the remaining vascular tissue in the axial stele supplies the bicarpellary ovary as one ventral and two dorsals for each carpel (Figs. 29-32).

Clerodendrum siphonanthus

The stele of a pedicel comprises a number of closely placed vascular bundles. With the approach of receptacular base the vascular mass increases in volume and sends off ten traces to the calyx tube (Fig. 33, only 9 traces are seen). Immediately after departure of calyx traces, five traces for petals depart. Simultaneously four stamen traces also leave the stele (Fig. 34). All these nine traces enter the corolla tube for vascular organisation of petals and stamens (Fig. 35). The stele again forms a continuous ring containing less amount of vascular tissue. From this stele each carpel receives one dorsal and two ventral traces (Fig. 35), each of which supplies the ovule of the same carpel (Fig. 36).

Strychnos nux-vomica

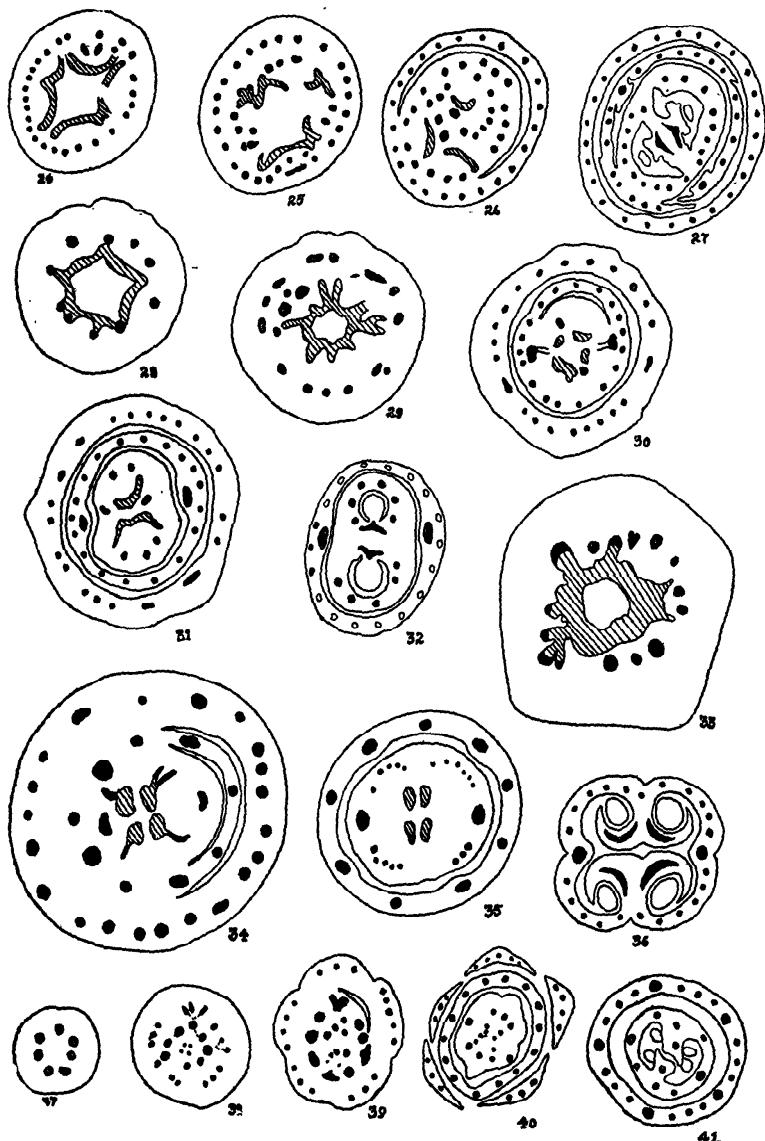
The stele in pedicel is composed of discrete bundles (Fig. 37). At first the stele gives off traces to bracteoles, one for each. At the receptacular base the stele gives rise to eleven traces of which few branch out and ultimately enter the calyx tube (Fig. 38). After departure of the calyx traces a set of ten traces diverge out from the stele and move to corolla tube (Fig. 39). Five of them give supplies to petal after branching out profusely while the other five remain unbranched and supply the five stamens (Figs. 40, 41). After the departure of the last traces to stamen, two dorsals from two opposite points move towards periphery. Later, three traces from either side of the dorsals, separate out as laterals while the remaining mass in the axis gives rise to two fused ventrals of adjacent carpels, which later supply the ovules (Figs. 40, 41).

Buddleja paniculata

The stele in pedicel is a dissected siphonostele. At first the stele gives off traces to bract and bracteoles respectively. Later, ten traces gradually

departing from the stele move to the calyx tube. With the departure of calyx traces, eight traces,

one by one, pass from the stele to corolla tube. Four of them branch out and give supplies to four



Figs. 24-41: 24-27. Floral anatomy of *Nyctanthes arbor-tristis* (see text for explanation). 28-32. Floral anatomy of *Jasminum grandiflorum* (see text for explanation). 33-36. Floral anatomy of *Clerodendrum infortunatum* (see text for explanation). In fig. 36 the two ventral bundles of each carpel are seen to supply the 2 ovules of the carpels. 37-41. Floral anatomy of *Strychnos nux-vomica* (see text for explanation).

petals while the other four remain unbranched and supply four stamens. After departure of last stamen

trace, the remaining vascular mass gives off traces to carpels as one dorsal and two ventrals for each.

The floral anatomy of few members of Oleaceae, Loganiaceae and Verbenaceae (*Kundu & Bose*, unpublished) studied suggests that *Nyctanthes* in no way resemble the members of Loganiaceae or Verbenaceae regarding the vascular organisation of flowers. The basic vascular organisation in flowers of *Nyctanthes* and *Jasminum* is similar in certain respects ; the distribution and behaviour of traces to calyx, corolla and carpels show, however, some differences and do not support their inclusions in the same family. In this connection it may be referred that the present observations on *Nyctanthes* and *Jasminum* corroborates the earlier findings of Fotidar (1939) and Joshi and Fotidar (1941) respectively.

The few members of Loganiaceae and Verbenaceae as studied here show more or less definite number of traces unlike *Nyctanthes*. Rao (1952) made an elaborate floral anatomical study of some members of the family Verbenaceae. His study does not indicate any similarity in the floral anatomical structures of the verbenaceous members and *Nyctanthes*.

V. Palynology

An account of the pollen morphology of *Nyctanthes*, of some members of Verbenaceae (*Phyla*, *Vitex*, *Clerodendrum* and *Tectona*) also of *Jasminum* (Oleaceae) is given below. Although pollen grains of only a few members have been studied, some definite differences are, however, observed.

Nyctanthes arbor-tristis

Pollen grains 3-colporate, spheroidal (both from polar and equatorial view), apocolpium diameter large. Exine thick to medium, reticulate, intectate. Sexine reticulate. Reticulum homobrochate, lumina hexagonal to irregular, simplibaculate. Bacula on the top united to form muri, nexine thinner than sexine.

Phyla nodiflora

Pollen-grains 3-colporate, equatorial view spheroidal, polar view triangular to subtriangular, $38.0 \times 30.0 \mu$. Exine thick, sexine perforated by minute pits (puncta), punctitegillate. Sexine united with nexine by very small bacula. Nexine as thick as sexine or slightly thicker.

Vitex negundo

Pollen grains 3-colporate, prolate to subprolate,

$30.0 \times 20.3 \mu$, polar area small. Exine thick, finely reticulate, intectate. Reticulum homobrochate, simplibaculate. Nexine thinner than sexine.

Tectona grandis

Pollen grains prolate, $37.7 \times 25.1 \mu$ (range $28.5-37.5 \mu \times 21.0-28.5 \mu$), 3-colporate, colpi free from each other, tenuimarginate. Exine 2.3μ thick, sexine 1.2μ thick, punctitegillate.

Clerodendrum infortunatum

Pollen grains 3-colporate, subprolate (both from polar and equatorial view). Exine medium. Sexine provided with small blunt processes on the surface of the grains (echinate), intectate, sexine united with nexine by very small bacula. Nexine as thick as sexine.

Jasminum grandiflorum

Pollen grains 4-colporoidate, spheroidal to subprolate, polar area small. Exine thick to medium, reticulate, intectate. Reticulum homobrochate, mostly hexagonal, simplibaculate. Bacula on the top slightly swollen and united to form the muri. Nexine thinner than sexine.

From the palynological point of view *Nyctanthes* appears to be somewhat related to *Jasminum* (Oleaceae) having grains with reticulate surface pattern. They are distinguished by aperture character, the former having 3-colpi whereas the latter having 4-colporoidate type.

Besides the scanty palynological data of 4 members of Verbenaceae presented here, the pollen grains of several genera of Verbenaceae have been studied by Erdtman (1952), Nair and Rehman (1962) and others. In most members of this family the pollen grains are 3-colporate or 3-4-colporate, the endocolpium showing variations in the various sporomorphs. The ornamentals of the surfaces are pasilate, granulate, areolate, tuberculate, spinulate and reticulate and forms a reliable character in the determination of genera and species. Wherever the surface pattern is found to be reticulate, the pattern of reticulation is however, different from that of the grains of *Nyctanthes* or *Jasminum*.

DISCUSSION

The present survey from all probable aspects of study gave a better understanding of the systematic status of the much debated genus *Nyctanthes*.

Except such character as lobate-dentate leaves

and quadrangular stem, the general morphological features of *Nyctanthes* particularly of the flowers show much resemblances to those of the members of Oleaceae.

The fruit of *Nyctanthes* is an orbicular capsule, compressed parallel to the septum, separating when ripe into two one-seeded cells. This type of fruit is characteristic of this genus and cannot be compared to the fruit of any other members of the family Oleaceae, Verbenaceae or Loganiaceae (*sens. lat.*). The fruit in most of the members of the family Oleaceae is either a drupe or a berry, rarely a capsule. In the different genera of the family Verbenaceae the fruit is mostly a drupe or a capsule which is very different from that of *Nyctanthes*. In Loganiaceae (*sens. lat.*) the fruit may be a capsule or a berry with many seeds.

Stant (1952) mentions the following differences which excludes the genus *Nyctanthes* from the Oleaceae. "Absence of conspicuous peltate secretory hairs and extra-floral nectaries. Sclerenchymatous idioblasts not present in the mesophyll of the leaf. Absence of crystals and of spiral thickening in the xylem fibres". She has not observed the presence of sclerosed palisade cells in *Nyctanthes* reported by Rao (1947).

The mesophyll of the leaves of many members of Oleaceae often include sclerenchymatous idioblasts. The occurrence of these sclereids seems to be very characteristic of many members of the family. Details of their shape and distribution are considered valuable in the identification of the species. Rao (1947) reported the presence of elongated sclereids in the palisade cell of *Nyctanthes*. Although these have not been observed by Stant (1952), this feature though not present always, has been observed by the authors in many cases. Sclerenchymatous idioblasts have been recorded in the mesophyll of several members of Loganiaceae with thick fleshy leaves and somewhat branched stone cells in some species of *Strychnos* (Metcalfe & Chalk, 1950). Such sclereids have not been recorded in the leaves of members of Verbenaceae studied so far.

Stant (1952) has not observed cystoliths, which are characteristic of certain Verbenaceae, in *Nyctanthes*. The absence of typical cystoliths has also been noted by the present authors, although they have observed cystolith-like bodies in certain epidermal cells. Metcalfe and Chalk (1950) have mentioned about the presence of white dots on the surface of the leaf of *N. arbor-tristis* and bodies resembling cystoliths

in the neighbouring cells. Cystoliths have not been recorded from the leaves of the members of Oleaceae.

Although Stant (1952) supported Airy Shaw regarding inclusion of *Nyctanthes* under Verbenaceae, she also presented some characters by means of which *Nyctanthes* differs from most genera of the Verbenaceae. "There are no extra-floral nectaries or multicellular peltate hairs in *Nyctanthes*. The rays are uni-or bi-seriate which are narrower than is common within the family with the exception of *Pseudocardpidium* where they are exclusively uniseriate. Wood fibres are non-septate as in *Avicennia* and *Peronema*, not typical septate as reported in many members of the family. Pits in the walls of wood fibres are conspicuously bordered, not simple as in the Oleaceae, though small borders have been reported in *Petrea arborea*". From our detailed comparatively studies on wood anatomy we have also observed these differential characters in *Nyctanthes*. It also appears, from the anatomical evidences obtained from the present study on petiole, nature of stomata, nodal structure, structure of secondary wood etc., that the genus is unrelated to Verbenaceae.

The floral anatomy further shows that though *Nyctanthes* possesses some affinity to *Jasminum* of Oleaceae in mode of departure of traces to calyx, corolla and stamens, its distribution of traces to carpels shows a marked difference. So, inclusion of these two genera under the same family has how far been justified is to be considered. Again *Nyctanthes* shows affinity neither to Verbenaceae nor to Longaniaceae regarding vascular organisation of flowers.

The cytological study on the other hand shows that *Nyctanthes* has some affinity for the genera of Oleaceae regarding somatic chromosome numbers which appear to be 46 in many cases (Darlington & Wylie, 1955).

It has been observed that from the palynological point of view *Nyctanthes* though somewhat related to Oleaceae, has characteristic differences. The pollen grains of members of the Verbenaceae so far studied are very different from those of *Nyctanthes* being much smaller in size and having entirely different surface pattern.

From the preliminary data of alkaloid fractions (Kundu & Chakravarty, 1966) it is observed that the leaves of *N. arbor-tristis* contain alkaloids of the indole group. Definite evidence of indole alkaloids have not been reported from any member of the family Oleaceae. The alkaloids of *Nyctanthes*

appear to bear likeliness to the alkaloids of *Strychnos nux-vomica* (Fam. Loganiaceae *sens. lat.*). These data as well as the informations obtained from the structure of leaves provided evidence suggesting the affinity of *Nyctanthes* to the members of the Loganiaceae (*sens. lat.*), especially to the family Strychnaceae (of Hutchinson), rather than to Verbenaceae or even to Oleaceae.

From all these evidences it could be stated that *Nyctanthes* though possesses much affinity to the members of Oleaceae, it has several special characteristics of its own, namely, morphological characters like lobate-dentate leaves, squared stem, structure of the fruit, different type of pollen grains, special anatomical features and the chemical constituent. Taking all these points into consideration it seems justified in shifting the genus *Nyctanthes* from Oleaceae and assigning it to a separate family —Nyctanthaceae.

Family Nyctanthaceae fam. nov.

Arbor parva

Folia opposita, ovata, saepe lobato-dentata, supra in facie scabrida vel hispida.

Inflorescentia terminalis, trichotomo-cymosa.

Flores sessiles, bracteati; Calycis tubus ovoideo-cylindricus, subtruncatus; Corolla salviformis, tubo cylindrico, luteo; lobis corollae 4-8, contortis in albastro, albis.

Stamina 2; Antherae subsessiles, faucilis insertae; Ovarium biloculare, loculis uniovulatis, ovulis basalis, erectes; Stylus cylindricus; Stigmate brevissime bifido.

Fructus capsularis, orbicularis, compressus, dispermus. Semina erecta, orbicularia, plana, pericarpio tenui, albumen nullum, cotyledonibus planis, radicula infera.

Nyctanthaceae fam. nov.

Small trees. Leaves opposite, ovate, often lobate-dentate, scabrid or hispid on the upper surface. Inflorescence terminal trichotomous cymes. Flowers sessile, bracteate. Calyx ovoid-cylindrical, subtruncate. Corolla salver-shaped, tube cylindrical, yellow, lobes 4-8, contorted in bud, spreading, white, deciduous. Stamens 2, anthers subsessile near the top of the corolla tube. Ovary bilocular, style cylindrical, stigma very shortly bifid, ovule 1 in each cell, erect, basal. Fruit an orbicular capsule, compressed parallel to the septum, separating when ripe into 2 one-seeded subdiscoid cells. Seeds erect, orbicular, flattened, testa thin, albumen absent, cotyledons flat, radicle inferior.

POSITION OF THE FAMILY

Hutchinson (1926) in his system of classification of Dicotyledons included Oleaceae and Loganiaceae under the same order Loganales. Again the members of these two families in comparison to those of Verbenaceae have some salient features related to those of *Nyctanthes*:

Recently the family Loganiaceae has been split up into six separate families (Hutchinson, 1959, 1964) where Strychnaceae comes as the last family prior to Oleaceae in the order Loganales. The proper phylogenetic position of Nyctanthaceae seems to be in between Strychnaceae and Oleaceae under the order Loganales of Hutchinson.

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REFERENCES

- AIRY SHAW, H. K. Note on the taxonomic position of *Nyctanthes* L. and *Dimetra* Kerr. *Kew Bull.* 271-272, 1952.
- BERTOLONI, Bertol. *Miscell. Bot.* 18 : 16-18, 1858 (cited by Airy Shaw. Original not seen).
- CRAIB, H. G. Contributions to the flora of Siam. Additamentum No. 9, *Kew Bull.* No. 10, p. 265, 1916.
- DARLINGTON, C. D. AND A. P. WYLIE. *The Chromosome Atlas.* London, 1955.
- ERDTMAN, G. *Pollen Morphology and Plant Taxonomy.* I. Angiosperms. Mass., U.S.A., 1952.
- FOTIDAR, A. N. The primary vascular system of the stem of *Nyctanthes arbor-tristis* Linn. *J. Indian. bot. Soc.* 18 : 43-45, 1939.
- HASSELBERG, G. B. E. The vascular system in the stem axis of the genus *Fagraea*. *Svensk. bot. Tidskr.* 25 : 229-237, 1931.
- HUTCHINSON, J. *The families of flowering plants.* I. Dicotyledons. Ed. I. Oxford, 1926.
- Ibid.* Ed. 2. Oxford, 1964.
- The genera of flowering plants (Angiosperme). Dicotyledons. Vol. I. Oxford, 1966.
- JOSHI, A. C. AND A. N. FOTIDAR. Floral Anatomy of *Olea fragrans* Thunb. *Proc. nat. Inst. Sci. India* 7 : 369-375, 1941.
- KUNDU, B. C. Studies on *Nyctanthes arbor-tristis* L. *Proc. Indian Sci. Congr.* III. 267, 1966.
- AND D. P. CHAKRABARTY. On *Nyctanthes arbor-tristis* L. *Proc. Symp. Indian Medicinal Plants.* Lucknow, 1966.
- AND S. BOSE. *Floral anatomical studies on some members of Verbenaceae* (unpublished).
- S. MITRA AND B. SAHA. A comparative study of the vegetative shoot apices in the Verbenaceae. *Proc. Indian. Sci. Congr.* III. 310-311, 1967.
- LINDLEY, JOHN. *The vegetable kingdom*, Ed. 3. London, 1858.
- MAJUMDAR, G. P. Anomalous structure of the stem of *Nyctanthes arbor-tristis* 2. *J. Indian. bot. Soc.* 20 : 119-122, 1941.
- MARODEN, M. P. F. AND I. W. BAILEY. A fourth type of nodal anatomy in dicotyledons illustrated by *Clerodendron trichotomum* Thunb. *J. Arnold Arbor.* 36 : 1-50, 1955.

- METCALFE, C. R. AND L. CHALK. *Anatomy of the Dicotyledons*, Vols. I & II. Oxford, 1950.
- NAIR, P. K. K. AND K. REHMAN. Pollen grains of Indian Plants—V. Verbenaceae. *Bull. nat. bot. Gdn.* No. 76, 1962.
- POPHAM, R. A. AND A. P. CHAN. Zonation in the vegetative stem tip of *Chrysanthemum morifolium* Bailey. *Amer. J. Bot.* 37 : 476-484, 1950.
- RAO, T. A. The occurrence of scleroid palisade cells in the leaf of *Nyctanthes arbor-tristis* L. *Curr. Sci.* 16 : 122-123, 1947.
- RAO, V. S. The floral anatomy of some Verbenaceae with special reference to the gynoecium. *J. Indian bot. Soc.* 31 : 297-315, 1952.
- STANT, M. Y. Anatomical evidence for including *Nyctanthes* and *Dimetra* in the Verbenaceae. *Kew Bull.* 275-276, 1952.