

EPIDERMAL STRUCTURES AND ONTOGENY OF STOMATA OF *NYCTANTHES ARBOR-TRISTIS* L.

B. S. TRIVEDI AND NIRMALA UPADHYAY

Botany Department, Lucknow University, Lucknow

ABSTRACT

The present paper deals with the epidermal structures and development of stomata in the *Nyctanthes arbor-tristis* L. of Oleaceae. Stomata are highly variable in structure and ontogeny. Anomocytic, paracytic and diacytic stomata have been observed in this genus. Epidermal structures at various stages of leaf development beginning from seedling stage have been observed and discussed in detail. Diacytic stomata which have not been reported so far in the family Oleaceae, have been observed in this genus. Systematic position of the genus *Nyctanthes arbor-tristis* has also been discussed in detail.

INTRODUCTION

Epidermal structures and ontogeny of stomata of *Nyctanthes arbor-tristis* have been worked out by INAMDAR (1967). Systematic position of the genus *Nyctanthes* has been discussed in detail by various workers, viz. AIRY-SHAW (1952), STANT (1952), INAMDAR (1967) and KUNDU (1968). Ontogeny of stomata and other epidermal structures of this plant were described by INAMDAR (1967) though he failed to note many important features. Therefore, epidermal structures and ontogeny of stomata of this plant have been studied by us in quite some detail. Investigations of epidermal structures from the seedling stages to mature leaves have been worked out. Detailed epidermal studies have shown that this plant is best placed in the family Oleaceae.

This family is characterized by the presence of foliar sclereids which occur in most genera. RAO (1947) stated that foliar sclereids occur in *Nyctanthes*. STANT (1952) stated that foliar sclereids are completely absent from the leaves of *Nyctanthes*. Our finding shows that sclereids are present in the cortical zone of root, being absent from the leaves.

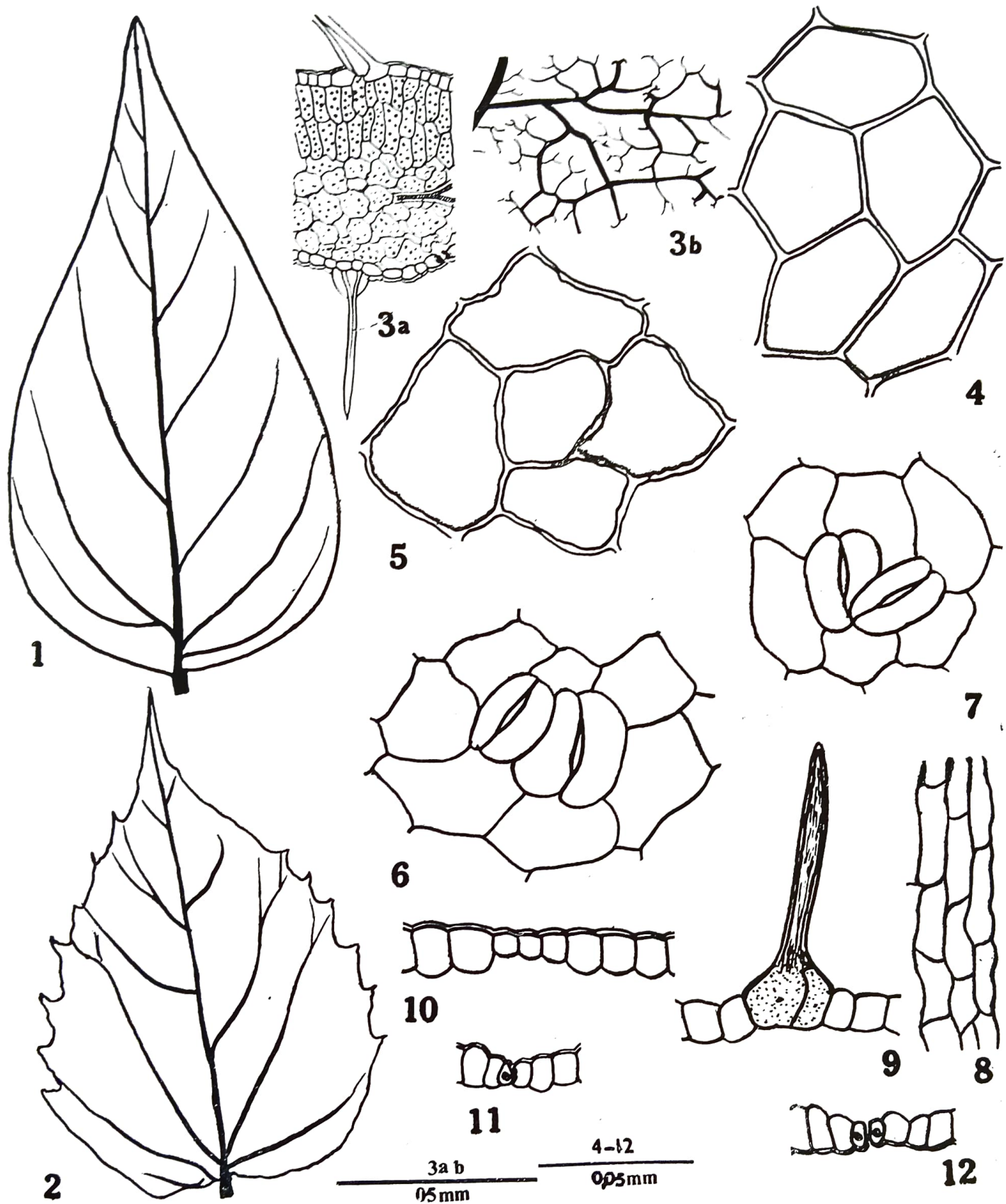
MATERIAL AND METHODS

Material for the present investigation was collected by the authors from the garden of Botany Department, Lucknow University. Fresh as well as preserved leaves at different stages of growth were collected for this study. Cuticular preparations were made by following the usual maceration procedure. Cuticles were stained with Sudan IV and mounted in glycerine jelly. For ontogeny of stomata cuticles obtained from fresh or preserved leaves were stained by Iron-Haematoxylin and finally mounted in Canada balsam. For stomatal frequency and stomatal index 10 counts for each specimen in that part of lamina, which lies between the midrib and leaf margin, were made. For the description of stomatal types and ontogeny the terminology used by PANT (1965), FRYNS-CLAESSENS & VAN-COTTHEM (1973) and DILCHER (1974) has been followed.

OBSERVATIONS

MORPHOLOGY OF LEAF

Leaves opposite, simple, often lobate, dentate or entire, shortly petiolate (Text-figs. 1-2), midrib prominently raised, secondary and tertiary veins also raised, nervation conspicuous (Text-fig. 3b).



- Text-figs. 1-2. Entire and dentate margined leaves respectively, x natural size.
 Text-fig. 3a. A part of leaf in t.s. showing mesophyll cells.
 Text-fig. 3b. A part of leaf showing venation.
 Text-figs. 4-5. Straight and sinuous cell walls respectively.
 Text-figs. 6-7. Contiguous stomata.
 Text-fig. 8. Marginal cells of the leaves.
 Text-fig. 9. Uniseriate trichome with bicelled trichome base seen in sectional view.
 Text-fig. 10. Cells of upper epidermis.
 Text-fig. 11. Single guard cell stomata in sectional view.
 Text-fig. 12. Normal stoma in sectional view.

Epidermal characters have been studied by cutting transverse sections of the leaf and by the maceration of the leaf or peeling off of the cuticle from the leaf surface.

ANATOMY OF LEAF

In sectional view, upper epidermal cells are larger in comparison to the lower ones as seen in transverse section (Text-figs. 10-11). Epidermal cells vary in size (Text-fig. 10). Mesophyll is differentiated into palisade and spongy parenchyma, palisade cells are elongated and are arranged in two rows. Sclereids have not been observed by us in the palisade zone of mesophyll as reported by RAO (1947). The palisade cells, however, show only thin walled parenchymatous cells (Text-fig. 3a).

EPIDERMAL CHARACTERS

Leaves of *Nyctanthes arbor-tristis* are bifacial. Epidermal cells have somewhat sinuate walls in young leaves and straight walls in mature leaves (Text-figs. 4-5). Marginal cells of the leaf are elongated and smaller than the remaining epidermal cells of the same leaf (Text-fig. 8). Stomata are confined to lower surface. METCALFE AND CHALK (1950) reported only anomocytic stomata in the leaf of *Nyctanthes*. INAMDAR (1967) reported two types of stomata viz. paracytic and anomocytic. We have observed three distinct types of stomata in this plant (Text-figs. 13, 24, 25, 26 & 27) viz., anomocytic, paracytic and diacytic. Frequency of all three types of stomata varies considerably in different pairs of leaves. For better understanding of stomatal structure in this plant, we observed them at various stages of leaf development beginning from the seedling stage. Stomatal frequency and stomatal index in various sets of leaves has been noted. It has been observed that the stomatal frequency and the stomatal index keeps on increasing up to the time till the seedlings have 10 pairs of leaves, the last pair of leaves have maximum stomatal frequency and stomatal index which becomes fixed in all subsequently formed leaves and is constant for the species (Text-fig. 29).

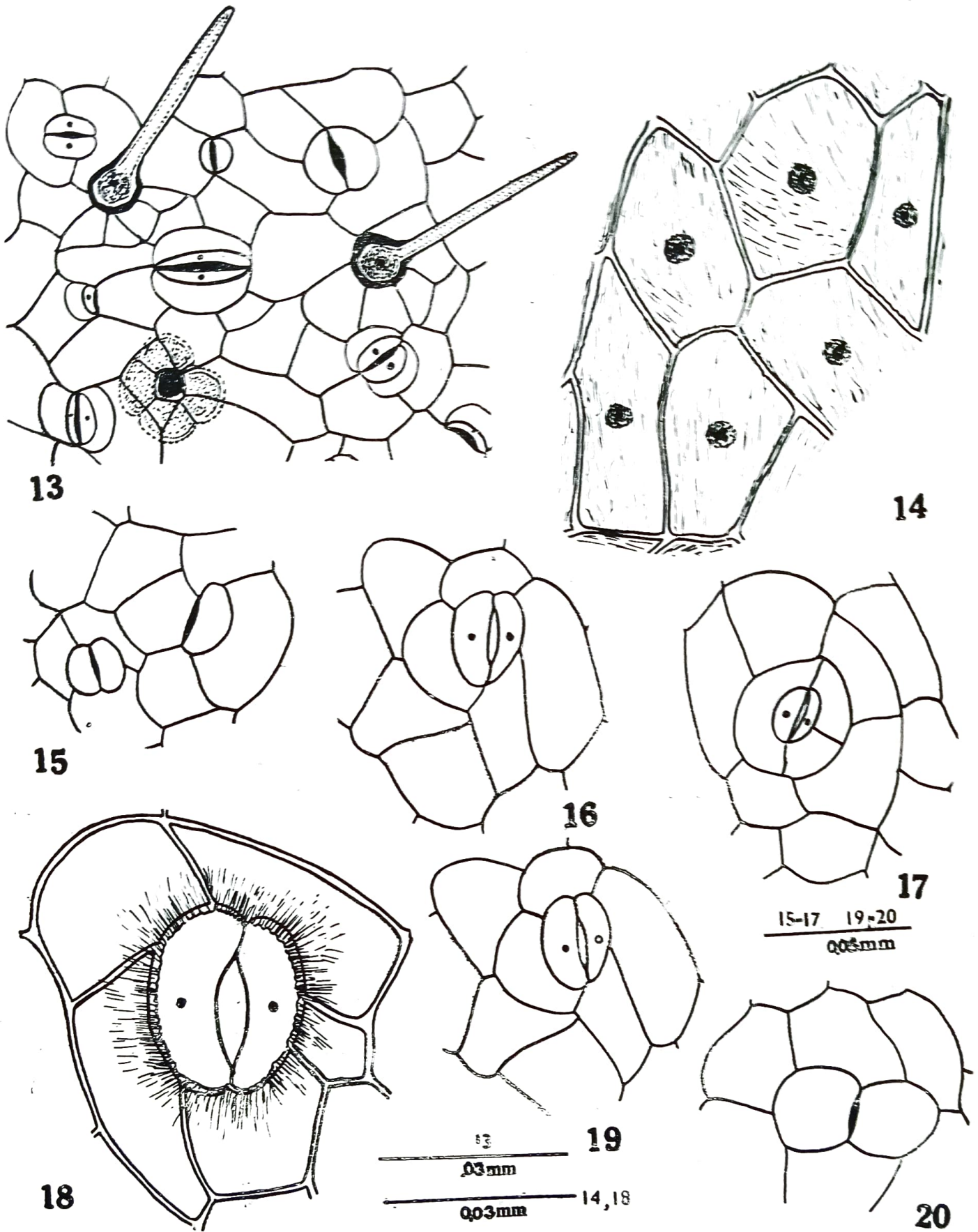
Different types of trichomes which occur in this plant have already been reported by INAMDAR (1967 a). In sectional view trichome bases are bicelled (Text-fig. 9).

ABNORMAL STOMATA

Stomata with a single guard cell, aborted guard cell, a single subsidiary cell and contiguous stomata have been noted in this plant (Text-figs. 6-7, 15, 20). In seedling stages contiguous stomata and aborted guard cells are more common (Text-figs. 6-7, 15). Stomata with single guard cell are commonly seen in mature leaves (Text-figs. 11, 15).

SIZE OF STOMATA AND CUTICULAR STRIATIONS

Stomata of two distinct sizes have been observed in this plant (Text-fig. 13). Stomata of two distinct sizes have also been observed in *Forestiera* (Oleaceae), METCALFE AND CHALK (1950), these authors include *Nyctanthes* also in this family. Larger stomata measure $33 \mu\text{m} \times 15 \mu\text{m}$, while smaller ones are $20 \mu\text{m} \times 10 \mu\text{m}$ (Text-fig. 13). However, earlier workers observed only one kind of stoma in this plant. Striated stomata and epidermal cells are commonly seen in mature leaves. Striated epidermal cells and stomata are more prominent on the fruit wall (Text-figs. 14 & 18) as compared to mature leaves. Stomata present on the fruit wall are highly variable and they are usually anomocytic (Text-figs. 16, 17, 19).



Text-fig. 13. Epidermal peeling of the leaf showing trichomes and different types of stomata.

Text-fig. 14. Epidermal cells of fruit wall showing striations.

Text-fig. 15. Stomata with single subsidiary cell.

Text-figs. 16-17 & 19. Different types of stomata on fruit wall.

Text-fig. 18. Large stoma on fruit wall showing striations arising from the guard cells.

Text-fig. 20. Degenerated stoma with subsidiary cells.

The following three patterns of stomatal development have been observed in this plant :

- (i) Anomo-mesoperigenous (Anomocytic stomata)
- (ii) Para-mesogenous (Paracytic stomata)
- (iii) Dia-mesogenous (Diacytic stomata).

(i) *Anomo-mesoperigenous*—In case of Anomo-mesoperigenous type the meristemoid divides into two unequal cells of which the larger becomes a neighbouring cell and the smaller daughter cell acts as g.m.c. (guard cell mother cell) (Text-fig. 23). Guard cell mother cell then divides to form two guard cells. This division is parallel to the first division of the meristemoid. Remaining subsidiary cells are perigenous in origin (Text-figs. 21, 26).

(ii) *Para-mesogenous*—In this type, the meristemoid as usual divides into two unequal cells of which the larger becomes a subsidiary cell, while the smaller cell divides again parallel to the first division forming two unequal cells. Thus, three cells are formed in a row of which the central cell functions as g.m.c. (Text-fig. 22). Guard cell mother cell divides parallel to the previous division forming two guard cells. Stomata formed this way have two guard cells and two subsidiary cells and at least one encircling cell which lies either at right angle to other cells or parallel to them (Text-figs. 24, 28).

(iii) *Dia-mesogenous*—Here the meristemoid divides into two unequal cells of which the larger becomes somewhat semicircular, the smaller cell divides again, opposite to the first one, which results in the formation of two unequal cells. The central cell acts as g.m.c. which divides at right angle to the subsidiary cells (Text-fig. 27). Stomata formed in this way have two subsidiary cells at right angle to the guard cells (Text-figs. 25, 26).

DISCUSSION

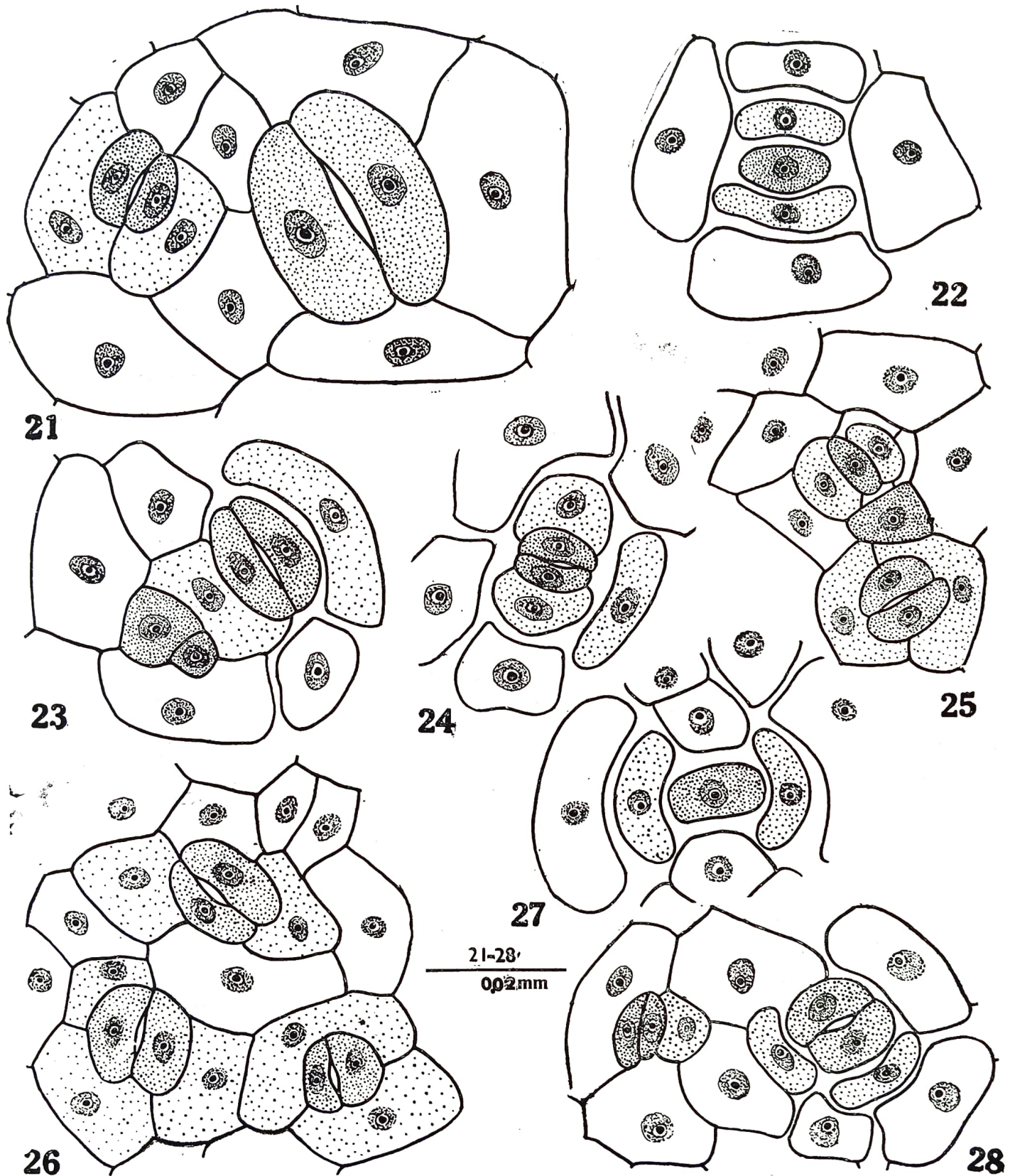
Leaf morphology, including epidermal structures and ontogeny of stomata, in *Nyctanthes* has been investigated in detail for all possible characters which may throw light on the systematic position of this plant. While investigating the epidermal structures, we came across many important and interesting features which are being reported here for the first time.

Systematic position of the genus *Nyctanthes* has been discussed from time to time by various workers. DE JUSSIEU (1789) assigned this genus to the family Oleaceae. LINDLEY (1853) split this family into Oleaceae proper and Jasminaceae. He was the first to suggest a verbenaceae affinity for the genus *Nyctanthes*. BENTHAM AND HOOKER (1873-76), EICHLER (1878) and ENGLER AND PRANTL (1897) placed *Nyctanthes* in the family Oleaceae as can be seen below :

Bentham and Hooker (1873-76)

Oleaceae

- Tribe I—Jasmineae —*Jasminum*, *Menodora*, *Nyctanthes*
- Tribe II—Syringaeae —*Schrebera*, *Forsythia*
- Tribe III—Fraxineae —*Syringa*, *Farxinus*, *Fontanesia*
- Tribe IV—Oleineae —*Forestiera*, *Phillyrea*, *Osmanthus*, *Chionanthus*, *Linociera*, *Notelaea*, *Noronhia*, *Olea*, *Lingustrum*, *Myxopyrum*



Text-fig. 21. Anomocytic stoma and large stoma.

Text-fig. 22. Guard cell mother cell and two subsidiary cells parallel to g.m.c.

Text-fig. 23. Meristemoid at dividing stage showing larger and smaller cells.

Text-fig. 24. Paracytic stoma with one encircling cell at right angle to other cells.

Text-fig. 25. Paracytic and diacytic stomata along with one meristemoid.

Text-fig. 26. Mature anomocytic, paracytic and diacytic stomata.

Text-fig. 27. Guard cell mother cell with two subsidiary cells at right angle to g.m.c.

Text-fig. 28. Paracytic stomata and anomocytic stomata.

Oleaceae

I. Oleoideae

- (a) Fraxineae —*Fontanesia, Fraxinus*
 (b) Syringeae —*Forsythia, Nathusia, Syringa*
 (c) Oleineae —*Hesperclaea, Phillyrea, Osmanthus, Forestiera, Mayepea, Notelaeae, Chionanthus, Tesarandra, Noronhia, Olea, Ligustrum, Myxopyrum*

II. Jasminoideae —*Jasmineae—Menodora, Nyctanthes, Jasminum*

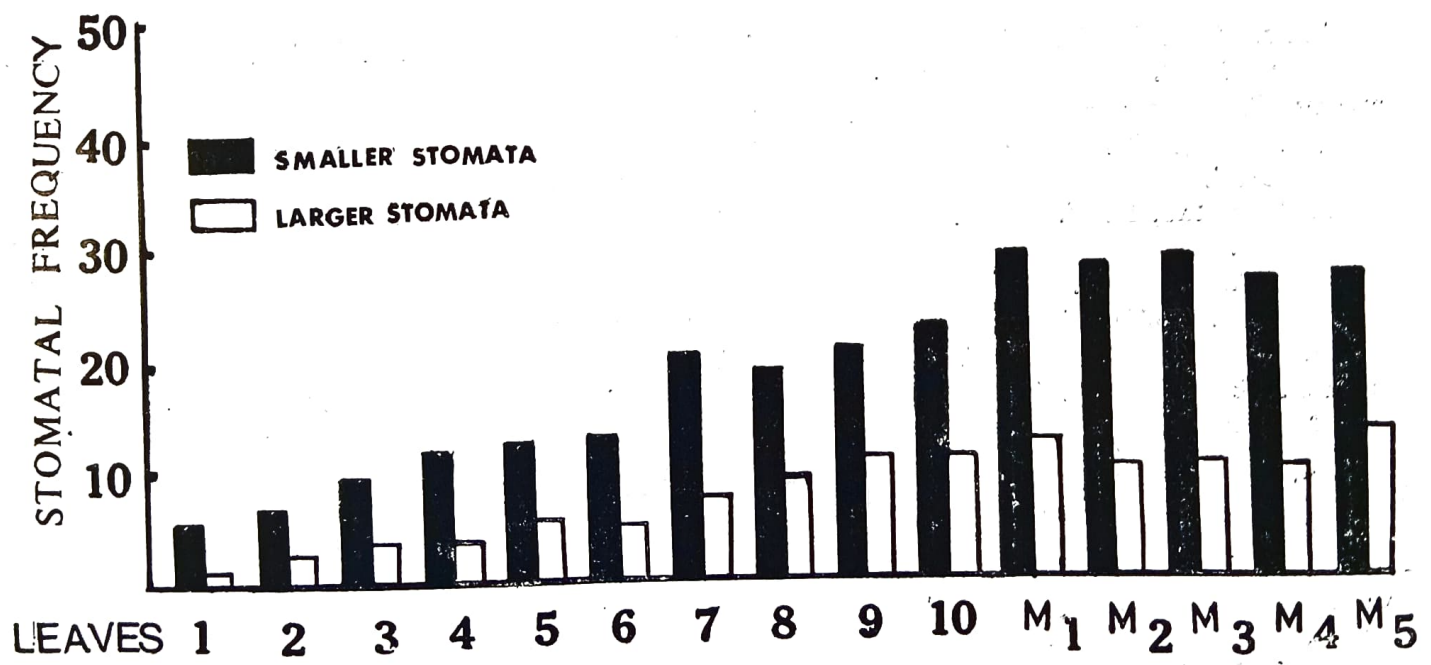
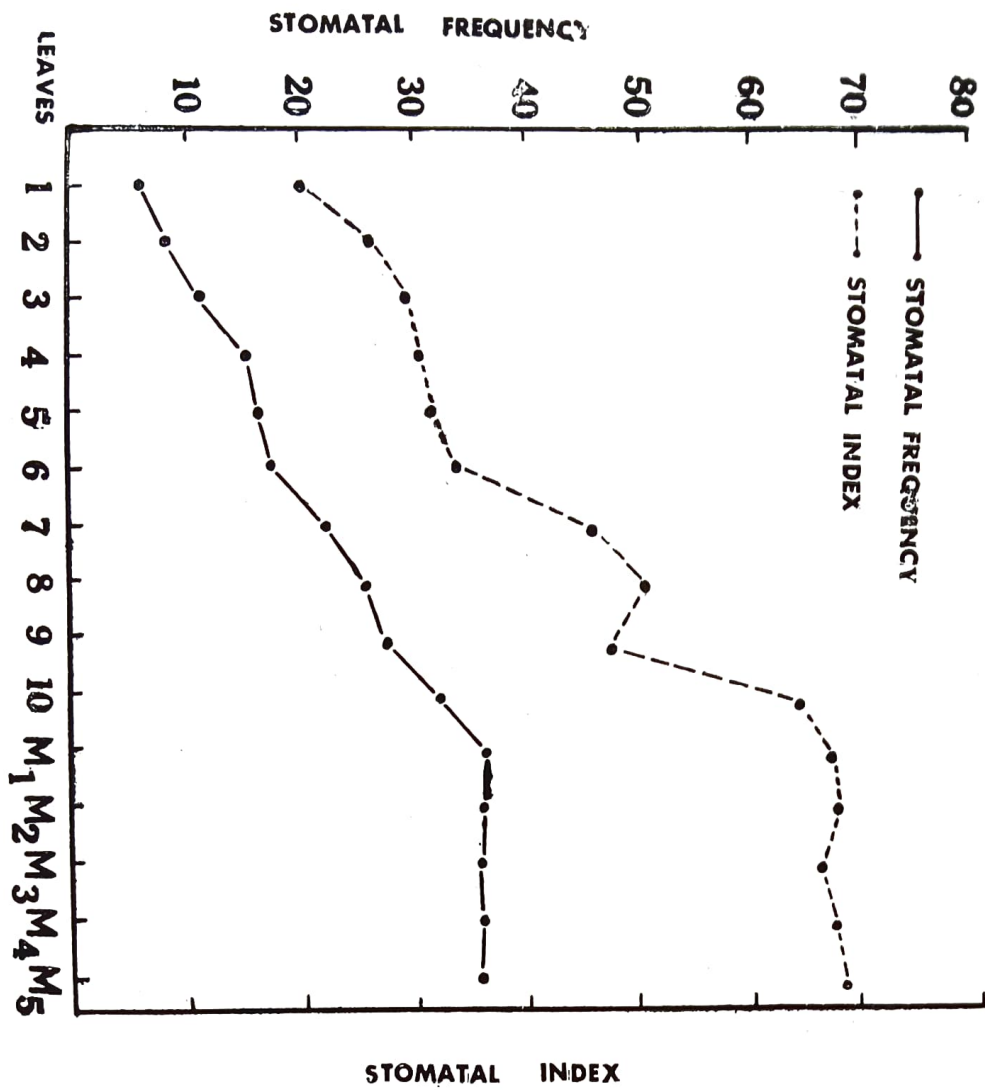
HUTCHINSON (1926, 1964 & 1966) places the family Oleaceae under the order Loganiales. AIRY-SHAW (1952) on the basis of morphological characters removed the genus *Nyctanthes* from Oleaceae and placed it in a new sub-family Nyctanthoideae of the family Verbenaceae. STANT (1952) on the basis of anatomical evidence included this genus under Verbenaceae. JOHNSON (1957) and MELCHOIR (1964) also support AIRY-SHAW and Stant's views. On the basis of embryological studies CRÉTÉ (1963) stated that the genus *Nyctanthes* and the family Oleaceae have identical embryogeny, which is shown by no other family of Gamopetalae. Evidence available from chemotaxonomy (DAS & RAO, 1966), morphology and embryology (KAPIL & VANI, 1967), trichome morphology and stomatal ontogeny (INAMDAR, 1967) supports inclusion of *Nyctanthes* within the family Oleaceae.

KUNDU (1966), on the other hand, thinks that the genus *Nyctanthes* is neither related to Oleaceae nor to Verbenaceae. KUNDU AND CHAKRAVARTY (1966), on the basis of anatomical and chemical constituents of *Nyctanthes*, believe that it should be kept in a separate family under Loganiaceae. KUNDU AND DE (1968) on the basis of comparative studies of *Nyctanthes* as well as of some other members of Oleaceae, Loganiaceae and Verbenaceae suggest removal of the genus *Nyctanthes* from Oleaceae and advocate placing it in a family of its own viz., Nyctanthaceae.

The genus *Nyctanthes* shows varied morphological features. Margin of leaves may be dentate or entire or intermediate. Flowers with stigma projecting out of the corolla tube or included, petals of 5 to 8 and highly variable in size. Cuticular structures of mature leaves of diverse shape and form were studied. All these, however, show almost similar cuticular structures. Epidermal structures including the appendages of the genus *Nyctanthes* have been compared to the other members of Oleaceae and to some taxa of Verbenaceae.

Our observation on the epidermal structures including ontogeny of stomata in the genus *Nyctanthes* reveal many features of interest which are indicated below :

- (1) It has been customary to say that the leaves of this plant are lobate, or lobate dentate. We have observed that they are either lobate dentate or with entire margins. Both these kinds of leaves appear at the seedling stage and persist in the adult plants.
- (2) The shape of epidermal cells varies at different stages of leaf development, these may be sinuate at seedling stages but becomes straight-walled in mature leaves.
- (3) INAMDAR (1967) reported that *Jasminum sambac* and *Nyctanthes* resemble each other in ontogeny and structures of stomata. Our observations on the other hand show that stomata in the leaves of *Jasminum sambac* and *Nyctanthes arbor-tristis* are quite different. The leaves of the two genera differ from each other in this character.



Text-fig. 29. Graph showing stomatal frequency and stomatal index of 15 pairs of leaves at different developmental stages.

Text-fig. 30. Histogram showing the frequency of the larger and the smaller stomata in 15 pairs of leaves at various stages of development.

- (4) Only two kinds of stomata have been recorded so far in this plant, viz. anomocytic and paracytic. We have recorded the occurrence of diacytic stomata also in this genus. Diacytic stomata are frequently present in the family Verbenaceae but are not reported from the family Oleaceae.
- (5) Abnormal stomata with a single guard cell, stomata with one subsidiary cell, degenerated and contiguous stomata have been observed. Abnormal stomata mostly occur at seedling stages.
- (6) Stomata of two distinct sizes occur throughout the foliar surface. Such a condition is known only in a species of *Forestiera* (Oleaceae). Ratio of the two types of stomata in different sets of leaves has been shown in a histogram (Text-fig. 30).
- (7) Striations in the guard cells as well as in epidermal cells are quite common in this genus. Epidermal cells of fruit wall have unbranched striations.
- (8) Sclereids are completely absent from the leaf of *Nyctanthes* though other members of the family Oleaceae are characterised by their presence. Sclereids, however, have been observed in the cortical zone of root of this plant. These are similar to root sclereids of *Rauwolfia* (MIA, 1964). Summing up the characters outlined above, it is seen that the genus *Nyctanthes* is unique in the family Oleaceae as its epidermal characters, ontogeny of stomata, leaf structure and venation of leaves differ from other common members of this family.

Nyctanthes does not fall in completely within the families Oleaceae and Verbenaceae, though it shares some characters of both. On the other hand, it has certain characters in which it differs from both. This genus, therefore, can best be placed on the basis of epidermal structures within the newly created family Nyctanthaceae of KUNDU AND DE (1968).

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