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PRELIMINARY LEAF EPIDERMAL STUDIES IN A FEW EPIPHYTIC ORCHIDS

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Abstract

During the present study, preliminary epidermal studies were made in ten species of epiphytic orchids belonging to seven genera *i.e., Acampe praemorsa* (Roxb.) Blatt. & McCann, *Coelogyne breviscapa* Lindl., *C. nervosa* A.Rich., *Cottonia peduncularis* (Lindl.) Reichb.f., *Flickingeria nodosa* (Dalz.) Seidenf., *Luisia macrantha* Blatt.& McCann, *Luisia zeylanica* Lindl., *Pholidota pallida* Lindl., *Rhynchostylis retusa* (L.) Bl., *and Sarcanthus pauciflorus* Wight. Hypostomatic condition was common in most of the selected orchids irrespective of the genus. Most of the orchids were having paracytic and anomocytic type of stomata. The stomatal frequency ranged from 490.12 to 16353.22 per mm^2; the highest stomatal frequency (16353.22) was recorded in *Flickengiria nodosa* and lowest was recorded in *Coelogyne nervosa* (490.12). Interestingly, a higher stomatal index was observed in *C. nervosa* (14.32) and lowest in *Rhynchostylis retusa* (3.45). Relationship between stomatal size and stomatal frequency was noticed in the present study. The stomatal size was inversely proportional to stomatal frequency.

Introduction

KAUSHIK (1983) HAS considered the taxonomic significance of stomatal features and classified the orchids into four sub families. Taxonomic importance of stomata was indicated by Mohana Rao and Khasim (1987). Leaf epidermal features in 43 species of epiphytic and ground growing orchids were studied by Vij *et al.*, (1991). In the present study, stomatal complex of ten species of epiphytic orchids belonging to seven genera was studied. Stomata are small pores, typically on the undersides of leaves that are opened or closed under the control of a pair of bean-shaped cells called guard cell. When open, stomata allow CO_2 to enter the leaf for synthesis of glucose, and also allow for water and free oxygen to escape. In addition to opening and closing the stomata, plants may exert control over their gas exchange rates by varying stomata density in new leaves when they are produced (such as in the spring or summer). The more the stomata per unit area (stomata density) the more the CO_2 can be taken up and the more water can be released. Thus, higher stomata density can greatly amplify the potential for behavioral control over water loss rate and CO_2 uptake (Wang *et al.*, 2007). There are three main types of stomata (Anisocytic, Paracytic, and Diacytic) in monocots (Fig. 1a-c) based on the arrangement of subsidiary cells (Abid, 2007). Each of these three groups does essentially the same job and is composed of the same cellular material, but these cells differ from the composition of the guard cells and the other normal epidermal leaf cells. Anisocytic cells are unequal in appearance to each other. An anisocytic cell group may be composed of three or more cells which surround the guard cells, buffering it from the other epidermal leaf cells. Paracytic subsidiary cells are arranged about the long axes of the stomata cells. The third group of subsidiary cells is called Diacytic, because they are arranged at right angles to the stomata cells (Ferry, 2008). But the stomata are anomocytic when the guard cells are not associated with any subsidiary cells (Metcalfe, 1961; Stebbins and Khush, 1961). The stomata was first studied by Strasburger (1866), followed by Vesque (1889) who recognized four broad categories of stomata based on the presence and arrangement of accessory cells as well as their mode of development. There are very few reports on stomata of Orchidaceae. Studies were therefore undertaken to provide the information for the stomatal type for the orchidaceae from the area under consideration.

Materials and Methods

Collection of the Research Material

The present investigation deals with 10 species of epiphytic orchids belonging to 7 genera *i.e., Acampe praemorsa* (Roxb.) Blatt. & McCann, *Coelogyne breviscapa* Lindl, *C. nervosa* A.Rich., *Cottonia peduncularis* (Lindl.) Reichb,f., *Flickingeria nodosa* (Dalz.) Seidenf., *Luisia macrantha* Blatt.& Mc Cann, *Luisia zeylanica* Lindl., *Pholidota pallida* Lindl., *Rhynchostylis retusa* (L.) Bl., *Sarcanthus pauciflorus* Wight (Table 1). These species were collected from the different regions of the Western Ghats of Shimoga and their type of inflorescence, floral position and phenology were recorded.

Peeling of Epidermal Layer

In the present study, stomatal complex of ten species of orchids belonging to seven genera was studied by using leaf peels; mature leaves were used to study the

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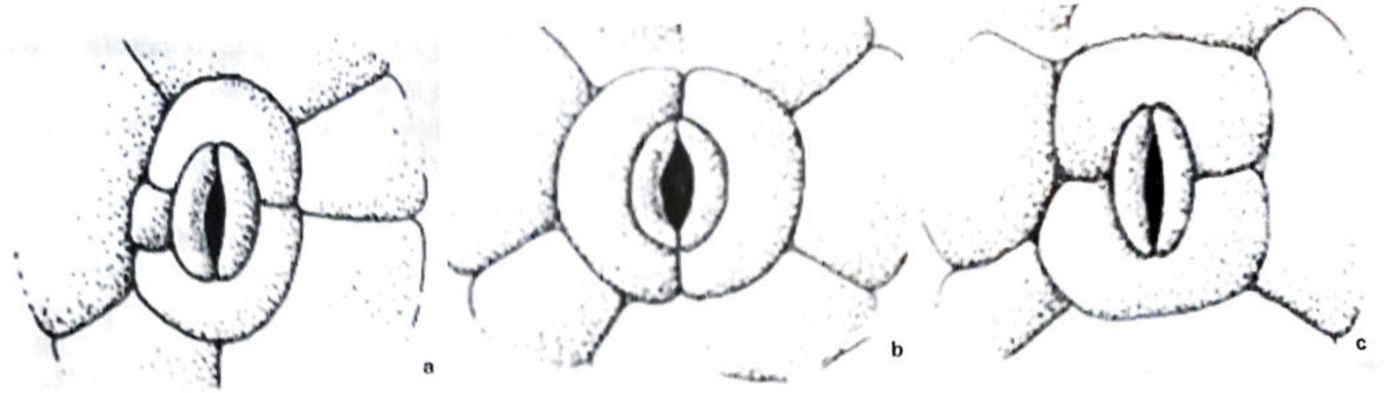


Fig. 1.a-c. Stomata types: a, Anisocytic; b, Paracytic; c, Diacytic.

dermal characters. The method suggested for orchids by Williams (1975) was adopted in the present investigation. The main characters analyzed during the study were: presence or absence of stomata on abaxial and adaxial surface; type of stomata; (paracytic, anomocytic, anisocytic, or tetracytic); and size of the stomata.

Determination of Stomatal Frequency

Using 10 fields of view at 10x and 40x objective as quadrats, the number of subsidiary cells per stoma was noted to determine the types of stomatal complex present in each specimen. Frequency of each stomatal complex type was expressed as % occurrence of each stomatal complex type based on all occurrences of stomatal complex types (Carr and Carr, 1990; Obiremi and Oladele, 2001). Terminologies used for stomatal complex types followed those of Dilcher (1974); Metcalfe and Chalk, (1988); and Saadu *et.al.*, (2009).

Determination of Stomatal Index

Stomatal Index (SI) was determined as follows:

$SI = S/E+S \times 100$

Where: SI = Stomatal Index

S = Number of stomata per mm^2

E = Number of ordinary epidermal cells per mm^2

Determination of Stomatal Size

The mean stomatal size or area of a species was determined by measuring length and breadth using a micrometer of a sample of 10 stomata using eye-piece micrometer.

Table 1. Locality and phenology of epiphytic orchids.

Species	Locality	Inflorescence	Floral position	Flower phenology
Acampe praemorsa (Roxb.) Blatt. & McCann	Tarikere	Raceme	Base of leaf sheath	January-April
Coelogyne breviscapa Lindl.	Niluguli-koppa	Raceme	Base of pseudobulb	April-September
C. nervosa A.Rich.	Mullayanagiri	Lateral raceme	Base of the bulb	June-September
Cottonia peduncularis (Lindl.) Reichb.f.	Kannangi	Panicle (branched raceme)	Base of leaf sheath	December-March
Flickingeria nodosa (Dalz.) Scidenef.	Kondlur	Solitary/auxillary	Axil of the leaf	June-October
Luisia macrantha Blatt.& McCann	Anegunda	Solitary cyme	Node of pseudo stem	December-January
L. zeylanica Lindl.	Bejjavalli	Raceme	Base of leaf sheath	April-May
Pholidota pallida Lindl.	Ulave-sringeri	Raceme	Base of pseudobulb	July-October
Rhynchostylis retusa (L.) Bl.	Kokkodu	Raceme	Base of leafy sheath	April-September
Sarcanthus pauciflorus Wight	Bejjavalli	Raceme	Arising from node	July-October

Results and Discussion

Present studies on phenological characteristics in 10 species of epiphytic orchids yielded interesting results (Table 1). The racemose type of inflorescence is very common, excluding *Flickingeria nodosa* and *Luisia macrantha*. Fig.1 shows that most of the orchids bloom in the extreme dry conditions (April). Generally blooming is continued, after the onset of rainy season, up to October in most of the species. All the leaves of selected orchids are leathery in texture. Earlier, Dressler and Dodson (1960) noted a strong association of leathery leaves with epiphytic habit and suggested their independent origin from the membranous ones, along several evolutionary lines in orchids. These plants were adopted for aerial habitats in order to avoid water loss. Stomata were absent in upper epidermis. The two important processes *i.e.* photosynthesis and transpiration were influenced by stomatal frequency (Inamdar *et al.*, 1991). *Acampe praemorsa, Coelogyne breviscapa, C. nervosa, Cottonia peduncularis, Flickingeria nodosa, Pholidota pallida* and *Sarcanthus pauciflorus* showed hypostomatic condition (Fig. 3a-j; Fig. 4k-t). Similar observations have been made by (Avadani *et al.*, 1982; Mulgaonkar, 2005 a,b,c; Singh, 1981). Hypostomatic condition was frequently

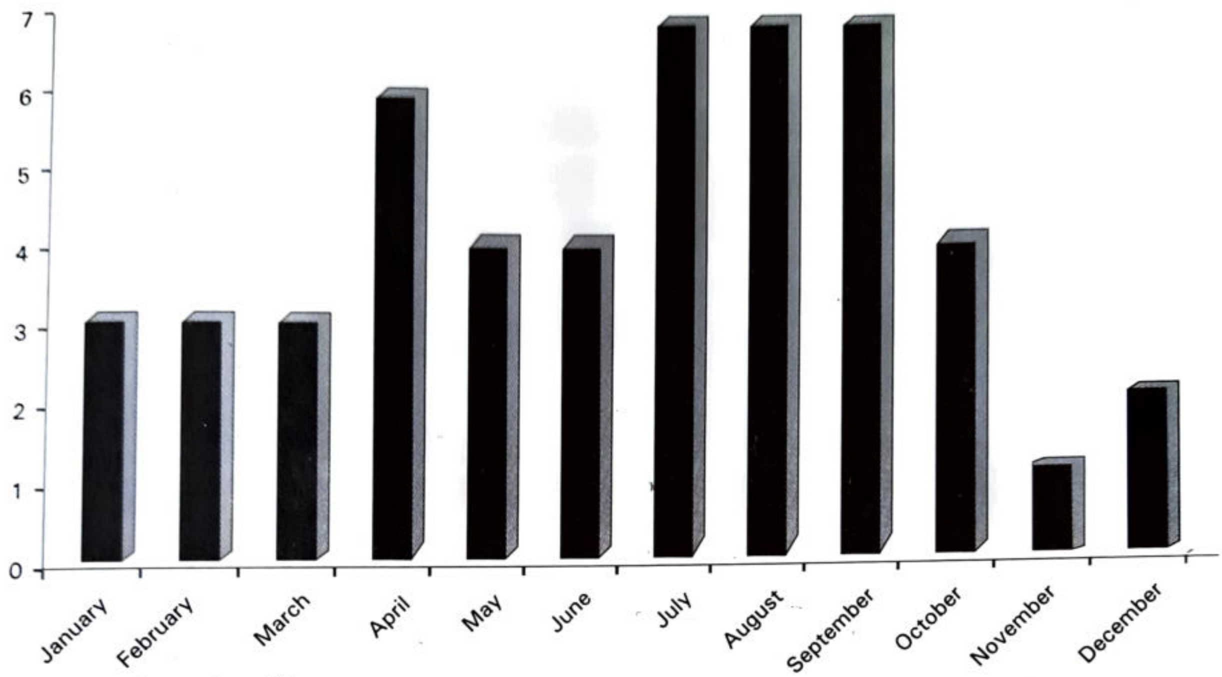


Fig.2. Floral phenology of orchids.

Table 2. Stomatal complex types in the investigated species.

Species	Leaf surface	Stomatal type	size	frequency	Index
Acampe praemorsa (Roxb.) Blatt. & McCann	Hypostomatic	Paracytic	4817.77	2998.12	6.64
Coelogyne breviscapa Lindl.	Hypostomatic	Paracytic	820.98	10084.48	10.57
C. nervosa A.Rich.	Hypostomatic	Paracytic	2203.18	490.12	14.32
*Cottonia peduncularis (*Lindl.) Reichb,f.	Hypostomatic	Anomocytic	2671.74	3270.51	7.02
Flickingeria nodosa (Dalz.) Scidenef.	Hypostomatic	Paracytic	554.56	16353.22	11.72
Luisia macrantha Blatt.& McCann	-	Paracytic	2581.38	6268.73	11.21
Luisia zeylanica Lindl.	-	Paracytic	1894.19	3270.63	11.72
Pholidota pallida Lindl.	Hypostomatic	Paracytic	2600.39	2998.08	11.00
Rhynchostylis retusa (L.) Bl.	Amphistomatic	Anomocytic	2203.42	4360.85	3.45
Sarcanthus pauciflorus Wight	Hypostomatic	Anomocytic	844.6	7086.39	6.98

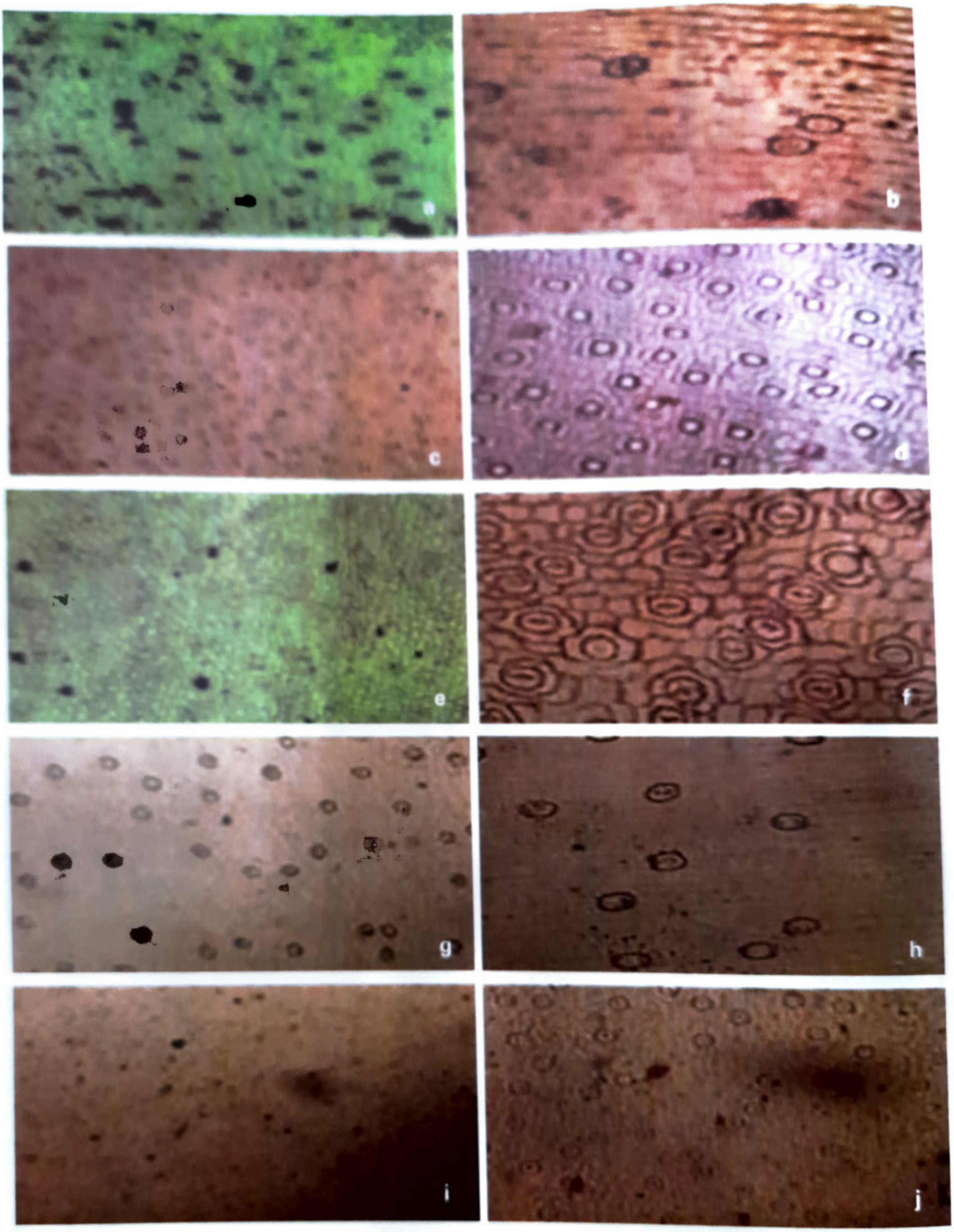


Fig. 3.a-j. Stomata at lower and higher magnifications: a-b, *Acampe praemorsa;* c-d, *Coelogyne breviscapa*; e-f, *Coelogyne nervosa;* g-h, *Cottonia peduncularis;* i-j, *Flickingeria nodosa*.

are generally associated with Crassulacean Acid Metabolism which has been considered an additional factor promoting amphistomaty in orchids. The present investigation clearly revealed the presence of paracytic and anomocytic type of stomata in orchids. Previously, these types were also reported in number of other monocots (Cheadle, 1953). Stebbing and Khush (1961) suggested that anomocytic type is limited only to the

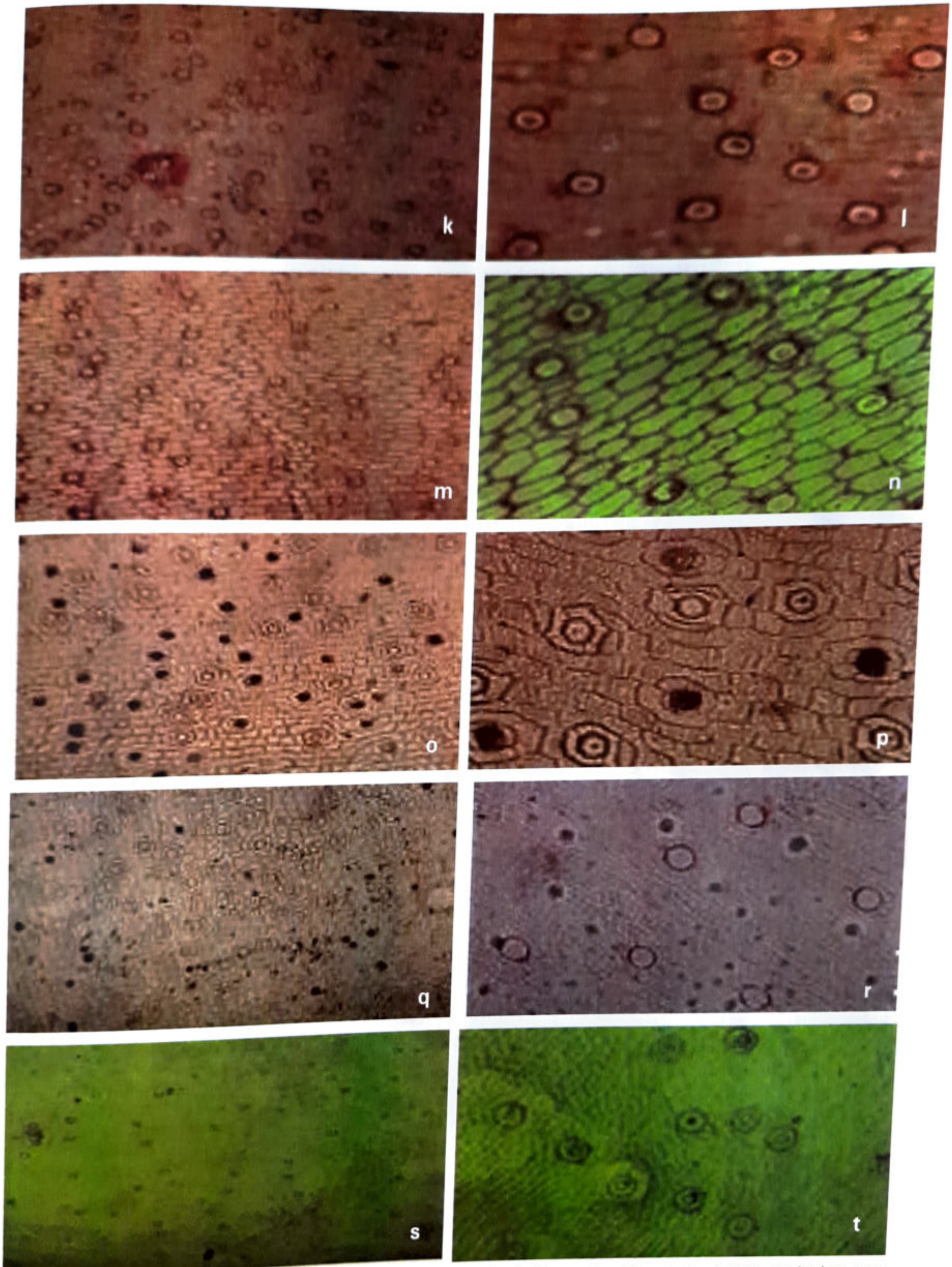
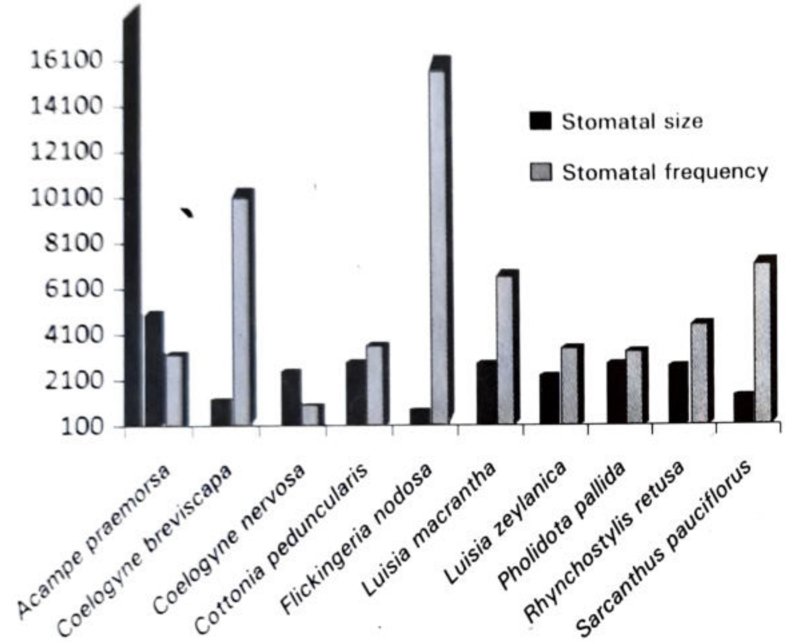


Fig.4.k-t. Stomata at lower and higher magnifications: k-l, *Luisia macrantha;* m-n, *Luisia zeylanica*; o-p, *Pholidota pallida;* q-r, *Rhynchostylis retusa*; s-t, *Sarcanthus pauciflorus*.

observed in mesophytic orchids. Amphistomatic condition is noticed only in *Rhynchostylis retusa*. Generally amphistomatic condition is noticed in dry and humid habitats. The association of amphistomata with more or less vertically oriented leaves of *Rhynchostylis retusa* confirms similar findings in several orchid species studied earlier (Mulgaonkar, 2005 a,b,c; Rasmussen, 1987). According to these authors, the thick leaves orders closely related to Liliales. For stomatal type, it is believed that paracytic type is primitive within the angiosperms (Cronquist, 1968; Takhtajan, 1969). However, present findings clearly indicated that paracytic type is most common, irresespective of genus. (Table 2, Figs. 3-4). Among the 10 orchids species, the highest stomatal size was noticed in *Acampe praemorsa* and followed by *Pholidota pallida*, and *Luisia macrantha;* stomatal size was very small in *Flickingeria nodosa* (Table 2). The stomatal frequency in the present study ranged between 490.12 to 16353.22 per mm^2; the highest stomatal frequency was noticed in comparatively thin membranous leaves of *Flickengeria nodosa* and lowest stomatal frequency was observed in *Coelogyne nervosa*. Vij *et al.* (1991) recorded lowest stomatal frequency in thick fleshly leaves and highest stomatal frequency in thin and membranous leaves. Similar observation was also made earlier by Mulgaonkar, (2005 a,b,c). Fig. 5 shows that there is direct relationship between stomatal size and stomatal frequency; if stomatal size is less, stomatal frequency is more and *vice versa*. Interestingly, a higher stomatal index was observed in *Coelogyne nervosa* (14.32) and lowest in *Rhyncostylis retusa* (3.45). This is because *C. nervosa* exposed to more sun light intensity than *R. retusa* (Fig. 6). Such observation is in line with earlier observations made by Vij. *et al.*, (1991). A direct positive co-relation of light intensity with the stomatal index in orchids was also indicated earlier by Rasmussen (1987).



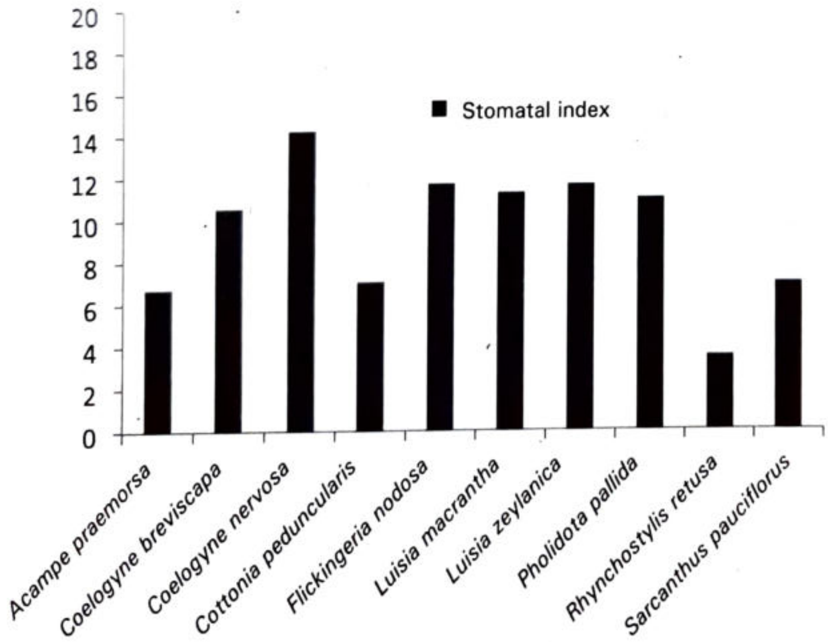


Fig.6. Stomatal Index in the investigated species.

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