

Stomatal Studies of Selected Epiphytic Orchids from Western Ghats- Goa, India

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ABSTRACT

In the present investigation leaf anatomy of fifteen epiphytic orchids belonging to nine genera *viz.*, *Acampe*, *Aerides*, *Bulbophyllum*, *Cottonia*, *Dendrobium*, *Pholidota*, *Porpax*, *Rhynchosstylis*, and *Smithsonia* from Western Ghats, Goa were studied. The study revealed differences with respect to leaf orientation, leaf texture, leaf surface and stomata. The stomatal types reported were anomocytic, tetracytic, pentacytic and paracytic. Maximum stomatal index was reported in *Porpax filiformis* while it was found to be minimum in *Rhynchosstylis retusa*. Stomatal frequency ranged between 25 mm² to 76.25 mm².

Key words: Epiphytic orchids, Stomata, Stomatal index, Stomatal frequency.

Introduction

Orchidaceae is one of the the largest family in monocotyledons (Dressler, 1993) with more than 25,000 species in 780 genera is widely distributed and occurs mainly in the tropical, subtropical and temperate regions. They have varied range of life forms *viz.*, terrestrial, epiphytic and saprophytic. Among these epiphytic orchids are more interesting because they are generally tolerant to changes in climate by their adaptive habits (Abraham and Vatsala, 1981). In India orchidaceae is represented by about 152 genera and 1300 species (Rao, 1979), Vartak, (1966) reported 65 species of orchids belonging to 30 genera from Karnataka and Maharashtra including a few orchids from Goa. Rao, (1986) reported 29 species from Goa belonging to 18 genera, Krishnaswamy *et al.*, (2004) reported 203 species and 59 genera of orchids in Karnataka state. In Goa the orchid distribution is mainly concentrated in the Western Ghats region,

which accounts for 86% of the total orchid diversity of the state (Jalal, 2019).

Leaf stomata which are found abundantly on the lower epidermal surface are the sites for gaseous exchange which occurs between intercellular spaces of subepidermal cells and the atmosphere. Transpiration water loss is mainly regulated by stomatal behavior (Meinzer *et al.*, 1997). On the basis of type of subsidiary cell the stomata are classified as Anomocytic, Anisocytic, Paracytic, and Diacytic type (Metcalf and Chalk, 1950). In taxonomic studies the leaf orientation pattern, shape and size of guard cell are of immense importance (Tomlison, 1974; Stace, 1984; Santhosh *et al.*, 2015). Studies are being carried out in different parts of the world on the distribution and taxonomic importance of stomata (Leela and Rao, 1996; Sahin, 1998; Santhosh *et al.*, 2015, Abraham *et al.*, 2016; Saensouk and Saensouk, 2020, Garvita and Wawangningrum, 2020). Present study was an attempt to carry out

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elaborate studies on stomata in epiphytic orchids from Western Ghats, Goa.

Materials and Methods

In the present investigation mature leaf from fifteen epiphytic orchids viz., *Acampe praemorsa* (Roxb.) Blatt. & McCann, *Aerides crispa* Lindl., *Aerides maculosa* Lindl., *Bulbophyllum sterile* (Lam.) Suresh, *Cottonia peduncularis* (Lindl.) Rchb. f., *Dendrobium barbatulum* Lindl., *Dendrobium macrostachyum* Lindl., *Dendrobium ovatum* (L.) Kranzl, *Pholidota imbricata*, *Porpax filiformis* (Wight) Schuit., Y.P. Ng & H. A. Pedersen, *Porpax jerdoniana* (Wight) Rolfe, *Porpax reticulata* Lindl., *Rhynchostylis retusa* L. Bl., and *Smithsonia straminea* C.J. Saldanha belonging to nine genera viz., *Acampe*, *Aerides*, *Bulbophyllum*, *Cottonia*, *Dendrobium*, *Pholidota*, *Porpax*, *Rhynchostylis*, and *Smithsonia* were collected from different parts of Goa which are used in stomatal studies (Table 1.). Details on leaf orientation pattern, texture, leaf surface was recorded for all the 15 selected orchids (Table 1). Mature leaf peelings were taken to study epidermal characteristics (Metcafe and Chalk, 1950, Williams, 1975). A portion of the peel was taken, stained in 1% aqueous solution of safranin and mounted in glycerine. Slides were viewed under 40x magnification of light mi-

croscope and noted the number of stomata, epidermal cells, type of stomata and also photographs were taken. For determination of stomatal index and stomatal frequency 10 different fields were viewed at 40X objective and average number of stomatal cells and epidermal cells present were noted and the stomatal index was calculated using the formula $SI = S/E+S \times 100$ (S = No. of stomatal cells; E = No. of ordinary epidermal cells.). $SF=S/$ Area of field of vision (S = No. of stomatal cells). The photography was taken with camera under 40x and 10x magnifications).

Statistical analysis

The data on stomatal index, stomatal frequency was subjected to One Way Analysis of Variance (ANOVA) using SPSS, Post Hoc test

Results and Discussion

the present study on phenological characteristics of leaf in fifteen species of epiphytic orchids revealed interesting results. The results on leaf phenology are depicted in Table 1. The selected epiphytic orchids revealed difference with respect to leaf orientation, leaf texture and leaf surface. The leaf texture was found to be thick and leathery in *Acampe praemorsa*, *Aerides crispa*, *Aerides maculosa*, *Cottonia peduncularis*,

Table 1. Leaf phenological characteristics and stomatal types of selected epiphytic orchids from Western Ghats, Goa.

Sr. No.	Name of the species	Leaf orientation	Leaf texture	Leaf surface	Type of stomata
1	<i>Acampe praemorsa</i> (Roxb.) Blatt. & McCann	S	L	Hypostomatic	Paracytic
2	<i>Aerides crispa</i> Lindl.	S	L	Hypostomatic	Paracytic
3	<i>Aerides maculosa</i> Lindl.	S	L	Amphistomatic	Paracytic
4	<i>Bulbophyllum sterile</i> (Lam.) Suresh	P	F	Amphistomatic	Paracytic
5	<i>Cottonia peduncularis</i> (Lindl.) Rchb.f.	V	L	Hypostomatic	Paracytic
6	<i>Dendrobium barbatulum</i> Lindl.	D	M	Hypostomatic	Anomocytic
7	<i>Dendrobium macrostachyum</i> Lindl.	D	M	Hypostomatic	Tetracytic
8	<i>Dendrobium ovatum</i> (L.) Kranzl.	D	M	Hypostomatic	Anomocytic, Tetracytic, Pentacytic
9	<i>Dendrobium peguanum</i> Kranzl	P	F	Hypostomatic	Anomocytic, Tetracytic
10	<i>Pholidota imbricata</i>	P	L	Hypostomatic	Anomocytic/Tetracytic
11	<i>Porpax filiformis</i> (Wight) Schuit., Y.P. Ng & H.A.Pedersen	S	M	Hypostomatic	Anomocytic
12	<i>Porpax jerdoniana</i> Wight) Rolfe	S	F	Hypostomatic	Anomocytic
13	<i>Porpax reticulata</i> Lindl.	S	F	Hypostomatic	Anomocytic
14	<i>Rhynchostylis retusa</i> (L.) Bl.	S	L	Amphistomatic	Paracytic
15	<i>Smithsonia straminea</i> C.J. Saldanha	S	L	Hypostomatic	Anomocytic/Tetracytic

S=Spreading; V= Vertical; P=Pseudobulb; D= Drooping; F=Fleshy; L= Leathery; M= Membranous

Rhynchostylis retusa. Of the four dendrobium species three species viz., *Dendrobium barbatulum*, *Dendrobium macrostachyum*, *Dendrobium ovatum* exhibited membranous type leaf texture while the leaf texture was found to be fleshy type in *Dendrobium peguanum*. All the three species of *Porpax* have shown fleshy type of leaf texture with leaves of *Porpax jerdoniana* having spreading type leaf orientation and reticulate ornamentation pattern on leaf surface. The leaves were found to be thick in *Bulbophyllum sterile* and *Smithsonia straminea* having fleshy type of texture, while *Pholidota imbricata* has shown membranous type of leaf texture. The leaves of other studied species exhibited presence of leathery leaf 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. In all the studied species the leaf surface was hypostomatic with the exception of *Bulbophyllum sterile*, *Aerides maculosa* and *Rhynchostylis retusa* where it was amphistomatic.

The results on stomatal studies revealed presence of different types of stomata viz., anomocytic, tetracytic, pentacytic and paracytic type (Fig. 1). In both the species of *Aerides* viz., *Aerides crispa* and *Aerides maculosa*, *Acampe praemorsa*, *Bulbophyllum sterile*, *Cottonia peduncularis*, *Rhynchostylis retusa* the stomata were found to be of paracytic type. Mulgaonkar, (2005) who has reported presence of paracytic stomata in her studies on dermal anatomy of some species of genus *Aerides* Lour. from

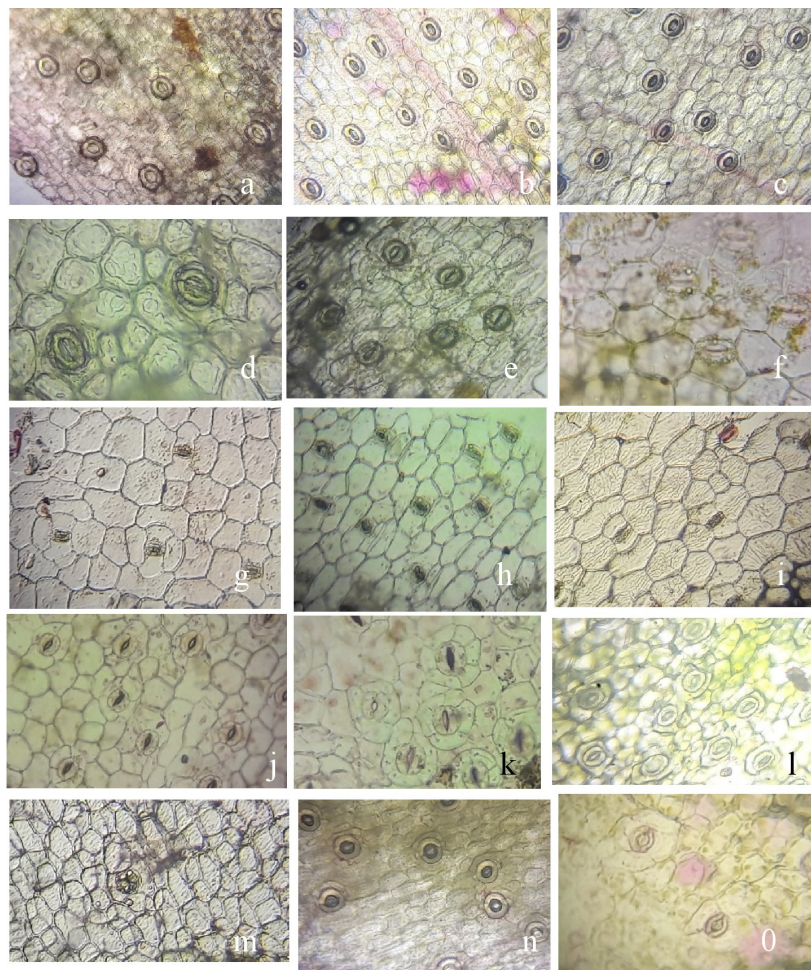


Fig. 1. Epidermal leaf anatomy of stomata of: (a) *Acampe praemorsa*, (b) *Aerides crispa*, (c) *Aerides maculosa*, (d) *Bulbophyllum sterile*, (e) *Cottonia peduncularis*, (f) *Dendrobium barbatulum*, (g) *Dendrobium macrostachyum*, (h) *Dendrobium ovatum*, (i) *Dendrobium peguanum*, (j) *Porpax filiformis*, (k) *Porpax jerdoniana*, (l) *Porpax reticulata*, (m) *Pholidota imbricata*, (n) *Rhynchostylis retusa*, (o) *Smithsonia straminea*.

Maharashtra. Among the dendrobium species *Dendrobium macrostachyum* and *Dendrobium peguanum* exhibited presence of tetracytic type of type while in *Dendrobium ovatum* and *Dendrobium barbatulum* the stomata were found to be both tetracytic and pentacytic type. In dendrobium species the number of subsidiary cells ranged between four to six. The presence of subsidiary cells is very common in Orchidales and this condition is more widespread than the absence of subsidiary cells, that is, anomocytic stomata (Williams, 1979). Angela *et al.*, (2015) have reported different types on stomata in their studies on six epiphytic orchids belonging to five genera. According to Stebins and khush (1961) anomocytic type of stomata is limited only to the orders closely related to liliales. In the present study most of the studied species showed hypostomatic condition. The amphistomatic condition of stomata was reported in *Aerides maculosa*, *Bulbophyllum sterile* and *Rhynchosstylis retusa*. The amphistomatic condition is related to the leaf thickness which in turn is

associated with the carbon fixation pathway (Mott, *et al.*, 1982).

The stomatal index and stomatal frequency varied significantly between the species (Table 3). The values of SI in the species of genus *Aeridis* were 3.75 % and 3.86 %, while in the genus *Dendrobium*, *viz.*, *Dendrobium barbatulum*, *Dendrobium macrostachyum*, *Dendrobium ovatum* and *Dendrobium peguanum* the SI values are 8.49 %, 6.93 %, 13.66 %, 8.48 % respectively. Variation in stomatal index in dendrobium species could possibly be due to variations in sun-light intensity. *Dendrobium ovatum* grow in more sun light intensity areas as compared to *Dendrobium barbatulum*, *Dendrobium macrostachyum* and *Dendrobium peguanum*. Rasmussen, (1987), has reported positive correlation between light intensity and stomatal index in orchids. The processes influenced by stomatal frequency are photosynthesis and transpiration (Inamdar *et al.*, 1991). The stomatal frequency ranged from a minimum of 25 mm² to maximum of 76.25 mm² (Table 2). Among the fifteen

Table 2. Dermal anatomical characteristics (mean ± SE, n = 10) of the epiphytic orchids from Western Ghats, Goa.

Sr. No.	Name of the species	Stomatal index (%)	Stomatal frequency (mm ²)
1	<i>Acampe praemorsa</i> (Roxb.) Blatt. & McCann	8.46 ± 0.546	28.75 ± 1.090
2	<i>Aerides crispa</i> Lindl.	3.75 ± 0.130	57.50 ± 2.041
3	<i>Aerides maculosa</i> Lindl.	3.86 ± 0.210	53.75 ± 1.909
4	<i>Bulbophyllum sterile</i> (Lam.) Suresh	4.70 ± 0.341	30.00 ± 2.412
5	<i>Cottonia peduncularis</i> (Lindl.) Rchb.f.	3.99 ± 0.713	25 ± 0.000
6	<i>Dendrobium barbatulum</i> Lindl.	8.49 ± 0.286	55.00 ± 2.041
7	<i>Dendrobium macrostachyum</i> Lindl.	6.93 ± 0.284	36.26 ± 1.250
8	<i>Dendrobium ovatum</i> (L.) Kranzl.	13.66 ± 0.522	68.75 ± 2.083
9	<i>Dendrobium peguanum</i> Kranzl	8.48 ± 0.376	40.00 ± 1.666
10	<i>Pholidota imbricata</i>	5.40 ± 0.284	33.75 ± 1.909
11	<i>Porpax filiformis</i> (Wight) Schuit., Y.P.Ng & H.A.Pedersen	23.66 ± 0.532	57.50 ± 2.041
12	<i>Porpax jerdoniana</i> (Wight) Rolfe	19.77 ± 0.511	76.25 ± 2.916
13	<i>Porpax reticulata</i> Lindl.	13.76 ± 0.834	32.50 ± 2.041
14	<i>Rhynchosstylis retusa</i> (L.) Bl.	3.38 ± 0.172	35.00 ± 1.666
15	<i>Smithsonia straminea</i> C.J. Saldanha	3.57 ± 0.541	37.5 ± 0.000

Table 3. One -way analysis of variance (ANOVA) Post Hoc test of the data on Stomatal Index, Stomatal frequency of fifteen orchid species.

		Sum of Squares	df	Mean Square	F	Significance
Stomatal Index	Between species	5511.867	14	393.705	244.642	**
	Within species	217.256	135	1.609		
	Total	5729.123	149			
Stomatal frequency	Between species	34556.250	14	2468.304	71.564	**
	Within species	4656.250	135	34.491		
	Total	39212.500	149			

Significant at ** P < 0.05; d.f- Degrees of freedom

orchids species the highest frequency was found in *Porpax jerdoniana* (76.25 mm²) followed by *Dendrobium ovatum* (68.75 mm²), *Aerides crispa* (57.50 mm²), *Porpax filiformis* (57.50 mm²), *Dendrobium barbatulum* (55.00 mm²), *Aerides maculosa* (53.75 mm²), and least stomatal frequency is recorded in *Cottonia peduncularis* (25 mm²). According to Goh, et al., (1977) more the succulence lesser the frequency of stomata. In the present investigation few species exhibited higher stomatal frequency and stomatal index but was not the case with other studied species.

In the present investigation the number of stomatal index varied between the genera and between the species. Low value of stomatal index is an adaptation of orchid plants to survive in different climatic conditions by conserving water.

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