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LEAF MICROMORPHOLOGY OF SOME *HABENARIA* WILLD. *SENSU LATO* (ORCHIDACEAE) SPECIES FROM WESTERN HIMALAYA

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Abstract

Leaf epidermal characteristics were investigated in twelve Western Himalayan species of *Habenaria* Willd. *sensu lato* with a view to assess their taxonomic and ecological importance. The leaves in all species investigated were soft, shiny and devoid of trichomes. The epidermal cells were polygonal in shape but quadrilateral on adaxial surface of *H. edgeworthii* J. D. Hook. Cell walls were straight except on abaxial epidermis of *H. commelinifolia* (Roxb.) Wall. ex Lindl. and *H. ensifolia* Lindl., where they were slightly undulated. The leaves were invariably hypostomatic and possessed anomocytic type of stomata. Additional presence of diacytic (*H. plantaginea* Lindl.) and twin (*H. marginata* Coleb.) stomata was of taxonomic implication. Stomatal frequency (per mm^2) was lowest (16.01±1.09) in *H. edgeworthii* and highest (56.84±3.50) in *H. marginata,* and stomatal index (%) ranged between 11.93±1.14 (*H. stenopetala* Lindl.) and 27.24±1.26 (*H. aitchisonii* Reichb. f.). Leaf epidermal features reflected no apparent relationship with species habitat. There were significant differences observed in many epidermal characteristics, which can ably supplement the data available on gross morphology to help in delimiting different *Habenaria* species.

Introduction

MICROMORPHOLOGICAL CHARACTERISTICS are in practice in plant taxonomy ever since high power microscopes became available. Even today, these are regarded as essential equipments to study microscopic structures. Amongest the non-reproductive plant organs, leaves are most widely used for systematic interpretations (Stace, 1980). As each taxon has its own surface characteristics, various leaf epidermal characters (size, shape and wall pattern of epidermal cells; size, type, frequency and index of stomata; presence/absence of trichomes, *etc*.) have been utilized for their taxonomic significance at family, subfamily, genus and species level (Adeniji and Ariwaodo, 2012; Akcin *et al*., 2013; Albert and Sharma, 2013; Angela *et al.,* 2015; Devi *et al*., 2013; Kowsalya *et al*., 2017; Ogundipe and Akinrinlade, 1998; Prashanta Kumar and Krishnaswamy, 2014; Solereder, 1908; Stace, 1980; Timonin, 1986; Tomlinson, 1974). Baruah (2017) studied epidermal features of peduncle, pedicel, and capsule in five orchid species and prepared an artificial taxonomic key based on useful taxonomic characters.

Mobius (1887) was the first to identify taxonomic markers in orchid leaf anatomy. Studies on epidermal characteristics of orchid leaves have been undertaken by many investigators (Banerjee and Rao, 1978; Carlsward *et al*., 1997; Cetzal-Ix *et al*., 2013; Chattopadhayay *et al.*, 2014; Cyge, 1930; Das and Paria, 1992; Endress *et al*., 2000; Inamdar, 1968; Kaushik, 1983; Khasim and Mohana Rao, 1986, 1990; Kowsalya *et al.*, 2017; Leitao *et al*., 2014; Mohana Rao and Khasim, 1986, 1987; Prashantha Kumar and Krishnaswamy, 2011; Rasmussen, 1981, 1986; Rosso, 1966; Sevgi *et al*., 2012a, b; Singh, 1981; Singh and Singh, 1974; Solereder and Meyer, 1930; Stebbins and Khush, 1961; Stern, 1997; Stern and Judd, 2000; Vij *et al*., 1991; Williams, 1975, 1976, 1979; Zanenga-Godoy and Costa, 2003), and many of them have highlighted their taxonomic significance. Furthermore, since leaf is the functional boundary layer between the plant and its environment, the ecological significance of dermal features has also been advocated (Kaushik, 1983; Mohana Rao and Khasim, 1986, 1987; Moreira *et al*., 2013; Ramudu *et al*., 2012; Sanford, 1974; Vij *et al*., 1991; Withner *et al*., 1974) in orchids. Atwood and Williams (1979) even suggested the use of epidermal characteristics of *Paphiopedilum* Pfitz. and *Phragmipedium* Rolfe in identifying sterile plants which were otherwise indistinguishable.

The Himalaya is about 2400 km long stretch of mountains with varying altitudes. Geographically, it has been divided into 3 sectors: i) Western Himalaya,

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comprising the northern part of Afghanistan, Pakistan and India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand) up to the western border of Nepal; ii) Central Himalaya, which falls in Nepal; and iii) Eastern Himalaya, extending from the North Bengal hills to Sikkim, Bhutan and Arunachal Pradesh. The Indian Himalayan Region (IHR) provides home to more than 850 orchid species (Singh, 2001). The leaf material for the present investigation was collected from populations growing in the state of Himachal Pradesh (Western Himalaya). There are only a few reports available on leaf epidermal features (Chattopadhayay *et al.*, 2014; Kaushik, 1983; Khasim and Mohana Rao, 1986; Mehra, 1989; Mohana Rao and Khasim, 1987; Shakya, 1999; Vij *et al*., 1991) of Himalayan orchids.

Habenaria Willd. is an orchid genus of about 600 species widely distributed throughout the tropical, subtropical and temperate regions of the world. In India, it is represented by 17 species (including *H. clavigera, H. edgeworthii, H. latilabris*) in Western Himalaya (Jalal and Jayanthi, 2015) and some of these are well known for their therapeutic properties (Chauhan, 1990; Vij *et al.*, 2013). The species can be easily identified when in bloom, but the vegetative characteristics (number and size of tubers and leaves, stem height) overlap in many of these. The mistaken identity of flowerless individuals many times results in collection of wrong plant material and poor quality of remedial formulations prepared from their tubers. The present paper reports the leaf epidermal characteristics (size, shape and wall pattern of epidermal cells; presence/absence of trichomes; size, type, frequency and index of stomata) of 12 species which will help identify them even if the available flowerless individuals are with green or withering leaves (or even leaf segments). All of these species were earlier included under genus *Habenaria* (Subfamily Orchidoideae, tribe Orchideae, subtribe Habenariinae) but three [*H. clavigera* Lindl. (Dandy)*, H. edgeworthii* J. D. Hook.*, H. latilabris* (Lindl.) J. D. Hook] have now (Govaerts *et al*., 2018) been included under genus *Herminium* L. (Subtribe Herminiinae). The results have been analyzed statistically, and photographs, both for abaxial and adaxial epidermal peels, are provided uniformly for each species.

Materials and Methods

Field trips were organized (2007-2015) in Western Himalayas to locate various orchid species. These were identified following standard flora (King and Pantling, 1898; Vij *et al*., 2013) using vegetative and floral characters. Leaf micromorphological features were investigated in 12 *Habenaria* species are included under the scope of present paper. Table 1 summarizes their collection details. Observations were made on various epidermal features such as size and shape of epidermal cells; presence/absence of trichomes; and size, type, stomatal frequency and stomatal index. For each species, 2-3 leaf segments (excised from the middle portion) of 1-2 cm width were sourced from different plants. They were fixed directly in FAA (1:1:18 of formalin, acetic acid and 50% ethyl alcohol)

Table 1. Collection details of presently studied *Habenaria* species.

Species	Collection details	
	Locality, District (altitude)	Habitat
Habenaria aitchisonii Reichb. f.	Khanog, Solan (1580 m)	Shady forest
H. clavigera (Lindl.) Dandy	Karsog, Mandi (1560 m)	Bushy grassland
H. commelinifolia (Roxb.) Wall. ex Lindl.	Ranital, Kangra (1080 m)	Bushy grassland
H. digitata Lindl.	Seri-Jatoli road, Solan (1580 m)	Bushy grassland
H. edgeworthii J. D. Hook	Nauradhar, Sirmaur (2500 m)	Bushy grassland
H. ensifolia Lindl.	Kaithalighat, Solan (1750 m)	Bushy grassland
H. intermedia D. Don	Forest road, Solan (1460 m)	Shady forest
H. latilabris (Lindl.) J. D. Hook.	Summer hill, Shimla (2120 m)	Bushy grassland
H. marginata Coleb.	Tihra, Mandi (960 m)	Bushy grassland
H. pectinata (J. E. Sm.) D. Don	Garhkhal, Solan (1760 m)	Bushy grassland
H. plantaginea Lindl.	Jwalaji, Kangra (820 m)	Shady forest
H. stenopetala Lindl.	Karol Tibba, Solan (1550 m)	Shady forest

Table 2. Leaf epidermal characteristics of the presently investigated *Habenaria* species.

Species	Epidermal cells					Stomata					
	Shape/ walls	Size (µm)				Type	Size (µm)			Frequency (per mm²)	Index (%)
		Abaxial surface		Adaxial surface			Abaxial surface		Adaxial surface	Abaxial surface	Adaxial surface
		length	width	length	width		length	width			
Habenaria aitchisonii	Pol/ Str	121.55±2.38f	107.52±1.42j	172.02±1.79e	147.02±1.74h	Ano	72.72±2.46f	55.95±2.05e	Absent	37.18±1.40f	27.24±1.26h
H. clavigera	Pol/ Str	64.18±1.28a	46.81±1.73c	134.56±4.22c	85.16±0.98b	Ano	74.10±1.22f	72.69±1.27h	Absent	34.12±1.75e	12.46±0.89a
H. commelinifolia	Pol/ Sun	122.32±2.52f	78.75±1.42f	221.35±4.40g	170.67±2.10i	Ano	68.85±2.18e	56.93±2.36e	Absent	40.89±1.75g	22.09±1.44f,g
H. digitata	Pol/ Str	78.28±2.47b	44.01±2.05b	112.11±3.28b	93.19±2.07d	Ano	37.68±1.58a	28.11±1.86a	Absent	20.69±1.78b	16.17±1.38b
H. edgeworthii	Pol/ Str	146.44±3.00h	85.44±2.64g	151.71±3.74d	89.59±4.08c	Ano	96.86±2.53i	92.99±1.70j	Absent	16.01±1.09a	19.07±1.32d,e
H. ensifolia	Pol/ Sun	106.85±3.22e	60.76±2.39d	153.44±3.31d	92.71±2.20c,d	Ano	62.47±1.92d	60.96±2.35f	Absent	30.31±2.06d	20.63±2.44e,f
H. intermedia	Pol/ Str	102.67±4.59d	84.64±1.71g	151.64±2.72d	101.17±2.76e	Ano	77.64±1.79g	68.28±1.77g	Absent	22.27±1.36b	18.34±1.36c,d
H. latilabris	Pol/ Str	151.76±3.67i	92.02±2.56h	182.60±2.87f	140.93±3.10g	Ano	85.22±1.51h	75.57±1.75i	Absent	25.12±1.36c	16.64±1.42b,c
H. marginata	Pol/ Str	102.64±1.32d	62.56±2.25d	154.69±2.26d	112.36±3.30f	Ano, Twin	62.32±1.52d	47.04±1.33c	Absent	56.84±3.50i	23.45±1.97g
H. pectinata	Pol/ Str	132.09±4.24g	98.45±1.73i	133.31±2.50c	101.59±3.93e	Ano	79.49±2.69g	73.98±3.37h,i	Absent	26.23±1.31c	19.91±1.54d,e
H. plantaginea	Pol/ Str	99.88±2.69d	73.60±2.05e	130.58±2.37c	115.36±2.47f	Ano, Dia	55.56±1.54c	52.68±2.07d	Absent	45.63±1.95h	20.01±2.12d,e
H. stenopetala	Pol/ Str	90.54±3.38c	35.01±1.33a	105.56±2.84a	54.52±1.69a	Ano	41.45±1.67b	35.06±1.46b	Absent	35.69±1.71e,f	11.93±1.14a

Data are shown as mean ± standard deviation. Values in a column with the same superscripts are not significantly different at P≤0.05. Ano, Anomocytic; Dia, Diacytic; Pol, Polygonal; Str, Straight; Sun, Slightly undulate.

in the field. These segments were later kept in 10% KOH solution for 12-24 hours following Kaushik (1983) with slight modification; the epidermis on both abaxial and adaxial surfaces were then gently removed with soft brush. The peels, so obtained, were stained with safranine, mounted in 10% glycerin on glass slides and observed under light microscope. Stomatal types were identified following Rasmussen (1987). The quantitative measurements [size of epidermal cells and stomata, stomatal frequency (number of stomata per square millimeter)] were made using standardized stage and ocular micrometers. The stomatal index was calculated by using following formula: i = [S/(S+E)]×100 where i = stomatal index, S = total number of stomata in a given area of leaf, and E = total number of epidermal cells in the same area of leaf. The data for each species were collected in 15 replicates. The quantitative results were subjected to one-way analysis of variance and post hoc tests to detect the significant differences ($P \leq 0.05$) in various characteristics among different species using SPSS 17.0 (SPSS Inc., USA).

Results

The leaf micromorphological characteristics of the presently studied Western Himalayan species of *Habenaria* showed significant differences. These species were found occupying two different habitats; eight were collected from bushy grasslands (plenty of sunlight), and the remaining four (*Habenaria aitchisonii* Reichb. f.*, H. intermedia* D. Don*, H. plantaginea* Lindl.*, H. stenopetala* Lindl.) from shady forest floors (lesser sunlight). The leaves were soft and shiny in each species, and their surfaces were devoid of any epidermal appendages (trichomes). The results are summarized in Table 2 and are presented here in detail.

The epidermal cells were polygonal in shape except on the adaxial surface of *H. edgeworthii*, which possessed quadrilateral cells (Figs. 1-2). Their walls were straight in ten and slightly undulated on the abaxial surface of two [*H. commelinifolia* (Roxb.) Wall. ex Lindl.*, H. ensifolia* Lindl.] species (Fig. 1). In each species, the cells on adaxial surface were comparatively larger than those on the abaxial one. Their length ranged between 64.18±1.28 µm (*H. clavigera*) and 151.76±3.67 µm (*H. latilabris*) on abaxial surface, and between 105.56±2.84 µm (*H. stenopetala*) and 221.35±4.40 µm (*H. commelinifolia*) on adaxial surface. Cell length showed significant differences in majority of the species (Table 2) irrespective of their habitats. Likewise, the cell width also showed variations. It was shortest in *H. stenopetala* (35.01±1.33 µm) and longest in *H. aitchisonii* (107.52±1.42 µm) on the abaxial, and in *H. stenopetala* (54.52±1.69 µm) and *H. commelinifolia* (170.67±2.10 µm) on the adaxial surface.

The stomata were confined only to the inter-costal (areas between parallel running leaf veins) regions of the abaxial leaf surface (hypostomaty). They were arranged longitudinally along the leaf axis. In *H. marginata* Coleb., same subsidiary cell was observed to be shared by two different stomata at few places (Fig. 2), such stomata sharing a common subsidiary cell were referred to twin (contiguous) stomata. The guard cells were kidney-shaped and were surrounded usually by 4-5 subsidiary cells. As size, shape and arrangement of subsidiary cells were not different from other epidermal cells, the stomata were invariably of anomocytic type. Additional presence of diacytic stomata, where guard cells were surrounded only by two larger sized subsidiary cells, was observed only in case of *H. plantaginea* (Fig. 2). The length and width of stomatal apparatus (whole stoma consisting of two guard cells) exhibited significant differences in many species. Their length was observed to vary between 37.68±1.58 and 96.86±2.53 µm, and the width between 28.11±1.86 and 92.99±1.70 µm in *H. digitata* Lindl. and *H. edgeworthii* respectively (Table 2). Both of these species were collected from bushy grasslands.

A marked variation was observed in stomatal frequency (per mm^2) ranging from 16.01±1.09 (*H. edgeworthii*) to 56.84±3.50 (*H. marginata*) and the differences were significant in majority of taxa. Since both of the above mentioned species were found distributed in bushy grasslands, therefore, stomatal frequency, reflected no relationship with species habitat. Simultaneously stomatal index also showed significant differences in many species. Its value (%) was lowest (11.93±1.14) in *H. stenopetala* and highest (27.24±1.26) in *H. aitchisonii*, both of which inhabited shady forest floors.

Discussion

Present investigation on various foliar micromorphological characteristics of twelve *Habenaria* species yielded interesting results. Different species shared more or less similar epidermal features probably due to their closer affinities. However, some of these also possessed one or more such character(s), which show significant differences and held good diagnostic value.

The subfamily Orchidoideae is known for its soft and shiny leaves, and the presently studied species were no exception. In presently studied species, both the leaf surfaces (abaxial, adaxial) were devoid of trichomes. Presence of such epidermal appendages is well documented in leaves of many Epidendroid

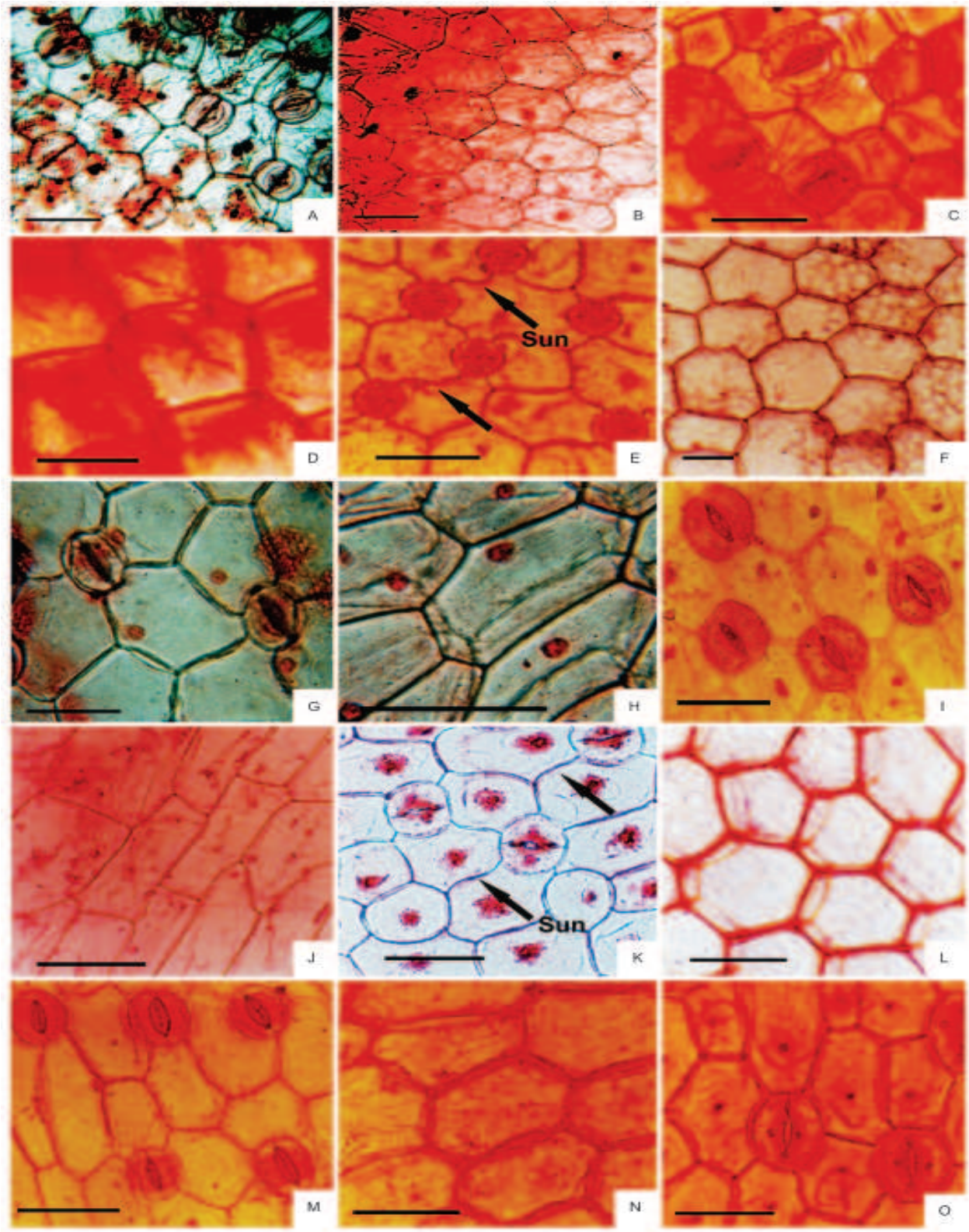

Fig. 1. A-O. Leaf micromorphological features of Western Himalayan *Habenaria* species: A-B, Abaxial and adaxial epidermis of *H. aitchisonii*; C-D, Abaxial and adaxial epidermis of *H. clavigera*; E-F, Abaxial and adaxial epidermis of *H. commelinifolia*; G-H, Abaxial and adaxial epidermis of *H. digitata*; I-J, Abaxial and adaxial epidermis of *H. edgeworthii*; K-L, Abaxial and adaxial epidermis of *H. ensifolia*; M-N, Abaxial and adaxial epidermis of *H. intermedia*; O, Abaxial epidermis of *H. latilabris*. Scale bars = 100 µm. (Sun, slightly undulate cell walls).

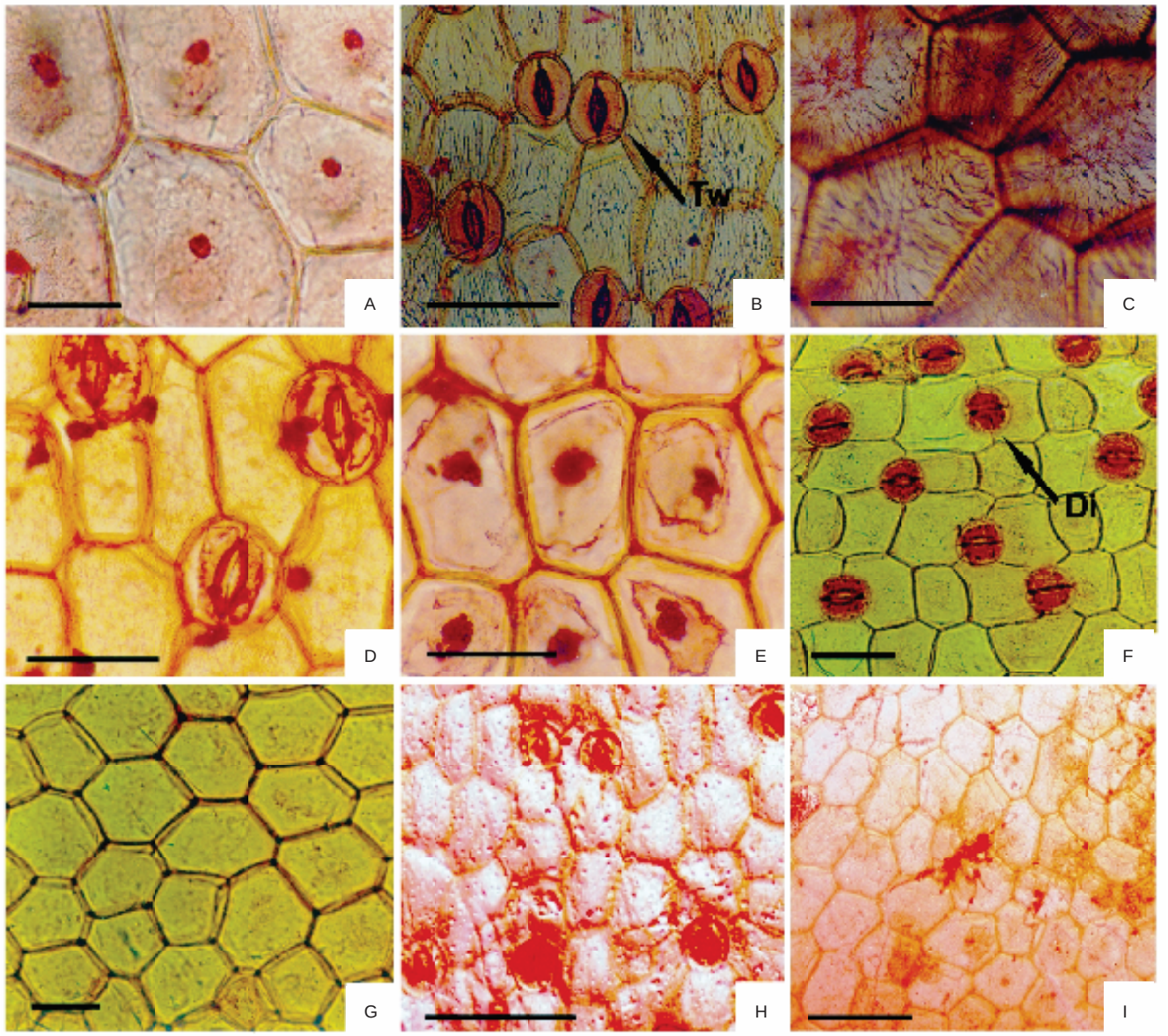

Fig. 2. A-I. Leaf micromorphological features of Western Himalayan *Habenaria* species: A, Adaxial epidermis of *H. latilabris*; B-C, Abaxial and adaxial epidermis of *H. marginata*; D-E, Abaxial and adaxial epidermis of *H. pectinata*; F-G, Abaxial and adaxial epidermis of *H. plantaginea*; H-I, Abaxial and adaxial epidermis of *H. stenopetala*. Scale bars = 100 µm. (Di, diacytic stomata; Tw, twin stomata).

orchids (Cardoso-Gustavson *et al*., 2014; Kaushik, 1983; Solereder and Meyer, 1930; Stpiczynska and Davies, 2009; Wagner, 1991; Yu *et al*., 2007), but there is no record of their occurrence in any *Habenaria* species. The epidermal cells were polygonal in shape. Quadrilateral cells, observed on the adaxial leaf surface of *H. egdeworthii* (Figs. 1-2) are of taxonomic implication. The cell walls were straight in majority of species, but slightly undulated in case of abaxial epidermis of *H. commelinifolia* and *H. ensifolia* (Fig. 1). The cell wall patterns have been reported to vary (straight, undulate, curved, repand) in different orchidaceous (Sevgi *et al*., 2012a; Vij *et al*., 1991) as well as non-orchidaceous (Adeniji and Ariwaodo, 2012; Akcin *et al*., 2013; Albert and Sharma, 2013) taxa, and have taxonomic inference. In each species, the adaxial leaf surface possessed comparatively larger sized cells than on abaxial side. These observations are in line with those of Withner *et al*. (1974), and Khasim and Mohana Rao (1986) that adaxial epidermal cells might be larger (sometimes up to 2-3 times) than the abaxial ones. Vij *et al*. (1991) suggested that the taxa with spreading leaves (like present ones) usually possess larger cells on adaxial surface; they are generally identical in dimensions on both the leaf surfaces in species with vertically orientated leaves. Ramudu *et al*. (2012), however, reported relatively larger epidermal cells on abaxial leaf surface of *Coelogyne nervosa*, an

epiphytic orchid species with vertically placed leaves. Presently, the epidermal cell size reflected no relation with the species habitat. The costal and inter-costal regions could readily be differentiated in all species; the former strictly had longer and narrower epidermal cells, and showed complete absence of stomata. Earlier, Rasmussen (1981) also ruled out the development of stomata in costal files in members of Orchidoideae.

The stomata were confined only to the abaxial leaf surface. Such an occurrence of hypostomaty is well reported in majority of orchid taxa. According to Cyge (1930), some orchid taxa may have amphistomatic leaves but the distribution of stomata is unequal in such cases with a lesser representation on the upper surface. Kaushik (1983) observed that stomata were present on both the leaf surfaces in conduplicate leaves of *Aerides multiflora* and *Rhynchostylis retusa,* in bilaterally compressed leaves of *Oberonia pachyrachis,* and all around the terete (*Aerides vandarum*, *Luisia trichorrhiza*) and subterete (*Cleisostoma gemmatum*) leaves. According to Parkhurst (1978) and Rasmussen (1987), hypostomatic leaves are predominant in the mesophytic species and amphistomaty is of common occurrence in species inhabiting very dry or humid locations. As all of the presently investigated species were mesophytic, present results are in conformity with these findings. Vij *et al*. (1991) suggested that the stomatal distribution in orchid leaves is essentially a genetic attribute whose manifestation varies with both the external (light, water) and internal (CO_2 metabolism) factors. In all species studied presently, the stomata were arranged longitudinally along the leaf axis. Solereder and Meyer (1930) earlier reported similar findings of guard cells being oriented parallel to the long axis of the leaf, in general. Rasmussen (1987) reported the occurrence of kidney shaped guard cells in various orchid taxa and the present species were no exception.

The occurrence of subsidiary cells has been debated in orchids. Stebbins and Khush (1961) reported lack of subsidiary cells in their stomata. According to Withner *et al*. (1974), the subsidiary cells are absent in orchids, but modified epidermal cells may occur adjacent to the guard cells. Williams (1975) followed the stomatal development in *Ludisia discolor* and clearly demonstrated the presence of subsidiary cells in its guard cells. Williams (1979) further suggested that the subsidiary cells are characteristically present in advanced orchids and their absence in plants is a primitive feature. Rasmussen (1987) studied the stomatal ontogeny in orchids and identified following six types of stomata in their leaves: i) anomocytic, where mature guard cells are surrounded by cells morphologically similar to other epidermal cells; ii) anisocytic, where mature guard cells are surrounded by subsidiary cells of unequal size; iii) diacytic, where mature guard cells are surrounded by a pair of subsidiary cells with their common walls at right angles to the long axis of guard cells; iv) paracytic, where mature guard cells are surrounded by 2 polar (smaller and broader) and 2 lateral (longer and narrower) cells; v) tetracytic, where mature guard cells are surrounded by four subsidiary cells of equal size; and vi) cyclocytic where mature guard cells are surrounded by an undetermined often large number of similar subsidiary cells radiating from the circumference of guard cells pair. In presently studied species, the guard cells were surrounded by 4-5 subsidiary cells of same size and shape (Figs. 1-2) as that of other epidermal cells; the stomata were of anomocytic type. Stern (1997) studied the vegetative anatomy of certain taxa of subtribe Habenariinae and observed uniform occurrence of anomocytic stomata in them. Dressler (1993) recognized three main patterns of stomatal development in orchids; the Epidendndroid pattern, usually with recognizable subsidiary cells that are perigenous in development with trapezoid cells; the Cranichid pattern, usually with recognizable subsidiary cells that are mesoperigenous in development; and the Orchidoid pattern, without recognizable subsidiary cells at maturity. According to Banerjee and Rao (1978), Shakya (1999) and Vij *et al*. (1991), there are very less differences in types of stomata in various members of subfamily Orchidoideae; the Epidendroid orchids, however, exhibit higher variability in this respect. An additional occurrence of diacytic stomata was observed in *H. plantaginea* (Fig. 2), which hinted at the genetic plasticity of this species. Furthermore, twin stomata, where a single subsidiary cell was shared by two different stomata were also observed in case of *H. marginata* (Fig. 2). Inamdar (1968) also reported the occurrence of similar kind of stomata in these species, thus confirming, the conservative nature of epidermal and stomatal characteristics. Twin stomata have earlier been reported in *Capsicum annuum* and *Lycopersicon esculentum* (Karatela and Gill, 1986).

Based on stomatal types, Kaushik (1983) attempted to classify Orchidaceae into 4 subfamilies, Anomocyticeae, Cyclocyticeae, Diacyticeae and Paracyticeae; of which the last one was considered as the most advanced having evolved from Anomocyticeae through Cyclocyticeae. However, such a classification, based purely on stomatal types,

appears quite premature, particularly in view of occurrence of multiple stomatal types at specific and/ or intra-specific level (*e.g.* occurrence of anomocytic as well as diacytic stomata in *H. plantaginea*). It may not be out of place to accept the taxonomic utility of stomatal types in orchids, but they might have evolved independently in several lines in this group of plants. Hence, a classification system where other characters (gross morphological, anatomical, cytological, *etc*.) will be taken in conjunction with epidermal features, would help in bringing naturally similar taxa much closer.

The length and width of stomatal apparatus showed variations of significant importance in many cases. Their length was observed to range between 37.68±1.58 and 96.86±2.53 µm, and width between 28.11±1.86 and 92.99±1.70 µm in *H. digitata* and *H. edgeworthii* respectively. As both of these are species of bushy grasslands, stomatal size reflected no relationship with plant habitat. Stomatal frequency was lowest in *H. edgeworthii* and highest in *H. marginata*. Carpenter and Smith (1975) suggested the taxonomic importance of variation in stomatal frequencies at generic levels. Paek and Jun (1995) demonstrated that stomatal density was higher in terrestrial orchids than their epiphytic counterparts. Ziegenspeck (1936), though, reported higher stomatal frequency in species from marshy habitats; in present taxa, higher frequencies were observed in species inhabiting open and well lighted situations (bushy grasslands) as reported earlier by Vij *et al*. (1991). More recently, Moreira *et al*. (2013) also reported higher stomatal frequency in an epiphytic orchid inhabiting comparitively more luminous sites. Presently, the value of stomatal index (%) was found to be lowest (11.93±1.14) in *H. stenopetala* and highest (27.24±1.26) in *H. aitchisonii*, both of which dwelled in shady forest floors. Sinclair (1987) suggested the importance of stomatal index in taxonomy, and highlighted that it remains relatively constant within a species. Paek and Jun (1995), however, suggested that the number of stomata may be increased with plant age in some orchid species.

Habenaria ensifolia was earlier treated as a synonym of *H. pectinata* (Govaerts *et al*., 2012). However, we have observed some morphological features (flower colour and arrangement, leaves, floral bracts) that make it distinct from the latter species. Flowers were greenish yellow in *H. ensifolia* and they were arranged laxly as compared to the white densely arranged flowers of *H. pectinata*. The leaves were linear lanceolate in *H. ensifolia* but ovate lanceolate in *H. pectinata*. Floral bracts were highly foliaceous and overtopped the flowers only in case of *H. pectinata*. Present study on leaf micromorphology also reflected many differences in epidermal characteristics of these taxa. Cell walls on abaxial surface, that were straight in *H. pectinata,* were slightly undulated in case of *H. ensifolia* (Figs. 1, 2). Stomata were oval in the former and round in the latter species. Significant differences were observed in size of their epidermal cells and stomata, and stomatal frequencies (Table 2). Therefore, in view of gross morphological and foliar micromorphological differences, *H. ensifolia* deserves to be treated as a distinct species and not as a synonym of *H. pectinata*. Govaerts *et al*. (2018), Jalal and Jayanthi (2015), and Vij *et al*. (2013) also treated them as separate species. Furthermore, there was no epidermal character (except cell shape and their wall pattern), which was shared only by *H. clavigera, H. edgeworthii* and *H. latilabris*, all of which are now treated under genus *Herminium* (Govaerts *et al*., 2018).

References

Adeniji, K. A. and J. O. Ariwaodo. 2012. Comparative foliar epidermal studies of genus *Pericopsis* (Papilionaceae) in Nigeria. *Phytol. Balcanica,* **18**: 37-41.

Akcin, O. E., G. Senel, and Y. Akcin. 2013. Leaf epidermis morphology of some *Onosma* (Boraginaceae) species from Turkey. *Turk. J. Bot.*, **37**: 55-64.

Albert, S. and B. Sharma. 2013. Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species. *Turk. J. Bot.,* **37**: 276-81.

Angela, Nengpilhing, H. Bishwajit Sharma, Krishna Chowlu, and N. Nageswara Rao. 2015. Foliar anatomy in some species of *Bulbophyllum* Thou. *J. Orchid Soc. India*, **29**: 67-74.

Atwood, J. T. and N. H. Williams. 1979. Surface features of the adaxial epidermis in the conduplicate-leaved Cypripedioideae (Orchidaceae). *Bot. J. Linn. Soc.,* **78**: 141-56.

Banerjee, A. K. and A. V. N. Rao. 1978. A preliminary epidermal studies in a few taxa in *Coelogyne* (Orchidaceae). *Curr. Sci.,* **47**: 630-32.

Baruah, A. 2017. Peduncle, pedicel, and capsule epidermal characters of certain orchid species from NorthEast India. *J. Orchid Soc. India,* **31**: 65-69.

Cardoso-Gustavson, P., L. M. Campbell, S. C. Mazzoni-Viveiros, and F. de Barros. 2014. Floral colleters in Pleurothallidinae (Epidendroideae: Orchidaceae). *Am. J. Bot.,* **101**: 587-97.

Carlsward, B. S., W. L. Stern, W. S. Judd, and T. W. Lucansky. 1997. Comparative leaf anatomy and systematics in *Dendrobium* sections *Aporum* and *Rhizobium* (Orchidaceae). *Int. J. Plant Sci.,* **158**: 332-42.

Carpenter, S. B. and N. D. Smith. 1975. Stomatal distribution and size in southern Appalachian hardwoods. *Can. J. Bot.,* **53**: 1153-56.

Cetzal-Ix, W., E. Noguera-Savelli, D. Jauregui, and G. Carnevali. 2013. Anatomía foliar comparada y sistemática del clado-*Trichocentrum* con énfasis en *Cohniella*

(Asparagales: Orchidaceae). *Rev. Biol. Trop.,* **61**: 1841-58.

Chattopadhyay, A., Promila Pathak, and K. C. Mahant. 2014. Foliar features in some Indian Orchids: Subtribe Habenariinae (Orchideae, Orchidoideae). *J. Orchid Soc. India*, **28**: 47-59.

Chauhan, N. S. 1990. Medicinal orchids of Himachal Pradesh. *J. Orchid Soc. India*, **4**: 99-105.

Cyge. T. 1930. Etudes anatomiques et ecologiques sur les feuilles des Orchidees indigenes. *Extr. Mem. Acad. Polon. Sci. Lett. Ser. B,* **12**: 16-32.

Das, S. N. and N. Paria. 1992. Stomatal structure of some Indian orchids with reference to taxonomy. *Bangladesh J. Bot.,* **21**: 65-72.

Devi, N. S., Y. Padma, C. L. Narasimhudu, and R. R. Venkata Raju. 2013. Diversity of stomata and trichomes in *Euphorbia* L. - I. *Bangladesh J. Plant Taxon.,* **20**: 27-38.

Dressler, R. L. 1993. *Phylogeny and Classification of the Orchid Family.* Cambridge University Press, Cambridge, UK.

Endress, P. K., P. Baas, and M. Gregory. 2000. Systematic plant morphology and anatomy - 50 Years of Progress. *Taxon,* **49**: 401-34.

Govaerts, R., P. Bernet, K. Kratochvil, G. Gerlach, G. Carr, P. Alrich, A. M. Pridgeon, J. Pfahl, M. A. Campacci, D. H. Baptista, H. Tigges, J. Shaw, P. J. Cribb, A. George, K. Kreuz, and J. J. Wood. 2012. *World Checklist of Orchidaceae.* The Royal Botanic Gardens, Kew, UK.

Govaerts, R., P. Bernet, K. Kratochvil, G. Gerlach, G. Carr, P. Alrich, A. M. Pridgeon, J. Pfahl, M. A. Campacci, D. H. Baptista, H. Tigges, J. Shaw, P. J. Cribb, A. George, K. Kreuz, and J. J. Wood. 2018. World Checklist of Orchidaceae. The Royal Botanic Gardens, Kew, UK.

Inamdar, J. A. 1968. Stomatal ontogeny in *Habenaria marginata* Coleb. *Curr. Sci.,* **37**: 24–25.

Jalal, J. S. and J. Jayanthi. 2015. An annotated checklist of the orchids of Western Himalaya, India. *Lankesteriana,* **15** (1): 7-50.

Karatela, Y. Y. and L. S. Gill. 1986. Observations on the developmental studies of stomatal differentiation in the epidermis of Solanaceae. *Feddes Repert.,* **97**: 303–11.

Kaushik, P. 1983. *Ecological and Anatomical Marvels of the Himalayan Orchids*. Today and Tomorrow's Printers and Publishers, New Delhi, India.

Khasim, S. M. and P. R. Mohana Rao. 1986. Anatomical studies in relation to habitat tolerance in some epiphytic orchids. *In: Biology, Conservation, and Culture of Orchids* (ed. S. P. Vij) pp. 49-57. Affiliated East West Press, New Delhi, India.

Khasim, S. M. and P. R. Mohana Rao. 1990. Anatomy in relation to taxonomy in some members of Epidendroideae (Orchidaceae). *Phytomorphology,* **40**: 243-50.

King, G. and R. Pantling. 1898. The Orchids of Sikkim Himalaya. *Ann. Roy. Bot. Gard. Calcutta,* **8**: 1-342.

Kowsalya, A., K. Rojamala, and T. Muthukumar. 2017. Comparative Vegetative Anatomy of South Indian Vandas (Orchidaceae). *Flora,* **235**: 59-75.

Leitao, C. A. E., M. A. H. Dolder, and A. L. Cortelazzo. 2014. Anatomy and histochemistry of the nectaries of *Rodriguezia venusta* (Lindl.) Rchb. f. (Orchidaceae). *Flora,* **209**: 233-43.

Mehra, P. N. 1989. *Ecology, Structural Adaptations and Cultural Aspects of Some Himalayan Orchids*. P. N. Mehra, Chandigarh, India.

Mobius, M. 1887. Uber den anatomischen bau der orchideen blatter und dessen bedeutung fur das system dieser familia. *Jahrb. Wiss. Bot.,* **18**: 530-607.

Mohana Rao, P. R. and S. M. Khasim. 1986. Leaf anatomy of three species of *Bulbophyllum* (Orchidaceae) with comments on their ecological adaptability and taxonomy. *Proc. Indian Acad. Sci. (Plant Sci.),* **91**: 391-97.

Mohana Rao, P. R. and S. M. Khasim. 1987. Evolutionary trends in growth habit and vegetative anatomy of Indian orchids. *J. Orchid Soc. India,* **1**: 57-70.

Moreira, A. S. F. P., J. P. de Filho, and R. M. dos Isaias. 2013. Structural adaptations of two sympatric epiphytic orchids (Orchidaceae) to a cloudy forest environment in rocky outcrops of Southeast Brazil. *Rev. Biol. Trop.,* **61**: 1053-65.

Ogundipe, O. T. and O. Akinrinlade. 1998. Epidermal micromorphology of some species of *Albizia* Durazz (Mimosaceae). *Phytomorphology,* **48**: 325-33.

Paek, K. Y. and E. S. Jun. 1995. Stomatal density, size and morphological characteristics in orchids. *J. Korean Soc. Hort. Sci.,* **36**: 851-62.

Parkhurst, D. F. 1978. The adaptive significance of stomatal occurrence on one or both surfaces of leaf. *J. Ecol.,* **66**: 367-83.

Prashantha Kumar, H. G. and K. Krishnaswamy. 2011. A study of phenology and stomatal complex of certain epiphytic orchids of Western Ghats of Karnataka, India. *Res. Rev. Biomed. Biotech.,* **2**: 21-29.

Prashanta Kumar, H. G. and K. Krishnaswamy. 2014. Preliminary leaf epidermal studies in a few epiphytic orchids. *J. Orchid Soc. India,* **28**: 91-97.

Ramudu, J., G. Ramesh, and S. M. Khasim. 2012. Molecular and morphological studies in *Coelogyne nervosa* A. Rich., an endemic orchid from southern India. *J. Orchid Soc. India,* **26**: 53-58.

Rasmussen, H. 1981. The diversity of stomatal development in Orchidaceae subfamily Orchidoideae. *Bot. J. Linn. Soc.,* **82**: 381-93.

Rasmussen, H. 1986. An aspect of orchid anatomy and adaptationism. *Lindleyana,* **1**: 102-07.

Rasmussen, H. 1987. Orchid stomata structure, differentiation, function and phylogeny. *In: Orchid Biology Reviews and Perspectives* Vol. IV (ed. J. Arditti) pp. 105-38. Cornell University Press, Ithaca, New York.

Rosso, S. W. 1966. The vegetative anatomy of Cypripedioideae (Orchidaceae). *Bot. J. Linn. Soc.,* **59**: 309-41.

Sanford, W. W. 1974. The ecology of orchids. *In: The Orchids Scientific Studies* (ed. C. L. Withner) pp. 1-67. John Wiley and Sons, New York.

Sevgi, E., E. Altundag, O. Kara, O. Sevgi, H. B. Tecimen, and I. Bolat. 2012a. Morphological, anatomical and ecological studies on some *Orchis* (Orchidaceae) taxa of Mediterranean region, *Turk. J. Environ. Biol.,* **33**: 343-53.

Sevgi, E., E. Altundag, O. Kara, O. Sevgi, H. B. Tecimen, and I. Bolat. 2012b. Studies on the morphology, anatomy and ecology of *Anacamptis pyramidalis* (L.) L. C. M. Richard (Orchidaceae) in Turkey. *Pak. J. Bot.,* **44**: 135-41.

Shakya, L. R. 1999. *Revision of the genus Oberonia Lindl. (Orchidaceae) in Himalayas*. Ph.D. Thesis, Tribhuvan University, Kathmandu, Nepal.

Singh, D. K. 2001. Orchid diversity in India. *In: Orchids: Science and Commerce* (eds. Promila Pathak, R. N. Sehgal, N. Shekhar, M. Sharma, and A. Sood) pp. 35-65. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Singh, H. 1981. Development and organization of stomata in Orchidaceae. *Acta Bot. Indica,* **9**: 94-100.

Singh, V. and H. Singh. 1974. Organization of the stomatal complex in some Orchidaceae. *Curr. Sci.,* **43**: 490-91.

Solereder, H. 1908. *Systematic Anatomy of Dicotyledons* Vol. 2. Clarendon Press, Oxford, UK.

Solereder, H. and J. F. Meyer. 1930. Systematische Anatomie der Monokotyledonen, Heft 6: Scitamineae-Microspermae. Gebruder Borntrager, Berlin.

Stace, C. A. 1980. *Plant Taxonomy and Biosystematics*. Edward Arnold Publishers, London, UK.

Stebbins, G. L. and G. S. Khush. 1961. Variations in organization of the stomatal complex in the leaf epidermis of the monocotyledons and its bearing on their phylogeny. *Am. J. Bot.,* **48**: 51-59.

Stern, W. L. 1997. Vegetative anatomy of subtribe Habenariinae (Orchidaceae). *Bot. J. Linn. Soc.,* **125**: 211-27.

Stern, W. L. and W. S. Judd. 2000. Comparative vegetative anatomy and systematics of orchid tribe Vanilleae excluding *Vanilla. Bot. J. Linn. Soc.,* **134**: 179-202.

Stpiczyñska, M. and K. L. Davies. 2009. Floral, resin-secreting trichomes in *Maxillaria dichroma* Rolfe (Orchidaceae: Maxillariinae). *Acta Agrobot.,* **62**: 43-51.

Timonin, A. K. 1986. Taxonomic value of stomatal characteristics and the evolution of stomatal complexes in flowering plants. *Byull Mosk O-va Ispyt Prir Old Biol.,* **91**: 72-81.

Tomlinson, B. B. 1974. Development of the stomatal complex as a taxonomic character in the monocotyledons. *Taxon,* **23**: 109-28.

Vij, S. P., P. S. Kaushal, and P. Kaur. 1991. Observations on leaf epidermal features in some Indian orchids: Taxonomic and ecological implications. *J. Orchid Soc. India,* **5**: 43-53.

Vij, S. P., J. Verma, and C. Sathish Kumar. 2013. *Orchids of Himachal Pradesh*. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Wagner, G. J. 1991. Secreting glandular trichomes: more than just hairs. *Plant Physiol.,* **96**: 675-79.

Williams, N. H. 1975. Stomatal development in *Ludisia discolor* (Orchidaceae): Mesoperigenous subsidiary cells in the Monocotyledons. *Taxon,* **24**: 281-88.

Williams, N. H. 1976. Subsidiary cell development in Catasetinae (Orchidaceae) and related groups. *Bot. J. Linn. Soc.,* **72**: 299-309.

Williams, N. H. 1979. Subsidiary cells in Orchidaceae: Their general distribution with special reference to development in Oncidieae. *Bot. J. Linn. Soc.*, **78**: 41-66.

Withner, C. L., P. K. Nelson, and P. J. Wejksonra. 1974. The anatomy of orchids. *In: The Orchids Scientific Studies* (ed. C. L. Withner) pp. 267-347. John Wiley and Sons, New York, USA.

Yu, Z. M., Y. Hong, D. C. Xi, M. Jun, and Z. S. Gang. 2007. Leaf epidermic characters of *Dendrobium* (Orchidaceae) and its taxonomic significance. *Southwest China J. Agric. Sci.,* **20**: 716-20.

Zanenga-Godoy, R. and C. G. Costa. 2003. Anatomia foliar de quatro espécies do gênero *Cattleya* Lindl. (Orchidaceae) do Planalto Central Brasileiro. *Acta Bot. Brasilica,* **17**: 101-18.

Ziegenspeck, H. 1936. Orchidaceae. *In: Lebensgeschichte der Blutenpflanzen Mitteleuropaspp* (eds. O. Kirchner, E. Loew, and C. Schaoter) pp. 1-740. Eugen Ulmer, Stuttgart, Germany.