



Received on 18 May 2018; received in revised form, 26 June 2018; accepted, 06 July 2018; published 01 January 2019

MORPHOLOGICAL AND ANATOMICAL VARIATIONS OF THE SELECTED SPECIES OF *OSBECKIA* L. (MELASTOMATACEAE)

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Keywords:

Osbeckia, Morphological, Anatomical, Habitat, Herkogamy

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ABSTRACT: *Osbeckia* L. of Melastomataceae are unique flowering plants showing diversity in their habit and morphological features adapted to their habitats. Limited studies were recorded with the anatomy and morphology of high elevation species. The micromorphology and anatomy of vegetative and reproductive structures of seven species and three varieties of *O. aspera*, representing diverse habitats such as altitude, availability of water and intensity of sunlight out of the twenty species of *Osbeckia* reported from India were narrated in the present study. An apparent type of herkogamy noticed in *Osbeckia* species was also recorded. Herkogamy in this family is an important feature of phylogenetic importance. This aspect of the flowers of *Osbeckia* appears to represent a special type of herkogamy. The most evident type of herkogamy present in Melastomataceae was represented by flowers that have styles exerted beyond the stamens. Styles and stamens that were about the same length but bent opposite from each other were noticed in some species. Based on the comparison of herkogamy in flowers of many species, it may be concluded that this type of herkogamy was unique in *Osbeckia* sp. only. The present results showed wide variations among the macromorphological features among the species. Similarly, the stomatal index showed a variation from 16.46 to 36.76 (abaxial side) and 3.98 to 32.19 (adaxial side). Stomata were diacytic to anomocytic type. Epidermal hairs also showed diversity. Further studies are planned for the electron microscopic analysis of pollen, stomata and trichome nature.

INTRODUCTION: Melastomataceae comprises about 4500 species which were diverse concerning their distribution, habit, and morphology. They occupy a variety of forests, and open vegetation types including lowland savannas, meadows and grasslands, vertical land cuttings, and some species were noticed in seasonally flooded habitats. The habit of the species varies from small annual herbs to medium-sized trees, although the majority of the species were shrubs or small trees.

The *Osbeckia* of Melastomataceae were characterized by the urceolate or subglobose calyx-tube with bulbous-based bristles and anthers which are oblong, truncate, attenuate or beaked. *Osbeckia* species also show much diversity in their habit and adaptive morphological structures developed concerning their habitats.

Few studies have accounted at the anatomy and morphology of high elevation species of the family, and none of the species of *Osbeckia* were traced in such studies. The plants are growing at high elevations, have adaptations for withstanding harsh climatic conditions such as increased solar radiation, high winds, and high ground temperature¹. The objective of this study was to describe the micromorphology and anatomy of vegetative and

	DOI: 10.13040/IJPSR.0975-8232.10(1).320-28
	The article can be accessed online on www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(1).320-28	

reproductive structures of seven species, and three varieties of *O. aspera*, representing different habitats like altitude, availability of water and intensity of sunlight among the twenty species of *Osbeckia* reported from India.

A study on the apparent type of herkogamy present in *Osbeckia* was also aimed. Herkogamy is the spatial separation of stamens and stigmas usually within flowers². In the Melastomataceae, herkogamy is supposed to avoid selfing and promote outcrossing³. Herkogamy in this family is an important phylogenetic feature⁴. Plant responses to environmental variations involve complex interactions of many morphological, anatomical, or physiological characteristics, which are responsible for adaptive success to a particular environment or habitat.

The morphological and anatomical identification of plant fragment when present in a crude or ethnic medicinal mixture was also targeted towards the improvement of this important medicinal plant⁵. Any effort on genetic improvement also begins with an assessment of morphological, anatomical and genetic variability in the local germplasm so that breeding strategies can be mapped out.

MATERIALS AND METHODS:

Study Area: Plants were collected from the different parts of Punmudi hills of Thiruvananthapuram district; Illikkal kallu and

Wagomon of Kottayam district; Padappakra in Kollam district; Munnar and Rajamala of Idukki district and Meppadi to Chembra peak region of Wayanad district of Kerala, India. The specimens were fixed in FAA in the field itself. Micromorphology analysis was carried out using simple light microscopy on leaves, hypanthia, ovaries, petals, stamens, and stigmas of all the species and varieties. Images were taken with a Nikon D5300 camera using 40 mm macro lens. For light microscopy of flowers and leaves, the samples were hand sectioned, viewed with Leica compound microscope and digitally photographed with a Sony digital camera. The herbarium was deposited to the University college herbarium, Trivandrum with serial numbers UCB 1212 UCB1220.

The stomatal density was determined as the number of stomata per square millimeter of the leaf. The index was determined as the number of stomata per square millimeter divided by the number of stomata plus number of epidermal cells per square millimeter multiplied by 100. The lengths of stomata were measured to determine the stomatal size. The stomatal index (SI) was intended using the equation described by Salisbury⁶, that is:

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

Where, S = the number of stomata per unit area and E = the number of epidermal cells in the same unit area.

TABLE 1: SPECIES OF OSBECKIA SELECTED FOR THE STUDY

S. no.	Name	Habitat	Altitude (feet)
1	<i>Osbeckia aspera</i> (L.) var. <i>aspera</i>	Tropical low land, evergreen forest and plains	400-700
2	<i>Osbeckia aspera</i> (L.) var. <i>wightiana</i>	Evergreen and moist deciduous forests and grasslands	3400-4000
3	<i>Osbeckia aspera</i> (L.) var. <i>travancorica</i>	Midland meadows	3000
4	<i>Osbeckia reticulata</i> Bedd.	High altitude grasslands	4000
5	<i>Osbeckia gracilis</i> Bedd.	Moist deciduous and shola forests	2100
6	<i>Osbeckia virgata</i> D. Don ex Wight & Arn.	Evergreen and moist deciduous forests, plains	400-700
7	<i>Osbeckia leschenaultiana</i> DC.	High altitude shola forests	3500
8	<i>Osbeckia wynaadensis</i> Clarke	Waterlogged semi-evergreen and evergreen forests	2100-4000
9	<i>Osbeckia parvifolia</i> Arn. Wight & Arn.	Mid land, Grass lands	3000

RESULTS AND DISCUSSION:

1. *Osbeckia gracilis* Bedd:^{7, 8} The plants were stout shrubs; branches glaucous and glabrous. Leaves were 3.5 × 2 cm, elliptic-oblong, obtuse at apex and base, cuspidate, yellowish green, densely covered with half-adnate hairs spreading from midrib **Fig. 2f**, 3-ribbed; petiole 3 mm long. Stomata were paracytic with two parallel and cap like subsidiary cells **Fig. 3f**.

Pedicelled flowers in terminal cymes, calyx tube 7 mm long, densely simple-hairy, lobes 4 × 3 mm, ovate-oblong, obtuse, ciliate; petals 15 × 12 mm, obovate, ciliate, pink; anthers 5 mm long.

2. *Osbeckia wynaadensis* Clarke:^{7, 8} Tall shrubs; branches densely scabrid. Leaves 18 × 3 cm, lanceolate, acute, 3 - 5 nerved, hairy; hairs adnate and spreading from the midrib. Stomata were with

four subsidiary cells. Flowers many, in terminal paniced cymes; pedicels 4 mm long; calyx tube 8 mm long, bristles 4 or 5 in a tuft, to 3 mm long; lobes 11×5 mm, lanceolate, ciliate; petals 20×10 mm, obovate, anthers 7 mm long. Seeds 0.5×0.3 mm obovoid, minutely muriculate **Fig. 2g**.

3. *Osbeckia leschenaultiana* DC: *Osbeckia leschenaultiana* DC.^{7, 8} were shrubs; branchlets terete, corrugate, young parts with rufous hairs. Internodes short. Leaves 2.5×1.5 cm, elliptic-ovate, acute at apex, base rounded, densely pubescent, 3-5 nerved; petiole to 3 mm. Flowers terminal, solitary or capitate, pink; calyx cup with compound rufous hairs, stalked hairs; petals to 2.5 cm, obovate; stamens 10, filaments 6 mm, anthers 6 mm, twisted.

4. *Osbeckia aspera* L.: *Osbeckia aspera* L. was the most extensively explored species of *Osbeckia*. Many reports were documented regarding their anti-oxidant, anti-diabetic anti-inflammatory and hepatoprotective effects⁹. Three different varieties were collected viz, *Osbeckia aspera*, *O. aspera* var. *aspera*, *O. aspera* var. *travancorica* Bedd ex

Gamble and *O. aspera* L. var. *wightiana* Benth. ex Wight & Arn.

4a. *O. aspera* L. var. *aspera*: Plants were shrubs growing erect; hairs were seen on the branches which were usually appressed to the stem or spreading. Leaves simple and arranged opposite, and size varies from 3.5 cm to 9 cm in length and 1.5 to 3.2 cm in breadth. Leaves were elliptic to lanceolate, acute or shortly acuminate at apex and base attenuate and five ribs were seen towards the base. On both sides of the leaves, short hairs were seen **Fig. 4a**. When dry, the leaves turned yellowish-green. Petiole was 1 cm long. Stomata were anomocytic **Fig. 3a, b**. Flowers were about 2 cm in diameter and pentamerous in terminal cymes or sometimes in elongated clusters. Calyx tube 6-8 mm long, ca 5 mm wide, cupular, with dense short bristle-like hairs; lobes 5, oblong, obtuse. Petals 5, pink, $1-1.5 \times 0.8-1$ cm, ovate, apex rounded. Stamens 10; anthers 5-6 mm long. Ovary hairy at apex; style exserted, 1.25-1.5 cm long; stigma curved, papillate. Capsules $0.8-1 \times 0.6-0.7$ cm, ovoid, ribbed; seeds many **Fig. 1c**.



FIG. 1: A- HABIT OF *OSBECKIA ASPERA* VAR. *ASPERA*, B- HERCOGAMY IN *O. ASPERA* VAR. *ASPERA*, POLLINATOR USING THE STYLE TO HOLD ON TO THE FLOWER; C- DRY FRUITS OF *O. ASPERA* VAR. *ASPERA*; D- HERCOGAMY AND HABIT OF *O. VIRGATA*, E AND F- TRICHOMES OF THE YOUNG AND MATURE FRUITS OF *O. VIRGATA*

4b. *O. aspera* L. var. *travancorica* (Bedd ex Gamble): Leaves of *O. aspera* var. *travancorica* were 15×5 cm, ovate-oblong, acuminate, 7-ribbed,

covered with adpressed hairs above and below; petiole 1 cm long. Mineral deposits were seen on the ventral epidermis of the leaves Flowers 6 cm

across, 5-15 together in terminal cymes, bracts 1 cm across, orbicular; calyx cup 13 mm long, densely covered with stalked bristles, lobes 7×4 mm, obovate, emarginated, bristled; intersepalar emergences with stellate bristles; petals 3.5×2.5 cm, obovate, pink; filaments 13 mm long, anthers 9 mm long, acuminate, constricted at base **Fig. 2h**.

4c. *Osbeckia aspera* (L.) var. *wightiana* (Benth. ex Wight & Arn.): Shrubs; stem 4-ribbed leaves

were $9-10 \times 3-4$ cm, ovate, rounded at base, acute at apex, 5-ribbed, densely hairy; hairs half adnate above and below **Fig. 10**; petiole 1 cm long. Flowers 3-5 together in terminal sessile clusters; bracts densely rufous hairy; calyx tube 8 mm long, densely tufted hairy, hairs rufous brown; lobes 3×2 mm, bristled at tip; anthers 9 mm long, acuminate **Fig. 2e**.

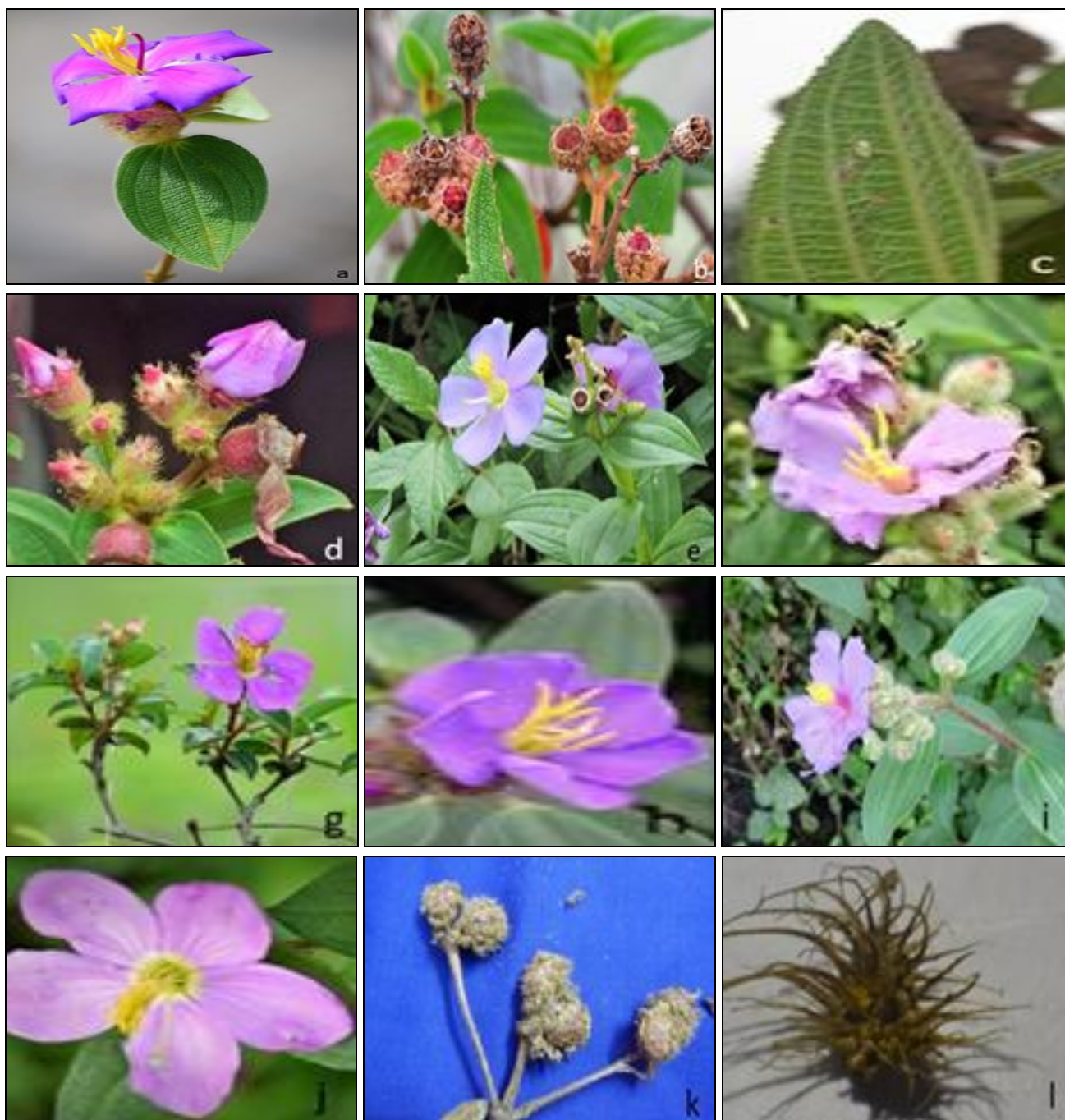


FIG. 2: A- *O. RETICULATE* FLOWER; B- HABIT; C- YOUNG AND MATURE FRUITS WITH DENSE TRICHOMES; D- ABAXIAL SURFACE OF LEAF; E- *O. ASPERA* VAR. *WIGHTIANA*; F- *O. GRACILIS*; G- *O. WYNAADENSIS*; H- *O. ASPERA* VAR. *TRAVANCORICA*, I, J- *O. LESCHENAUTIANA*; K- *O. PARVIFOLIA*; L- DRY FRUITS OF *O. ASPERA* VAR. *WIGHTIANA*; P- A SINGLE TRICHOME COMPLEX

5. *Osbeckia reticulata* Bedd.: The plants were shrubs; branchlets rusty. Leaves were 5-8 × 2.5-4.5 cm in size, coriaceous, strigose, dark green above, paler below, 5-7-nerved, base obtuse, margin serrulate, apex acute; petiole 1 cm. Dense hairs on both the surfaces **Fig. 4c-f**. Stomata were diacytic **Fig. 3c, d** Cymes terminal, to 6(8) cm, 4-6 flowered; peduncle 0. Flowers 5 merous with 6 cm wide. Hypanthium urceolate, 1.5 × 1.4 cm; intersepalal emergences stalked, cupular, 7 × 3 mm; lobes 5, oblong, 9 × 4 mm; bristle hairs both simple and stellate. Petals 5, purple-rose, orbicular, 4 × 3 cm. Stamens 10, to 2 cm; filaments 1 cm; anthers equal to, or slightly longer than, filaments, oblong, slightly beaked. Ovary 5-celled, 10-lobed; style 2.5 cm. Capsule urceolate, 1.8 × 1.8 cm; seeds numerous **Fig. 2a, d**.

6. *Osbeckia virgata* D. Don ex Wight & Arn.: *Osbeckia virgata* was a small shrub with leaves measuring 1.5-3.3 × 0.5 - 1.8 cm, elliptic to linear-lanceolate, base attenuate, apex acute, hairy below

on nerves, prominently 3-ribbed; petiole up to 5 mm long. Hairs on the leaves are very short. Flowers in terminal few-flowered clusters, pentamerous, c. 2 cm across. Calyx tube 3-4 mm long, subglobose with stalked stellate and simple bristles; lobes 5, lanceolate, acute. Petals pink, 6-7 × 3-4 mm, obovate. Stamens 10; anthers 4-5 mm long. Ovary 5-locular, with stiff tufted hairs **Fig. 2f**.

7. *Osbeckia parvifolia* Arn. Wight & Arn.: *Osbeckia parvifolia* Arn. Wight & Arn. Syn. *O. cupularis* D. Don ex Mal: Cherukadali, a small herb distributed along the Southern montane wet scrub and Southern montane wet grasslands. Commonly grow in marshy areas; favors sandy loam soils, medium acidic low in potash and phosphate and high in organic carbon. Leaves were simple, opposite ovate or elliptic-ovate, acute, 3-ribbed, up to 4 × 2 cm. Flowers white or pink in capitate heads. Fruit ovate-oblong, obscurely ribbed **Fig. 2k**.

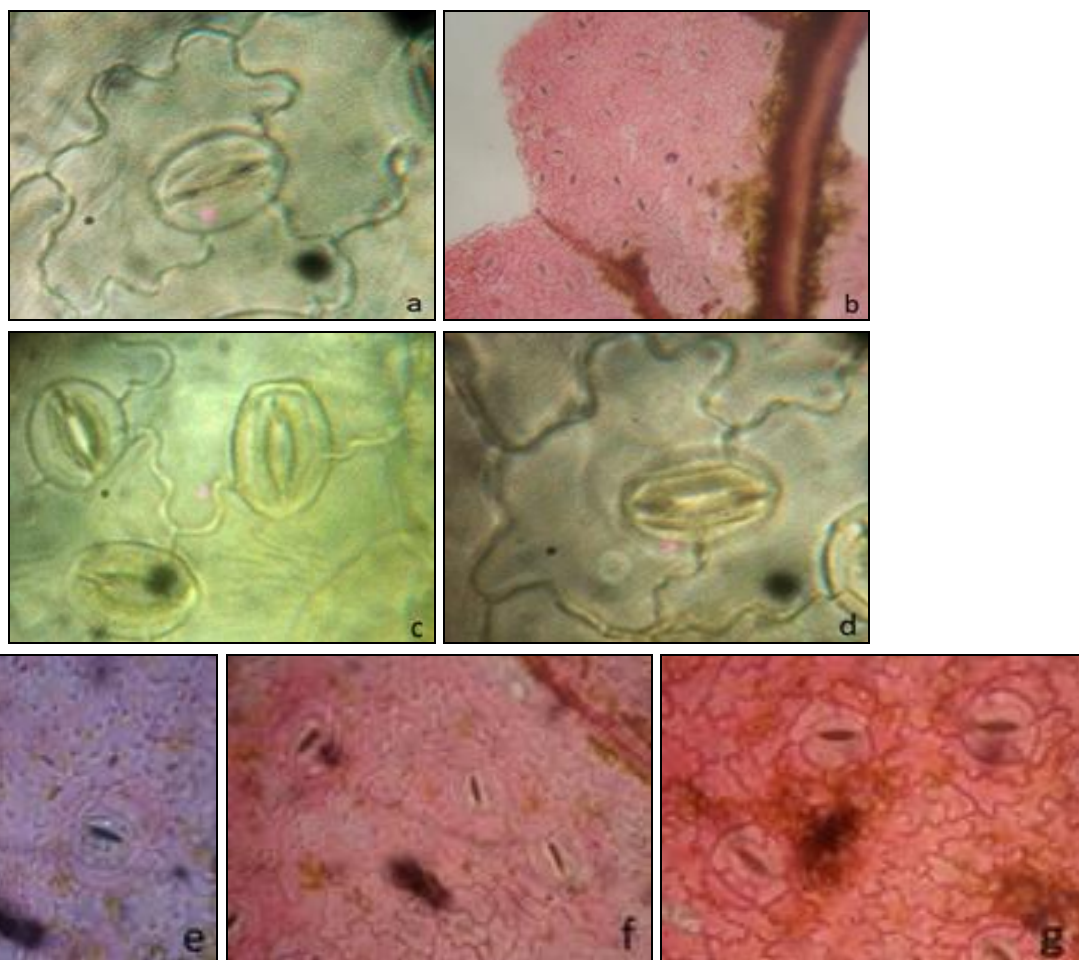


FIG. 3: A, B- ANOMOCYTIC STOMATA OF *O. ASPERA* VAR. *ASPERA*; C, D- DIACYTIC STOMATA OF *O. RETICULATE*; E- STOMATA OF *O. GRACILIS*; F- STOMATA OF *O. LESCHENAULTIANA*; G- STOMATA OF *O. WYNAADENSIS*

TABLE 2: STOMATAL INDEX

S. no.	Name of the species	Stomatal Index	
		Abaxial Surface	Adaxial Surface
1	<i>Osbeckia gracilis</i>	27.23	6.01
2	<i>Osbeckia wynaadensis</i>	23.54	4.82
3	<i>Osbeckia leschenaultiana</i>	27.55	9.31
4a	<i>O. aspera</i> var. <i>aspera</i>	34.23	14.23
4b	<i>O. aspera</i> var. <i>travancorica</i>	36.76	11.79
4c	<i>Osbeckia aspera</i> var. <i>wightiana</i>	44.02	23.86
5	<i>Osbeckia reticulata</i>	47.83	32.19
6	<i>Osbeckia virgata</i>	16.46	8.40
7	<i>Osbeckia parvifolia</i>	21.94	3.98

The maximum number of stomata was found in *O. reticulata* with a stomatal index of 47.83 and minimum was recorded in *Osbeckia virgata*, i.e. 16.46 **Table 2**. Stomata were minute and varied from diacytic to anomocytic **Fig. 3a-f**. Transverse sections of the leaves of *Osbeckia aspera* varieties show a thick cuticle, above the one cell thickened

epidermis. The epidermis also covers the leaf margins and finishes of the abaxial leaf surface. The epidermis on the abaxial surface is much thinner than that of the adaxial surface. Whereas in *O. virgata*, both the epidermis were thickened equally. Glands were common on both the epidermis of all the species **Fig. 4, a-b**. In *O. reticulata* basal globular glands were seen on the abaxial surface and in *Osbeckia aspera* var. *aspera* glands were seen serially on the lower portion of the glandular trichome. The hypodermis layer was absent. The palisade layer consists of a thin compact, elongated cells deposited with druse crystals in a scattered pattern **Fig. 5a-c** which make the adaxial surface rough. Transverse sections of the leaves of *O. wynaadensis* confirmed with the deeply sunken primary and secondary veins. Also, a thin cuticle, as well as one cell layer thickened epidermis. The glandular trichomes of the *O. virgata* were small with a globular gland at the base **Fig. 4b**.

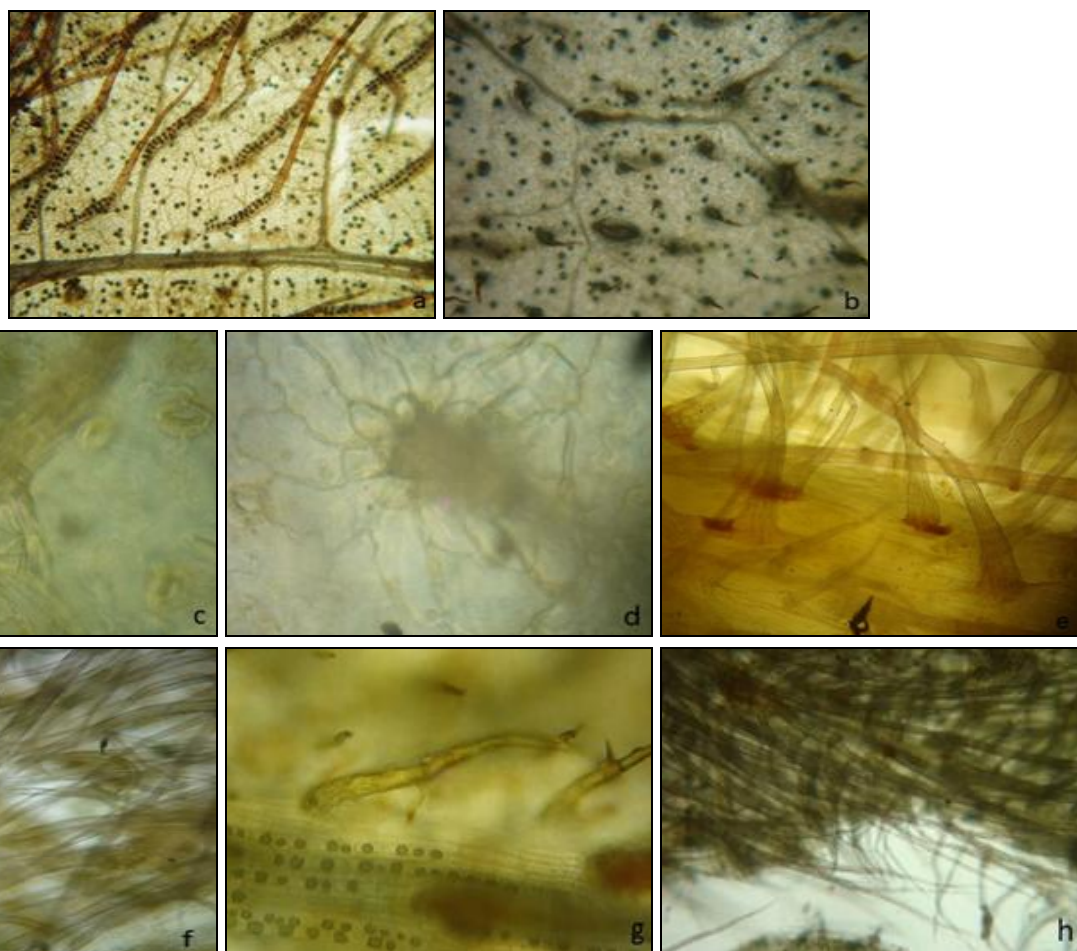


FIG. 4A- GLANDULAR TRICHOMES ON THE DORSAL SURFACE OF *OSBECKIA ASPERA* VAR. *ASPERA*; **B-** SMALL GLANDULAR HAIRS OF *O. VIRGATA*; **C, D-** GLANDULAR BASE OF THE LONG TRICHOME ON VENTRAL SURFACE *O. RETICULATE*; **E, F-** LONG NON-GLANDULAR TRICHOMES OF *O. RETICULATE*; **G-** NON GLANDULAR TRICHOME OF *O. LESCHENAULTIANA*; **H-** DENSE NON GLANDULAR HAIRS OF *OSBECKIA ASPERA* VAR. *WIGHTIANA*

The hypanthium was glabrous in *O. aspera* except with the scattered inconspicuous glands on the surface, and the calyx lobes, which were ciliate. Between each pair of calyx lobes, there is was large seta. The apex of the ovary was pubescent with simple trichomes, and the style was surrounded by larger, erect, simple trichomes **Fig. 1, c, f**.

The petals were violet to purple, ovate and with an acuminate apex, and their surface consists of elevated cells, which were laterally contracted **Fig. 2a**. The stamens were totally yellow, isomorphic and lack appendages on the filament connective junction **Fig. 2f- k**.

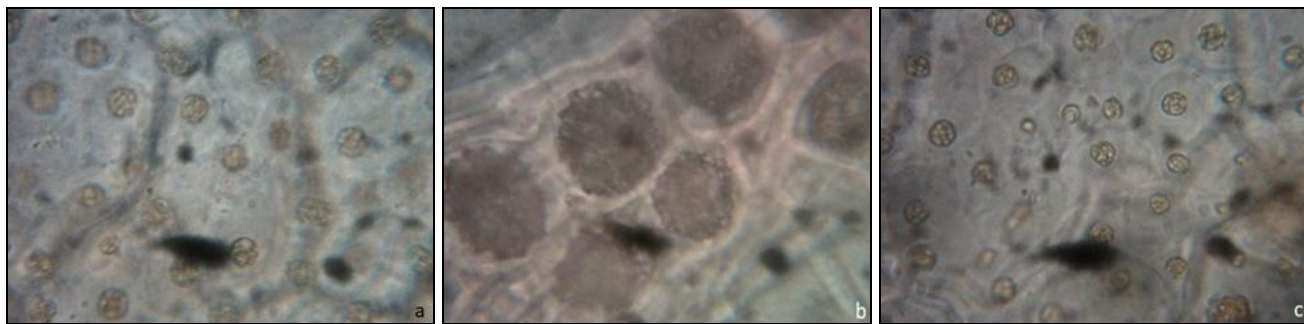


FIG. 5: A- MINERAL DEPOSITS ON THE VENTRAL SURFACE OF THE LEAF FROM *O. ASPERA* VAR. TRAVANCORICA; B- SESSILE GLANDS ON THE ADAXIAL SURFACE OF *O. RETICULATE*; C- CRYSTALS ON THE SURFACE OF *O. VIRGATA*

Amphistomatic leaves were not common among Melastomataceous species and were absent in the closely related Melastoma. Presence of stomata on both surfaces of leaves in *Osbeckia* was documented for the first time. Stomatic index was high on both the surfaces in the high altitude species such as *O. aspera* var. wightiana and *O. reticulata*. The adaptive significance of amphistomatic leaves has been suggested as to maximum leaf conductance to CO₂, and their occurrence has been linked to plants adapted in full-sun environments, and experiencing rapidly fluctuating or continuously available soil water as opposed to seasonal or long-term soil water reduction¹⁰. Amphistome was a derived feature within angiosperms, and the same was likely to be the case in Melastomataceae. An additional unusual aspect of the leaves of *O. reticulata* was the sessile glands on the adaxial leaf surface.

Alchemilla procumbens, *Geranium seemannii*, *Acaena elongata*, *Lupinus montanus*, and *Symphoricarpos microphyllus*, distributed along an altitudinal gradient in the Sierra Nevada of central Mexico, were evaluated concerning their morpho-anatomical modifications vs. elevation and other physic-chemical features. Interestingly, the elevation was related to anatomical changes in the leaf and wood of the species. Canopy density and potassium content in the soil also contributed to explaining the variation in anatomical features¹¹.

Anatomical, morphological, and architectural characters for selected plant species of Madagascar was statistically compared by Azizan *et al.*, (2017)¹². Barthélémey & Caraglio (2007) applied a multilevel and comprehensive mode to plant form, structure, and ontogeny of species¹³. Castellanos, Kolterman, and Vester (2011) analyzed *Buxus vahlii* in two different environments in Puerto Rico¹⁴. Isnard *et al.*, (2012) compared growth form evolution in Piperales and its relevance for understanding angiosperm diversification: an integrative approach by architecture, anatomy, and biomechanics¹⁵. Abubakar, Mua 'zu, Khan, & Adamu, (2011) analyzed morpho-anatomical variation in *Moringa oleifera* accessions from Northern Nigeria¹⁶.

Serebryanaya and Shipunov, (2009) suggested that changes in plant morphological features have resulted in small-scale evolutionary events in different species of plants¹⁷. Xu, Guo, Xu, and Wang, (2008), analyzed leaf size variation forms a basis for evolutionary changes in plants and also leads to modifications of the distribution of leaf biomass between support and functional tissues¹⁸. Plants are generally adapted to high phenotypic plasticity, and therefore the observed differences regarding leaf characters being caused by the direct influence of environmental conditions. But, Widén and Schiemann, (2003) noticed that phenotypic plasticity from differences in morphological sizes

can coincide with genetic variation¹⁹. Gill and Nyawuame, (2008) established phylogenetic relationships between the bicarpillatae following the analysis of the stomatal complexes and its ontogeny²⁰. Similarly, study reports revealed that ecotypes could be differentiated based on epidermal characters due to the adaptation of the environment in which they grow. Mbagwu, Nwachukwu, and Ubochi, (2007) confirmed closer affinity among Citrus species based on leaf epidermal cell wall variations²¹. Sharma, Sehrawat, Singhrot, and Tele, (2010) compared morphological chemical characterization of *Psidium species*²².

Rosatto, Takahashi, Silva, and Franco, (2010) observed that, in leaves growing in the bright sun, the regulation of stomatal opening was the main limiting factor of CO₂ assimilation, emphasizing the role of stomatal conductance²³. Dahlgren, Eriksson, Bolmgren, Strindell, and Ehrlén, (2006) documented that the leaf area is a highly plastic characteristic that depends on environmental conditions, mainly light intensity²⁴. In *Aechmea distichantha* plants exposed to different environmental conditions seems to be a trade-off among photosynthetic leaf area for light capture and water use and mechanical support²⁵. Rodrigues Pereira *et al.*, (2013) compared leaf morpho-anatomical variations in *Billbergia elegant* and *Neoregelia mucugensis* exposed to low and high solar radiation²⁶. Thus, the overall study supports the variations noticed among the *Osbeckia species*, *i.e.*, the habitats of the plants play a role in their phenotypic evolution supported by their morpho-anatomical features.

Another aspect of the flowers of *Osbeckia* was there *i.e.*, stamens were bent away opposite from the style **Fig. 1b** and **d**. This aspect of the flowers of *Osbeckia* appears to represent a special type of herkogamy. The most evident type of herkogamy present in Melastomataceae was represented by flowers that have styles clearly exerted beyond the stamens. Styles and stamens that were about the same length but bent opposite from each other were noticed in *O. virgata*, *O. aspera*, and *O. leschenaultiana*. Based on the comparison of herkogamy in flowers of many species, it may be concluded that this type of herkogamy was unique in *Osbeckia sp.* only.

CONCLUSION: The micromorphology and anatomy of vegetative and reproductive structures of seven species and three varieties of *O. aspera* was carried out in the present study, and it reveals that the species were evolved to cope up with the habitat variations like altitude, availability of water and intensity of sunlight. The diversity of the genus *Osbeckia* was due to this adaptive morphological evolution concerning their habitats.

The anatomy and morphology of high elevation species were delimited them to a unique group as compared other species. The study on the herkogamy noticed in *Osbeckia* is also important in their phylogenetic context. Further studies on the phenology and reproductive behavior of all the species are warranted

ACKNOWLEDGEMENT: The authors acknowledge Kerala State Council for Science, Technology, and Environment (KSCSTE), Government of Kerala for funding the project and also UGC, Bangalore for providing FIP fellowship for doing Ph. D. (No.FIP/12th Plan/KLKE 028/ TF 10 dated 19/01/2016).

CONFLICT OF INTEREST: No conflict of interest among the authors.

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How to cite this article:

Lawrence B and Murugan K: Morphological and anatomical variations of the selected species of *Osbeckia* L. (Melastomataceae). *Int J Pharm Sci & Res* 2019; 10(1): 320-28. doi: 10.13040/IJPSR.0975-8232.10(1).320-28.

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