Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 07, pp.54432-54440, July, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

CUTICULAR FEATURES OF SELECTED SPECIES OF *AMMANNIA, ROTALA* AND *NESAEA* (LYTHRACEAE) IN SOUTH INDIA

***Lemiya, K. M. and Pradeep, A.K.**

Department of Botany, Interuniversity Centre for Plant Biotechnology, University of Calicut, Kerala-673 635, India

ARTICLE INFO

Article History:
Received 20th April, 2017
Received in revised form
06th May, 2017
Accepted 09th June, 2017
Published online 31st July, 2017

Key words:

Ammannia,
Leaf epidermis,
Lythraceae,
Nesaea,
Rotala,
stomata.

ABSTRACT

The circumscription and delimitation of *Ammannia* L., *Rotala* L. and *Nesaea* Comm. ex Kunth. have long been confused due to their similar vegetative morphology, floral and seed structure and shared habitats. The delimitation of these genera based on traditional morphological characters was found to be perplexing. Various characters have been used in describing and delimiting species of these three closely related genera, but little attention has been drawn to the leaf cuticular features and stomatal complex. Present study deals with both qualitative and quantitative characterization of stomata of four species of *Ammannia*, two species of *Nesaea* and thirteen species of *Rotala* from South India. Except some variations such as relative distribution percentage of stomata in abaxial and adaxial surface, other significant epidermal characteristics that could contribute to the taxonomy of these three genera have been revealed. Characters such as relative distribution percentage of stomatal index, stomatal and epidermal cell size, presence or absence of some unique type of stomata were observed to play a key role in species delimitation. Hypostomatic leaves were reported here for the first time for *R.occultiflora* and *R. densiflora.* A key for the identification of South Indian taxa based on cuticular features are also provided.

Citation: Lemiya, K. M. and Pradeep, A.K., 2017. "Cuticular features of selected species of *Ammannia, Rotala* and *Nesaea* (Lythraceae) in South India", *International Journal of Current Research*, 9, (07), xxxxxxxxxx.

INTRODUCTION

The loosestrife family, Lythraceae has received considerable scientific attention in both taxonomic and economic prospective since long. It comprises 32 genera and c. 600 species (Graham 2005) with a worldwide distribution. The members of this family show extreme variation in habit, ranging from tall trees and woody shrubs to small aquatic herbs. The relationships and generic limits of Ammannia L., Rotala L., Nesaea Comm. ex Kunth have long been poorly understood. Their fairly generalized floral morphology has resulted in confused inferences of relationships making generic delimitation, based on traditional morphological characters problematic. Many species of these genera are found to be endemic to South India. Apart from a few scattered papers (Prasad *et al.,* 2012; Sunil *et al.,* 2013; Prasad and Raveendran 2013a, 2013b; Gaikwad *et al.,* 2013; Ratheesh Narayanan *et al.,* 2014; Anto *et al.,* 2014; Lemiya and Pradeep 2015) dealing with the description of new taxa, there has been no significant studies on South Indian endemic species of Ammannia, Nesaea and Rotala. Various characters have been used in describing and delimiting species for these three closely related genera. However, no much attention has been drawn to leaf epidermal characters that are of taxonomic importance. It has been demonstrated that cuticular features offer valuable information on phytotaxonomy, particularly for plants that are difficult to classify or identify (Nishida *et al.,* 2016). Stomatogenesis has long been studied by morphologists, physiologists and taxonomists (Kazama and Mineyuki 1997; Rao and Ramayya 1977; Volponi 1999). Stomata are small openings, which are found in the epidermal layers of plants, allowing access to CO_2 and egress for water. These structures are surrounded by guard cells, which control the pore size. The first scientific studies about stomata were done by Stresburger (1886) followed by Vesque (1989) who recognized 4 broad categories of stomata based on the presence and arrangement of accessory cells as well as their mode of development. Generally there is diversity in stomatal types, even on the same surface of an organ, which can often limit the practice of using stomatal features as a taxonomic character (Pant and Kidwai 1964). Regardless of diversity, the most frequent stomata type can be used as taxonomic character (Gopal 1980). Metcalfe and Chalk (1979) reported more than 25 main types of stomata in dicots on the basis of arrangement of epidermal cells neighbouring the guard cells. However, Stace (1984) recognized 31 different types of stomata among angiosperms. But the present study is based on Prabhakar (2004) who described 11 stomatal types, on the basis of their structure rather than on its ontogenetic pathways.

****Corresponding author:*** **Lemiya, K. M.**
Department of Botany, Interuniversity Centre for Plant Biotechnology, University of Calicut, Kerala-673 635, India.

MATERIALS AND METHODS

Fresh materials of different species representing three genera of Lythraceae were collected from their natural habitats from different regions of South India. Both fresh and dried specimens were used for the study. Fresh leaves were taken from collected specimens representing 13 species of *Rotala*, four species of *Ammannia* and two species of *Nesaea.* Leaf samples from living plants were fixed immediately after collection in formalin-acetic acid-alcohol (FAA) solution for a minimum of 24 hours and then submerged and washed with distilled water for 2 hours. Leaves from herbarium specimens were fully rehydrated. Epidermal layers were isolated either by simple hand peeling or by maceration in 40% nitric acid and were stained in 1% aqueous solution of saffranin for about 3 to 5 minutes. Excess stain was rinsed off with distilled water. The specimen was then placed in droplets of glycerin on a glass slide and covered with a cover glass for microscopic study to determine the stomatal complex types, stomatal density, stomatal index, and stomatal size. All these reading were based on average obtained from observations of ten microscopic fields under Leica DM2500 Phase contrast microscope (40X). Photomicrographs of good preparations were taken at a magnification of ×400 objective. The length and breadth of epidermal cells and stomata apparatus were measured with micrometer eyepiece graticule. The number of stomata and epidermal cells were taken and recorded. Stomatal index was calculated by the formula described by Wilkinson (1979). Based on different qualitative epidermal characterestics, a key for the identification of South Indian species of *Ammannia*, *Nesaea* and *Rotala* were also constructed.

RESULTS

The major charactrestics of leaf epidermis of three genera are illustrated (Figure 1- 6) and summarized (Table. 1 and 2). The foliar epidermal characters such as type, number and size of stomata, shape and size of epidermal cells for each species of three genera are described below.

***Ammannia* L.**

***Ammannia baccifera* L.**

Amphistomatic; largely isotricytic, often tetracytic and occasionally staurocytic and diacytic on abaxial and largely isotricytic, often tetracytic and staurocyctic and occasionally anisotricyctic on adaxial surface. Stomatal size ranges from 485.1 to 706.4 μm^2 on abaxial surface and from 545.2 to 783.6 μm^2 on adaxial surface. Stomatal index is 19.5% on abaxial and 11.3% on adaxial surfaces. Epidermal cells polygonal or irregular, iso and anisodiametric on abaxial surface, 25−73μm long and 17− 46 μm wide; Polygonal, with 5−7 sides,iso or anisodiametric on adaxial surface, 38− 94μm long and 32−51μmwide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and straight to curvedon adaxial surface [Figure 1. (A. 1 and A. 2)].

***Ammannia baccifera* subsp. *aegyptiaca* (Willd.) Koehne**

Amphistomatic; largely isotricytic, often tetracytic and occasionally anisotricytic on both abaxial and adaxial surface. Stomatal size ranges fom 585.8 to 713.6 μm^2 on abaxial surface and from 803.9− 853.8 μm^2 on adaxial surface. Stomatal index is 14.3 % on abaxial and 10.3 % on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 22− 63 μm long and 16− 37 μm wide; Polygonal, iso or anisodiametric on adaxialsurface, 29−77 μm long and 20−47 μm wide. Anticlinal cell wall thin and wavy to sinuate on abaxial surface and thick, straight to curved on adaxial surface [Figure 1. (B1 and B2)].

***Ammannia multiflora* Roxb.**

Amphistomatic; largely isotricytic, often tetracytic and anomocytic and occasionally staurocytic and on abaxial and largely isotricytic, often tetracytic and anomocytic and occasionally staurocytic and anisotricytic on adaxial surface. Stomatal size ranges from 343.2 to 572.6 μm^2 on abaxial surface and from 544.8 to 790.2 μm^2 on adaxial surface. Stomatal index is 14.7% on abaxial and 10.3% on adaxial surfaces. Epidermal cells irregular, iso and anisodiametric on both abaxial and adaxial surfaces, 31−78μm long and 14−36 μm wide on abaxial and 34−97μm long and 19−56μm wide. Anticlinal cell wall thin and sinuate on both abaxial and adaxial surfaces [Figure 1. (C1 and C2)].

***Ammannia octandra* Cham. and Schltdl**

Amphistomatic; largely Isotricytic, often anomocytic and staurocytic, occasionally diacytic and anisotricytic on abaxial and largely isotricytic often tetracytic and occasionally staurocytic on adaxial surface. Stomatal size ranges from 334.8 to 504.9 μm^2 on abaxial surface and from 617.7 to 1175μm^2 on adaxial surface. Stomatal index is 20.4% on abaxial and 11.5% on adaxial surfaces. Epidermal cells polygonal or irregular , iso and anisodiametric on abaxial surface, 36−79 μm long and 17−35 μm wide; Polygonal, iso or anisodiametric on adaxial surface, 29−77μm long and 30− 63μm wide. Anticlinal cell wall, thick and wavy to sinuate on abaxial surface, thick and curved to wavy on adaxial surface [Figure 2 (D1 and D2)].

***Nesaea* Kunth**

***Nesaea brevipes* Koehne**

Amphistomatic; largely Isotricytic, often anomocytic and tetracytic, occasionally staurocytic and anisotricytic on abaxial surface and largely isotricytic and often tetracytic on adaxial surface. Stomatal size ranges from 494.8 to 1004.4 μm^2 on abaxial surface and from 309.4 to 405.9 μm^2 on adaxial surface. Stomatal index is 14.3% on abaxial and 15.7% on adaxial surfaces. Epidermal cells polygonal or coarsely irregular, iso and anisodiametric on both abaxial and adaxial surfaces, 31− 78μm long and 22− 42 μm wide on abaxial surface, 35− 105 μm long and 22− 42 μm wide on adaxial surface. Anticlinal cell wall thick and wavy to sinuate on both abaxial and adaxial surfaces [Figure 2 (E1 and E2)].

***Nesaea prostrata* (Dillwyn) Suresh**

Amphistomatic; largely Isotricytic, often tetracytic and occasionally staurocytic on both abaxial and adaxial surfaces. Stomatal size ranges from 258.7 to 464.5 μm^2 on abaxial surface and from 373.5 to 570.9 μm^2 on adaxial surface. Stomatal index is 10% on abaxial and 22.3 % on adaxial surfaces. Epidermal cells irregular, iso and anisodiametric on both abaxial and adaxial surfaces, 23− 73 μm long and 17−41μm wide on abaxial and 23−87 μm long and 15−34 μm wide on adaxial surfaces. Anticlinal cell wall thin and sinuate on both abaxial and adaxial surfaces [Figure 2 (F1 and F2)].

Table 1. Leaf epidermal characteristics on abaxial surface of *Ammannia, Nesaea* and *Rotala*

Sl No:	Taxa	Average stomatal size (μm²) ±SE	Average stomatal index (%) ±SE	Stomatal type	Average epidermal size (μm²) ±SE	Epidermal cell shape	Anticlinal cell wall type
1	*Ammannia baccifera* subsp. *baccifera*	571.3±15.41	19.5±0.047	ISO	1406±64.184	P	WS
2	*Ammannia baccifera* subsp. *aegyptiaca*	674±36.95	14.3±0.294	ISO	1042.5±38.93	P	WS
3	*Ammannia multiflora*	475±48.78	14.7±0.286	ISO	1196.3±120.15	I	Si
4	*Ammannia octandra*	397.8±34.34	20.4±2.205	ISO	1240±95.825	P	WS
5	*Nesaea brevipes*	693.5±79.12	14.3±0.726	ISO	1871.4±118.17	P	WS
6	*Nesaea prostrata*	384.4±49.88	10±0.471	ISO	1161.±81.43	I	Si
7	*Rotala densiflora*	558±31.68	21±1.699	ISO	1264.±289.185	P	WS
8	*Rotala rosea*	512.8±37.78	13.3±0.356	TET,ISO	805.1±73.783	P	WS
9	*Rotala fimbriata*	574.2±24.02	13.4±0.711	ISO	894.6±61.84	I	WS
10	*Rotala macrandra*	544±11.39	14.3±1.152	ISO	1092.2±31.092	P	WS
11	*Rotala indica*	326.7±15.41	24.3±1.901	ISO	854.4±67.57	P	WS
12	*Rotala rotundifolia*	606.5±17.75	9.5±1.042	ISO,TET	1030.7±50.42	P	WS
13	*Rotala ritchei*	247.7±3.62	8.6±1.451	TET,ISO	825.7±56.27	P	WS
14	*Rotala malampuzhensis*	512.6±5.93	19.5±0.787	TET,ISO	766.4±43.0	P	WS
15	*Rotala tulunadensis*	561.3±43.44	8.9±0.638	ISO,TET	1275.1±239.97	P	WS
16	*Rotala malabarica*	337.5±68.06	18.2±1.152	ISO,TET	581.4±139.94	P	CW
17	*Rotala occultiflora*	478.7±37.13	20.2±0.356	TET	703.1±62.972	P	WS
18	*Rotala mexicana*	478.7±9.94	10.8±0.748	TET	707.7±72.529	P	CW
19	*Rotala juniperina*	613.1±10.55	15.6±1.389	ISO	887.1±35.96	P	WS

Table 2. Leaf epidermal characteristics on adaxial surface of *Ammannia, Nesaea* and *Rotala*

	Taxa	Average stomatal size (μm²) ±SE	Average stomatal index (%) ±SE	Stomatal type	Average epidermal size (μm²) ±SE	Epidermal cell shape	Anticlinalcell wall type
1	*Ammannia baccifera* subsp. *baccifera*	635±51.07	11.3±0.51	ISO	2627±389.46	P	SC
2.	*Ammannia baccifera* subsp. *aegyptiaca*	840±8.74	10.3±0.72	ISO	1570±177.14	P	SC
3.	*Ammannia multiflora*	659.1±28.09	10.3±1.970	ISO	1872.6±409.5	I	Si
4.	*Ammannia octandra*	866±96.49	11.5±0.21	ISO	2345.2±445.9	P	CW
5	*Nesaea brevipes*	749±14.95	15.7±0.54	ISO	1663.2±76.18	P	WS
6	*Nesaea prostrata*	460.4±12.06	22.3±0.53	ISO	1296.4±147.9	I	Si
7	*Rotala densiflora*	1782±56.79	1.7±0.356	ISO	3328.4±933.3	P	C
8	*Rotala rosea*	458.7±47.23	10±0.236	TET	1612.1±403.3	P	W
9	*Rotala fimbriata*	536.3±8.50	8.3±0.726	ISO	874.6±58.99	P	W
10	*Rotala macrandra*	564.3±7.08	12.3±0.97	ISO	1170±41.76	P	SC
11	*Rotala indica*	271.2±43.39	14.3±0.82	ISO	964.6±81.684	P	SC
12	*Rotala rotundifolia*	591.7±34.28	8.3±0.356	ISO	1359.7±145.5	P	CW
13	*Rotala ritchiei*	378±10.13	9.7±1.01	TET	683.5±40.42	P	W
14	*Rotala malampuzhensis*	610.6±20.84	14.1±1.16	ISO	1211.7±56.5	P	W
15	*Rotala tulunadensis*	455.7±6.061	9±0.707	ISO	1329.3±61.1	P	WSi
16	*Rotala malabarica*	340.1±16.48	12.3±1.90	ISO	668.1±84.43	P	CW
17	*Rotala occultiflora*	0	0	0	307.3±24.93	P	SC
18	*Rotala mexicana*	387.04±16.72	6.1±1.327	TET,STAU	748.7±67.36	P	CW
19	*Rotala juniperina*	661.3±18.81	17.9±0.36	ISO	1010.2±28.4	I	Si

***Rotala* L.**

***Rotala densiflora* (Roth) Koehne**

Hypostomatic to amphistomatic (with a reduced amount of stomata on adaxial surface); largely isotricytic and tetracytic often anomocyctic on abaxial surface. Stomatal size ranges from 488 to 577 μm², Stomatal index 21% on abaxial surface. Epidermal cells polygonal or rectangular, iso or anisodiametric with 4-8 sides on adaxial surface, 53− 94μm long and 35− 60μm wide; irregular and anisodiametric on abaxial surface, 30−52μm long and 24−36 μm wide. Anticlinal cell wall, thick and curved on adaxial surface, thin and wavy to sinuate on abaxial surface [Figure 3 (G1 and G2)].

***Rotala rosea* (Poir.) C. D. K. Cook**

Amphistomatic; largely tetracytic and isotricytic often anomocytic and occasionally staurocytic on abaxial and largely tetracytic often anomo and isotricytic and occasionally staurocytic on adaxial surface.

Stomatal size ranges from 382.5 to 628.1 μm² on abaxial surface and from 328 to 618.9 μm² on adaxial surface. Stomatal index is 13.3% on abaxial and 10% on adaxial surfaces. Epidermal cells polygonal or irregular , iso and anisodiametric on abaxial surface, 25.9− 59μm long and 15− 20μm wide; Polygonal or rectangular, iso or anisodiametric on adaxial surface, 42−66μm long and 16-42μm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and wavy on adaxial surface [Figure 3 (H1 and H2)].

***Rotala fimbriata* Wight**

Amphistomatic; largely isotricytic often tetracytic occasionally staurocytic on both abaxial and adaxial surface. Average stomatal size ranges from 450.5 to 670 μm² on abaxial surface and from 417.5 to 594.9 μm² on adaxial surface. Stomatal index is 13.4% on abaxial and 8.3% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 25− 48 μm long and 18− 35 μm wide; Polygonal or rectangular or squarish, iso or anisodiametric on adaxial surface, 25− 48μm long and 18− 35 μm wide. Anticlinal cell wall thin and wavy to sinuate on abaxial surface, thick and wavy on adaxial surface [Figure 3 (I1 and I2)].

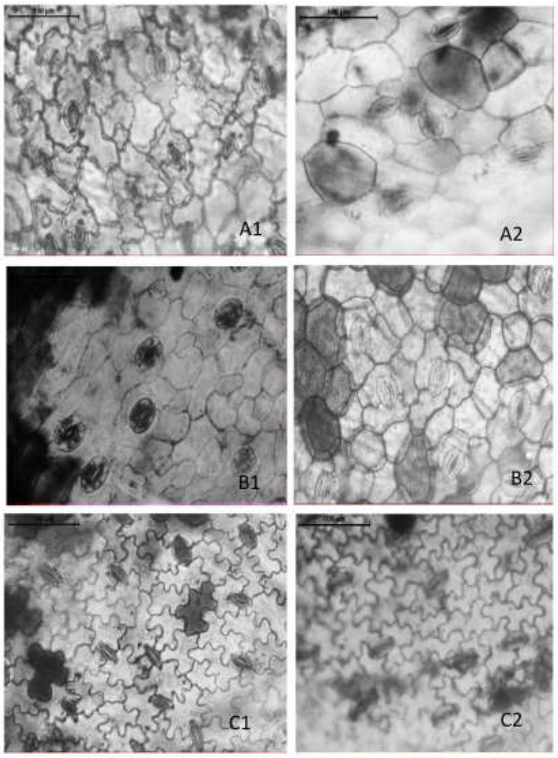

Figure 1. A- ***Ammannia Baccifera*** **subsp.** ***baccifera*** **(A1.abaxial surface with polygonal or irregular epidermal cells and wavy to sinuate anticlinal cell wall & A2. adaxial surface with polygonal epidermal cells and straight to curved anticlinal cell wall); B-** ***Ammannia baccifera*** **subsp.** ***aegyptiaca*** **(B1. abaxial surface with polygonal epidermal cells and wavy to sinuate anti clinal cell wall & B.2. adaxial surface with polygonal epidermal cells and straight to curved anticlinal cell wall); C-** ***Ammannia multiflora*** **(C1. abaxial & C2. adaxial surfaces- both surfaces with irregular epidermal cells and sinuate anticlinal cell wall)**

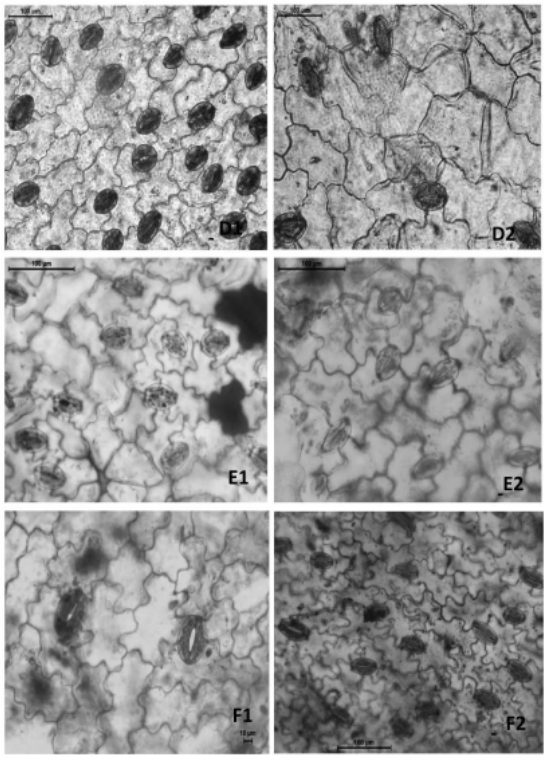

Figure 2. D- ***Ammannia octandra*** **(D1. abaxial surface having polygonal or irregular epidermal cells with wavy to sinuate anticlinal cell wall & D2. adaxial surface having polygonal epidermal cells with curved to wavy anticlinal cell wall); E-** ***Nesaea brevipes*** **(E1. abaxial & E2. adaxial surfaces both having polygonal or coarsely irregular epidermal cells with wavy to sinuate anticlinal cell wall); F-** ***Nesaea prostrata*** **(F1. abaxial & F2. adaxial surfaces- both surfaces having irregular epidermal cells with sinuate anticlinal cell wall)**

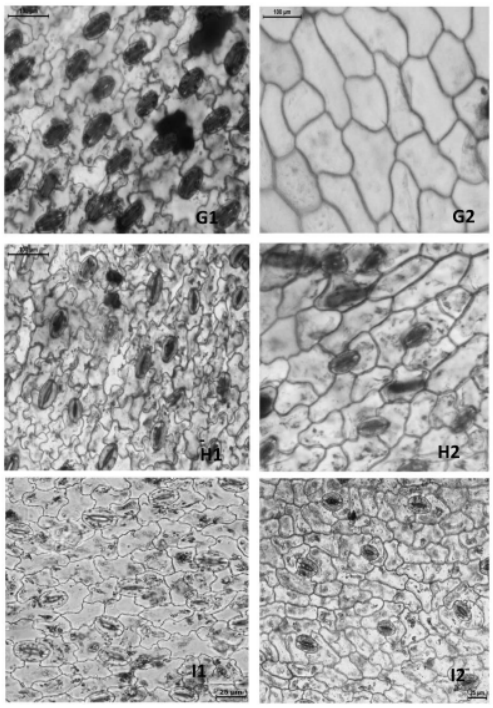

Figure 3. G- ***Rotala densiflora*** **- hypostomatic (G1. abaxial surface having irregular epidermal cells with wavy to sinuate anticlinal cell wall & G2. adaxial surface having polygonal epidermal cells with curved anticlinal cell wall); H-** ***Rotala rosea*** **(H1. abaxial surface having polygonal or irregular epidermal cells with wavy to sinuate anticlinal cell wall & H2. adaxial surface having polygonal epidermal cells and wavy anticlinal cell wall); I-** ***Rotala fimbriata*** **(I1. abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & I2. adaxial surfaces having polygonal or rectangular or squarish epidermal cells with wavy anticlinal cell wall)**

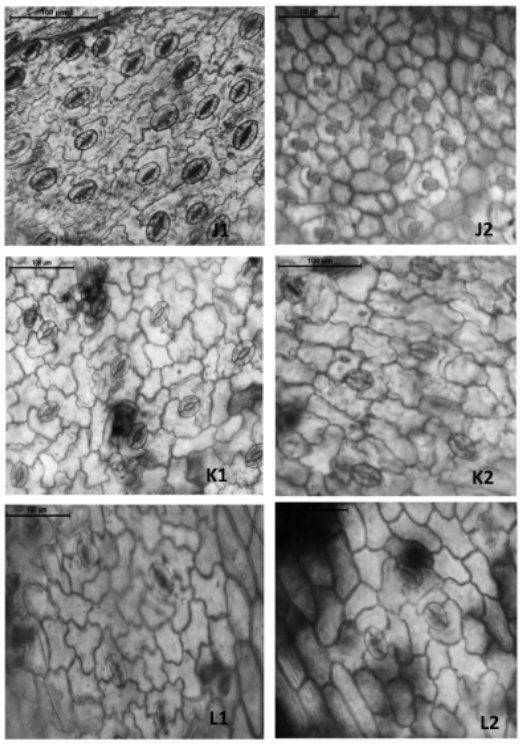

Figure 4. J- ***Rotala indica*** **(J1. abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & J2. adaxial surface having polygonal epidermal cells with straight to curved anticlinal cell wall); K-** ***Rotala macrandra*** **(K1. abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & K2. adaxial surface having polygonal epidermal cells and straight to curved anticlinal cell wall); L-** ***Rotala rotundifolia*** **(L1. abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & L2. adaxial surfaces having polygonal epidermal cells with curved to wavy anticlinal cell wall)**

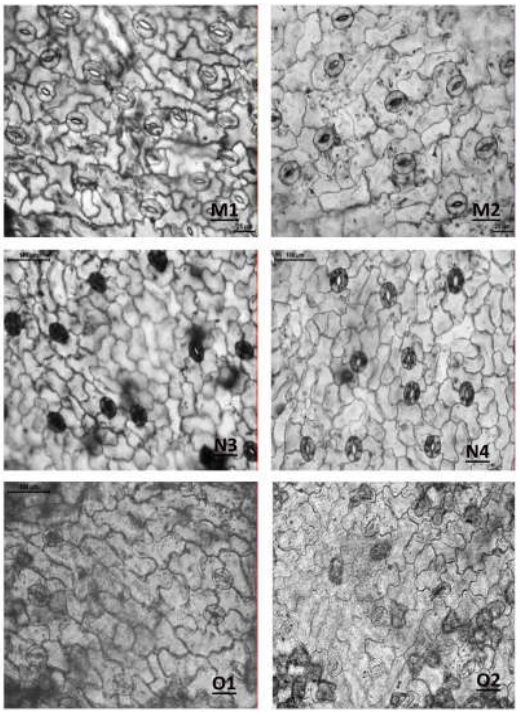

Figure 5 M- ***Rotala malampuzhensis*** **(M1- abaxial surface having polygonal or irregular epidermal cells with wavy to sinuate anticlinal cell wall & M2. Adaxial surface having polygonal epidermal cells with wavy anticlinal cell wall); N-** ***Rotala ritchiei*** **(N1. abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & N2. Adaxial surface having polygonal epidermal cells and wavy anticlinal cell wall); O-** ***Rotala tulunadensis*** **(O1- Abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & O2- adaxial surfaces having polygonal or irregular epidermal cells with wavy to sinuate anticlinal cell wall)**

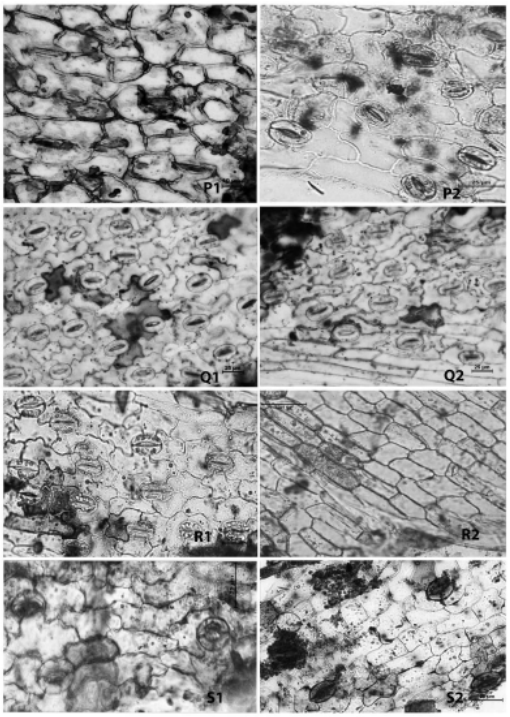

Figure 6. P- ***Rotala malabarica*** **(P1 & P2. abaxial & adaxial surface having polygonal epidermal cells with curved to wavy anticlinal cell wall); Q-** ***Rotala juniperina*** **(Q1. abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & Q2. adaxial surface having irregular epidermal cells and sinuate anticlinal cell wall); R-** ***Rotala occultiflora*** **(R1. abaxial surface having polygonal epidermal cells with wavy to sinuate anticlinal cell wall & R2 adaxial surfaces having polygonal epidermal cells with straight to curved anticlinal cell wall); S-** ***Rotala mexicana*** **(S1 & S2. abaxial & adaxial surface having polygonal epidermal cells with curved to wavy anticlinal cell wall)**

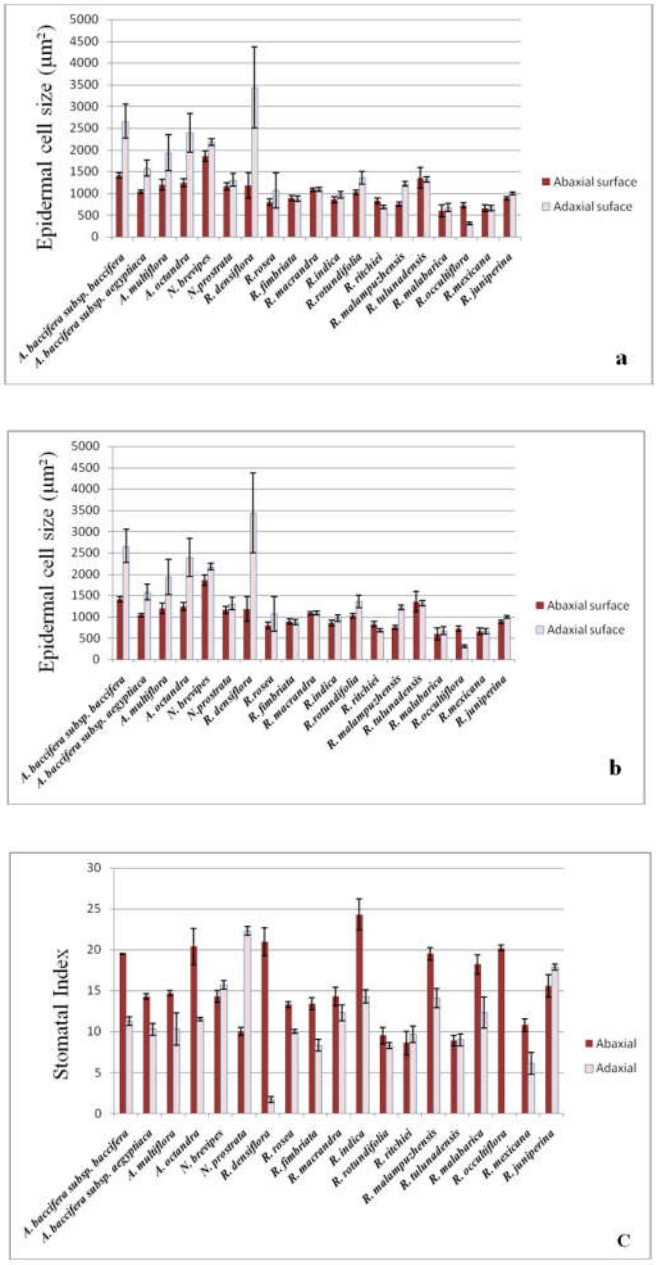

Figure 7. Graphical representation of variation of stomatal size (a), epidermal Size (b) and stomatal Index (c) in different species of ***Ammannia***, ***Nesaea*** **and** ***Rotala***

Rotala indica (Willd.) Koehne

Amphistomatic; largely isotricytic, often tetracytic and occasionally staurocytic and anomocytic on both abaxial and adaxial surfaces. Stomatal ranges from 260 to 367 µm^2 on abaxial surface and from 208.8 to 382.5 µm^2 on adaxial surface. Average stomatal index is 24.3% on abaxial and 14.3% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 28− 69 µm long and 15− 33.5 µm wide; Polygonal with 5−7 sides, iso or anisodiametric on adaxial surface, 25.7− 54µm long and 18− 33.5µm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and straight to curved on adaxial surface [Figure 4 (J1 and J2)].

Rotala macrandra Koehne

Amphistomatic; largely isotricytic, often tetracytic and occasionally staurocytic and anomocytic on both abaxial and

adaxial surfaces. Stomatal size ranges from 447 to 665.2 μm^2 on abaxial surface and from 562.8 to 622.1 μm^2 on adaxial surface. Stomatal index is 14.3% on abaxial and 12.3% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 28− 70μm long and 16-35μm wide; Polygonal with 5- 7 sides, iso or anisodiametric on adaxial surface, 25.7− 54 μm long and 18− 33.5 μm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and straight to curved on adaxial surface [Figure 4 (K1 and K2)].

***Rotala rotundifolia* (Buch.- Ham. ex Roxb.) Koehne**

Amphistomatic; largely isotricytic and tetracytic, often anomocytic and occasionally anomocytic on abaxial and largely isotricytic, occasionally tetracytic on adaxial surfaces. Stomatal size ranges from 497.7 to 612.9 μm^2 on abaxial surface and from 497.8 to 628.6 μm^2 on adaxial surface. Stomatal index is 9.5% on abaxial and 8.3% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 29− 63μm long and 13 − 42μm wide; Polygonal with 5− 7 sides, iso or anisodiametric on adaxial surface, 36− 69 μm long and 15-41μm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and curved to wavy on adaxial surface [Figure 4 (L1 and L2)].

***Rotala malampuzhensis* R.V. Nair ex C. D. K. Cook**

Amphistomatic; largely tetracytic and isotricytic often anomocytic and occasionally staurocytic and anisocytic on abaxial and largely isotricytic, often anomo and tetracytic and occasionally staurocytic and anisocytic on adaxial surfaces. Stomatal size ranges from 342.8 to 620.8 μm^2 on abaxial surface and from 621.8 to 732.2 μm^2 on adaxial surface. Stomatal index is 19.5% on abaxial and 14.1% on adaxial surfaces. Epidermal cells polygonal or irregular, iso and anisodiametric on abaxial surface, 21−56 μm long and 14−32 μm wide; Polygonal or rectangular with 4− 6 sides, iso or anisodiametric on adaxial surface, 27− 82 μm long and 14-40μm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and wavy on adaxial surface [Figure 5 (M1 and M2)].

***Rotala ritchiei* (Clarke) Koehne**

Amphistomatic; largely tetracytic often anomo and isotricytic and occasionally staurocytic on abaxial and largely tetracyctic and Isotricytic, often anomocytic and occasionally staurocytic on adaxial surfaces. Stomatal size ranges from 220.1 to 266.9 μm^2 on abaxial surface and 353.1− 427.5 μm^2 on adaxial surface, Average stomatal index is 8.6% on abaxial and 9.7% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 23− 64 μm long and 13−32 μm wide; Polygonal, iso or anisodiametric on adaxial surface, 21− 55 μm long and 14− 28 μm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and wavy on adaxial surface [Figure 5 (N1 and N2)].

***Rotala tulunadensis* K. S. Prasad, P. Biju, Raveendran and K. G. Bhat**

Amphistomatic; largely tetracytic and isotricytic often anomocytic on abaxial and largely isotricytic, often staurocytic and tetracytic and occasionally anomocytic on adaxial surfaces. Average stomatal size ranges from 400 to 773.6 μm^2 on abaxial surface and from 362.1 to 538.36 μm^2 on adaxial surface, Average stomatal index is 8.9% on abaxial and 9.0% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 30− 83 μm long and 14− 49μm wide; Polygonal or irregular, iso or anisodiametric on adaxial surface, 22−91 μm long and 12−42 μm wide. Anticlinal cell wall thick and wavy to sinuate on both abaxial and adaxial surfaces [Figure 5 (O1 and O2)].

***Rotala malabarica* Pradeep, K. T. Joseph and Sivar**

Amphistomatic; largely tetracytic and isotricytic often anomocyticand staurocytic on abaxial and largely isotricytic, often anomocytic, tetracytic and staurocytic and on adaxial surfaces. Stomatal ranges from 194.5− 479.3 μm^2 on abaxial surface and 306−375.4 μm^2 on adaxial surface. Stomatal index is 18.2% on abaxial and 12.3% on adaxial surfaces. Epidermal cells polygonal iso and anisodiametric on abaxial and adaxial surface, 29−53 μm long and 12−18 μm wide on abaxial and 34−45 μm long and 14−19μmwide. Anticlinal cell wall thick and curved to wavy on both abaxial and adaxial surface [Figure 6 (P1 and P2)].

***Rotala juniperina* A. Fern**.

Amphistomatic; largely isotricytic, often tetracytic and occasionally anomocytic and anisocytic on both abaxial and adaxial surfaces. Stomatal size ranges from 605.6 to 640.0 μm^2 on abaxial surface and 615.4− 690μm^{2}on adaxial surface. Stomatal index is 15.6% on abaxial and 17.9% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 25−67μm long and 14−28μm wide; Irregular, iso or anisodiametric on adaxial surface, 24 −69μm long and 14−36μm wide Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and sinuate on adaxial surface [Figure 6 (Q1 and Q2)].

***Rotala occultiflora* Koehne**

Hypostomatic, largely tetracytic, often isoticytic and anomocyctic and occasionally staurocytic on abaxial surface. Stomatal size ranges from 304.3 to 668.9 μm^2. Stomatal index 20.2% on abaxial surface. Epidermal cells polygonal on both abaxial and adaxial surfaces, 20.8− 71μm long and 13−21μmwide; 14−34.1μm long and 9.2−15μm wide. Anticlinal cell wall, thick and and wavy to sinuate on abaxial and straight to curved on adaxial surface [Figure 6 (R1 and R2)].

***Rotala mexicana* Cham. and Schltdl**

Amphistomatic; largely tetracytic, often anomocytic and isotricytic, occasionally staurocytic on abaxial and largely isotricytic and staurocytic, often anomocytic and isotricytic on adaxial surfaces.Stomatal size ranges from 485.3 to 504 μm^2 on abaxial surface and from 378 390 μm^2 on adaxial surface. Stomatal index is 10.8% on abaxial and 6.1% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on both abaxial and adaxial surfaces, 23 − 64.3μm long and 9.5−20.8μm wide on abaxial surface; 27−50μm long and 10−28.6 μm wide. Anticlinal cell wall thick and curved to wavy on both abaxial surface and adaxial surfaces [Figure 6 (S1 and S2)].

Combined key to the South Indian species of *Ammannia, Nesaea* and *Rotala*

1a. Leaves hypostomatic ……..*R. occultiflora*
1b.

2a. Leaves both hypostomatic and amphistomatic……..*R.densiflora*
2b. Leaves strictly amphistomatic ……3
3a. Epidermal cell polygonal…….....5
3b. Epidermal cell irregular…….....4
4a. Stomatal index high on adaxial surface…….*N. prostrata*
4b. Stomatal index high on abaxial surface…...*A. multiflora*
5a. Anticlinal cell wall wavy on adaxial surface……...6
5b. Anticlinal cell wall otherwise on adaxial surface…….....9
6a. Isotricytic stomatal percentage high on both surfaces 7
6b. Isotricytic stomatal percentage low on both surfaces…....8
7a. Stomatal size larger on abaxial surface……..*R. fimbriata*
7b.Stomatal size larger on adaxial surface...*R. malampuzhensis*
8a. Stomatal index high on adaxial surface…….....*R. ritchiei*
8b. Stomatal index high on abaxial surface……*R.rosea*
9a. Stomatal size larger on adaxial surface…… ……..12
9b. Stomatal size larger on abaxial surface…… ……10
10a. Anticlinal epidermal cell wall straight to curved…..*R. indica*
10b. Anticlinal epidermal cell wall curved to wavy……11
11a. Adaxial surface with high percentage of isotricytic stomatal percentage..... *R. rotundifolia*
11b. Adaxial surface with high percentage of both tetracytic and staurocytic stomata.....*R. mexicana*
12a. Stomatal index high on abaxial surface…….....14
12b. Stomatal index high on adaxial surface……..13
13a. Anisocytic stomata is present……*R. juniperina*
13b. Anisocytic stomata is absent……*N. brevipes*
14a. Diacytic stomata present……15
14b. Diacytic stomata absent…… …….…….....16
15a. Anisotricytic stomata present……...*A. octandra*
15b. Anisotricytic stomata absent…....*A. baccifera* subsp. *baccifera*
16a. Anisocyctic stomata present……...*A. baccifera* subsp. *aegyptiaca*
16b. Anisocyctic stomata absent…….....17
17a. Staurocytic stomata present on abaxial surface…… ...*R. malabarica*
17b. Staurocytic stomata absent on abaxial surface ……*R. macrandra*

DISCUSSION

The present study revealed the presence of amphistomatic leaves in South Indian species of *Rotala, Ammannia* and *Nesaea* except in *Rotala occultiflora* which is hypostomatic. This best supports the studies of Rajagopal (1979), in which species of Lythraceae were considered to be either amphistomatic or hypostomatic. The hypostomatic character of the mentioned species may be due to the adaptation to water loss which is in agreement with Metacalf and Chalk (1950) and Mbagwu and Edeoga (2006). Presence of hypostomatic leaf in *R. occultiflora* is the first report, since earlier workers (Kshirsagar and Vaikos, 2013) reported amphistomatic leaf for the same. Here, we described *R. densiflora* as hypostomatic to amphistomatic, as some population is found to be strictly hypostomatic, while a very few others to be hypostomatic with maximum of only a 10 number of stomata per unit area. This hypostomatic population is observed to posses largest stomata on their adaxial surface among all species considered here, which is in corroboration with some previous studies, where an inverse relationship between stomatal size and and stomatal number was reported (Muenscher, 1915; Camargo and Marenco, 2011; Ajayan *et. al* 2015; Zoric *et. al* 2009). In all species, isotricytic stomata are distributed in large proportion on both abaxial and adaxial surfaces except in *R. rosea* and *R. ritchiei*, where tetracytic stomata are distributed in large proportion. In earlier studies, 'anomocytic' stomata were reported in Lythraceae (Panigrahi 1981; Thanki *et. al* 2000) which is now considered as synonymn of 'Isotricytic and tetracytic' type according to latest classification of Prabhakar (2004). Strangely, staurocytic stomata were found to be distributed in major proportion along with tetracytic stomata in *R. mexicana*. Presence of trifling percentage of diacytic stomata was observed to be species specific to *A. baccifera* subsp. *baccifera* and *A. octandra*.

Ahmad *et al.,* (2010) noted valuable intergeneric and interspecific variations in the pattern of epidermal cells that can be used to as an important taxonomic tool to identify many species. Almost all species of three genera possess polygonal epidermal cells with either wavy to sinuate or straight to curved anticlinal cell wall. However, in *Ammannia multiflora* and *Nesaea prostrata*, the epidermal cells are irregular with sinuate anticlinal cell wall on both of their abaxial and adaxial surfaces and in *Rotala juniperina*, the same on their adaxial surface. The anticlinal cell wall on the adaxial surface shows much diversity among the species of three genera. Based on the studies of Barthlott (1981) on anticlinal cell wall of epidermal cells as important characteristics of taxonomic character, the combination of adaxial diversity and abaxial uniformity of anticlinal cell wall were used for the delimitation of three genera under study. Relatively larger epidermal cells are distributed on adaxial surface compared to abaxial surfaces of all species studied here. Still, in the case of *Rotala ritchiei, Rotala rosea* and *Nesaea brevipes*, the abaxial surfaces possess larger epidermal cells. The epidermal cell size was observed to be maximum in adaxial surface of *R. densiflora* and minimum in the adaxial surface of *R. ritchiei.* Stomatal index is considered as one of the useful tools in order to distinguish species, since it is fairly constant for a particular species (Salisbury 1928) than stomatal density. There was a great variation in stomatal index, in between different species of *Ammannia*, *Rotala* and *Nesaea*. Highest index was observed in abaxial surface of *R. indica* and lowest in adaxial surface of *R. densiflora*. Generally high stomatal index was observed on abaxial surfaces of leaf compared to adaxial surface in all species of *Ammannia* studied and in majority species of *Rotala*, which is the characteristics of herbaceous species (Willmer and Fricker 1996), but the same was observed on adaxial surface in the case of genus *Nesaea*. Also in *R. ritchei, R.tulunadensis* and *R. juniperina*, stomatal index is slightly more on the adaxial surfaces than abaxial surface which is the characteristics of floating leaves of aquatic plants. Considering the genus *Rotala*, the amphibious or terrestrial species show a noticeable difference between abaxial and adaxial stomatal index, while in *R. macrandra* and *R. rotundifolia*, which are more aquatic in nature, showed negligible difference between adaxial and abaxial stomatal index.

Significant comparison of stomatal size between abaxial and adaxial leaf surfaces has been reported by Zoric *et al.,* (2009). Similar types of comparative studies in this group revealed, species of *Ammannia* possess relatively larger stomata on their adaxial surfaces compared to abaxial surface. Besides, in most of the species of *Rotala* (*R.macrandra*, *R. ritchei*, *R. malampuzhensis, R. densiflora* and *R. juniperina*) and in *Nesaea prostrata* the similar condition is observed. When Compared to *Ammannia* and *Nesaea*, different species of *Rotala* shows more diversity in relative stomatal size

difference between abaxial and adaxial surfaces. The stomatal size is observed maximum for *Rotala densiflora* on its adaxial surface and minimum for *Rotala ritchei* on its abaxial surface. There is only a narrow range differences in the value of stomatal size except in (*Rotala densiflora*), merging stomatal size among all species within the three genera and hence was not to be taken for consideration for the species delimitation.

Conclusion

In conclusion, most of the South Indian species of *Ammannia, Rotala* and *Nesaea* under the present study shares some generic qualitative characters such as type of stomata present in large proportion, shape of epidermal cell and pattern of anticlinal wall on abaxial surface. Characters like relative distribution percentage of stomatal index, stomatal and epidermal cell size, presence or absence of some unique type of stomata were observed to play a key role in species delimitation. The shape of epidermal cells, types and arrangement of stomata and stomatal index are found to be important in delimiting species in *Rotala, Ammannia* and *Nesaea*.

Acknowledgments

Authors are grateful to the Interuniversity Centre for Plant Biotechnology, Department of Botany, University of Calicut for providing facilities and financial assistance.

REFERENCES

Ahmad, K., Khan, M. A., Ahmad, M., Shaheen, N., and Nazir, A. 2010. Taxonomic diversity in epidermal cells of some sub-tropical plant species.. *International journal of Agricultural and Biology,* 12(1), 115-118.

Ajayan, K. V., Babu, R. L., and Patil Bayakka, B. 2015. Variability of Stomatal Index and Chlorophyll Content in four species of Solanaceae members. *International Journal of Biological Sciences,* 4(2), 16- 20.

Anto, P. V., Jacob, C.S., Abraham, P., Varghese, C. D., and Antony, I. 2014. A new species of *Rotala* L. (Lythraceae) from the lateritic hills of Thrissur district, Kerala, India. *International Journal Advanced Research,* 2(11), 532-535.

Barthlott, W. 1981. Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nordic Journal of Botany,* 1(3), 345-355.

Camargo, M. A. B. and Marenco R. A. 2011. Density, size and distribution of stomata in 35 rainforest tree species in Central Amazonia. *Acta Amazonica,* 41(2), 205-212.

Gaikwad, S. P., Sardesai, M. M., and Yadav, S. R. 2014. *Rotala sahyadrica* sp. nov.(Lythraceae) from Western Ghats, India. *Nordic Journal of Botany,* 32(5), 575-577.

Gopal, B. V., and Mishra, A. K. 1980. A new technique for plant epidermal studies, *Kenya Science and Tech.,* (B) 1, 99-100.

Graham, S. A., Hall, J., Sytsma, K., and Shi, S. H., 2005. Phylogenetic analysis of the Lythraceae based on four gene regions and morphology. *International Journal Plant Sciences*, 166(6), 995-1017.

Kazama, H., and Mineyuki, Y. 1997. Alteration of division polarity and preprophase band orientation in stomatogenesis by light. *Journal Plant Research*, 110(4), 489-493.

Kshirsagar, A. A., and Vaikos, N. P. 2013. *Asian Journal of Plant Sciences,* 3(3), 117-120.

Lemiya, K. M., and Pradeep, A. K. 2015. A new species of *Rotala* (Lythraceae) from Kerala, India. Rheedea, 25(2), 159-163.

Prabhakar, M. 2004. Structure, delimitation, nomenclature and classification of stomata. Acta Botanica Sinica-english edition-, *46*(2), 242-252.

Mbagwu, F. N., and Edeoga, H. O. (2006). Observations on the vegetative and floral morphology of some Vigna species (Leguminosae-Papilionoideae). *Pakistan Journal of Biological Sciences*, 9(9), 1754-1758.

Metcalfe, C. R., and Chalk, L. 1979. Anatomy of the Dicotyledons, Systematic Anatomy of the leaf and stem.Vol. I. 2nd Ed. Clarendon Press, Oxford.

Muenscher, W. L. 1915. A study of the relation of transpiration to the size and number of stomata. *American Journal of Botany*, 2(9), 487-504.

Nishida S, De Kok R, Yang Y. 2016. Cuticular features of *Cryptocarya* (Lauraceae) from Peninsular Malaysia, Thailand and Indo-China and its taxonomic implications. *Phytotaxa*, 244(1), 26-44.

Panigrahi SG. 1981. Anatomical studies on the genus *Rotala* L. (Lythraceae). Indian Scince Congress Association Proceedings*,* 68(3), 71-72.

Pant, D. D, Kidwai, P. F. 1964. On the diversity in the development and organization of stomata in Phyla nodiflora Michx. *Current Sciences,* 33 (21), 653-654.

Prabhakar, M. 2004. Structure, delimitation, nomenclature and classification of stomata. Acta Botanica Sinica -english edition-. 46 (2), 242-252.

Prasad, K.S., Biju, P., Raveendran, K and Bhat, K. G. 2012. *Rotala tulunadensis* sp. nov.(Lythraceae) from Kerala, India. *Nordic Journal of Botany,* 30(1), 58-60.

Prasad, K. S., and Raveendran, K. 2013a. A New Species of *Rotala* L. (Lythraceae) from Kerala, India. Taiwania 58 (2), 104−107.

Prasad, K. S., and Raveendran, K. 2013b. Rotala kasaragodensis (Lythraceae), a new species from Kerala, India. *Edinburgh Journal of Botany,* 70 (3), 451–454.

Rajagopal, T. 1979. Distributional patterns and taxonomic importance of foliar stomata. *Indian Journal of Botany,* 2, 63-9.

Ramayya, N., and Rajagopal, T. 1971. Foliar" Dermotypes" of the Indian Aizoaceae and their use in identification. *Journal of the Indian Botanical Society*, 50, 355-62.

Rao, S., and Ramayya, N. 1977. Stomatogenesis in the genus Hibiscus L. (Malvaceae). *Biological Journal of the Linnean Society,* 74 (1), 47-56.

Ratheesh Narayanan, M. K., Sunil, C. N., Shaju, T, Nandakumar, M. K., Sivadasan, M., and Alfarhan, A. H. 2014. *Rotala dhaneshiana*, a new species of Lythraceae from India. *Phytotaxa*, 188(4), 227–232.

Salisbury, E. J. 1928. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character. 216, 1- 65.

Stace C. A. 1984. The Taxonomic Importance of the Leaf Surface, in Current concepts in plant taxonomy. Heywood V H and Moore D M (Eds.). Academic Press. London, 67-94

Stresburger, E. 1886. Ein Beitrag zur Entwicklungs geschichte der Spaltoff nungen. Jahrbücher für Wissenschaftliche Botanik, 5, 297- 342.

Sunil, C. N., Ratheesh Narayanan, M. K., Nandakumar, M. K, Jayesh, P.J, Abdul Jaleel, V., and Anil Kumar, N. 2013.

Rotala khaleeliana sp. nov. (Lythraceae), a new species from lateritic hills of Kannur, Kerala, India. *International Journal of Advanced Research*, 1 (2), 14−16.

Thanki, Y. J., Shah, K. and Garasia, K. K. 2000. Stomatal ontogeny in some Lythraceae. *Journal of Phytological Research*, 13 (2), 187-189.

Vesque, M. J. 1889. De l'emploi des caractères anatomiques dans la classification des végétaux. Bulletin de la Société botanique de France. 36 (10), 41- 77.

Volponi, C. R. 1999. Stomatogenesis in Caryophyllaceae. *Phytomorphology*, 49 (3), 253-259.

Wilkinson, H. P. 1979. The plant surface (mainly leaf). In: Metcalfe CR and Chalk L, Anatomy of dicotyledons: systematic anatomy of the leaf and stem. 2nd ed. Oxford: Claredon Press. pp. 97-165.

Willmer, C., and Fricker, M. 1996. The distribution of stomata. In Stomata. Springer Netherlands. pp. 12-35.

Yadav, S. R, Malpure, N. V., and Chandore, A. N. 2010. *Rotala belgaumensis* (Lythraceae) from the Western Ghats, India. *Nordic Journal of Botany,* 28, 499–500.

Zoric, L., Merkulov, L., Lukovic, J., Boza, P., and Polic, D. 2009. Leaf epidermal characteristics of Trifolium L. species from Serbia and Montenegro. Flora-Morphology, Distribution, *Functional Plant Biology*, 204(3), 198-209.

****