International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 12, Issue 10-2025

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2025.12.10.003

Re-visiting the taxonomy of *Rotala rosea* (Lythraceae) in South India

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Abstract

Rotala rosea is an aquatic plant distributed in India, Indonesia, China, etc. that displays significant phenotypic plasticity, particularly in its colouration, leaf structure, and petal morphology. This study examines the morphological, anatomical, and palynological characteristics of *R. rosea* across various regions of South India, revealing substantial phenotypic diversity that overlaps with traits of several recently described species.

The findings indicate that the observed variations represent polymorphic variants of *R. rosea*, suggesting that traditional classification methods based solely on morphology are insufficient. Additionally, analyses of seed coat patterns and pollen morphology further support the conclusion that these are phenotypic variants of *R. rosea*. Consequently, the recently described taxa—such as *R. dhaneshiana*, *R. khaleeliana*, and *R. meenkulamensis*—should be regarded as synonyms of *R. rosea*.

Keywords: Rotala, Anatomy, Morphology, Palynology, Phenotypic Plasticity.

1. Introduction

The genus *Rotala*, first described by Linnaeus in 1771, encompasses around seventy-five accepted species distributed globally (POWO, 2023). This genus is particularly distinguished by its aquatic and amphibious growth habits, contributing to its ecological versatility and adaptation to diverse habitats. *Rotala* exhibits significant phenotypic plasticity, allowing it to thrive in varying environmental conditions, a trait that has been documented extensively (Cook, 1979).

Morphologically, *Rotala* is characterized by its herbaceous structure, which includes a quadrangular stem and simple leaves (Graham *et al*. 2011). The inflorescences are typically axillary and can present as solitary cymes or racemes, usually with brightly colored flowers. The reproductive strategy of *Rotala* involves capsule fruits that dehisce regularly through their valves, releasing numerous seeds characterized by a plano-convex shape (Cook 1979; Joseph and Sivarajan 1989; Thomas Mathew 1993).

The comprehensive foundational work on *Rotala* was initiated by Koehne in 1880, followed by a global revision of the genus conducted by Cook in 1979. Among the species within this genus, *Rotala rosea* (Poir.) C.D.K. Cook is particularly noteworthy due to its wide distribution across India, Indonesia, and China. This species exhibits considerable variations in colouration, leaf morphology, petal and bracteole characteristics (Cook, 1979).

In the context of South Indian flora, extensive field studies have led researchers to collect various *Rotala* species from diverse locations. Upon conducting meticulous taxonomic and morphological analyses, comparisons have been made between R. rosea and recently described species such as *R. meenkulamensis* Prasad & Ravindran, *R. dhaneshiana* Sunil, Ratheesh & Sivad., and *R. khaleeliana* Sunil, Ratheesh & Nandakumar. Preliminary findings suggest that these species exhibit close morphological affinities with *R. rosea*, prompting further investigation into their taxonomic classifications.
The current study employs a range of taxonomic tools and methodologies to refine and redefine the specific boundaries of *R. rosea* by integrating morphological, anatomical, and palynological data. This research aims better to understand the relationships among these closely related species, thereby contributing to the broader classification framework within the Lythraceae family.

2. Materials and Methods

Taxon sampling: The materials are examined manually through different herbaria such as MH, CALI, KFRI, MSSRF, CMPR, MBGH, TBGT, SNMH, and the high-resolution images from virtual herbaria of CAL, K, FI, M, L, BR, U, and TUB. Herbarium code follows Thires (continuously updating). The protologues, literature (Cook, 1979; Joseph and Sivarajan 1989; Thomas Mathew 1993; Lemiya 2017; Arun *et al.* 2022), and related floras are also carefully examined. *Rotala* members are collected from parts of South India such as Kerala, Karnataka and Tamil Nadu. The recently described materials are collected from the type localities. For micro-morphological analysis, detailed examinations were done using Leica Stereozoom microscope. The Fresh samples of the stem used for anatomical studies; slides are prepared by hand sectioning followed by mild safranin staining and mounted with 1% glycerin (Ruzin, 1999). Photographs are taken by Lieca compound microscope. The specimens were processed using standard herbarium techniques (Bridson and Forman 1998) and deposited in herbarium at Bishop Abraham Memorial College (BAM).

Scanning electron microscopy: The dried seeds were placed directly on galvanized metal stubs by adhesive tape, labeled and coated with gold-palladium alloy at a thickness of 30 mm (Panigrahi, 1986). Pollen grains are collected and prepared by Erdtman (1952) Acetolysis method modified by Melham *et al*. (2003). The acetolyzed pollen grains were dehydrated in an ascending series of ethanol 10, 50, 75, 90, and 100%, staying approximately 10 min at each step. A small sample of the pollen grains from the absolute alcohol step was pipetted onto double-sided carbon tape fixed in 12.5 mm diameter aluminum stubs and air-dried at room temperature. The micrographs were obtained using TESCAN BRONO S.R.O Czech/ MAIA3 XMH SEM at an accelerating voltage of 200 V to 30 KV under magnifications of 4x to 1,000,000x at SAIF, MG University, Kottayam, Kerala. For the description of terminology for pollen Erdtman (1952) followed.

3. Results and Discussion

Morphological observation: As Cook (1979) noted, phenotypic plasticity is prevalent in *R. rosea*. Field studies reveal various phenotypic variations, particularly in the colouration, leaf apex, petal shape, and apex structures. The number of sepals and petals can vary from three to five; in some accessions, petals may be absent. The shape and apex of the petals exhibit considerable variation both within a single population and across different populations. It is possible to observe both the presence and absence of petals within a single population, although this occurrence is rare in the same plant.

The phenotypic variations of *R. rosea* overlaps with the recently described species of *Rotala* from South India, specifically *R. dhaneshiana*, *R. khaleeliana*, and *R. meenkulamenis*. Variations in leaves and petals are illustrated in Figures 1 and 2, respectively. A comparison of *R. rosea* with its related species is provided in Table 1.

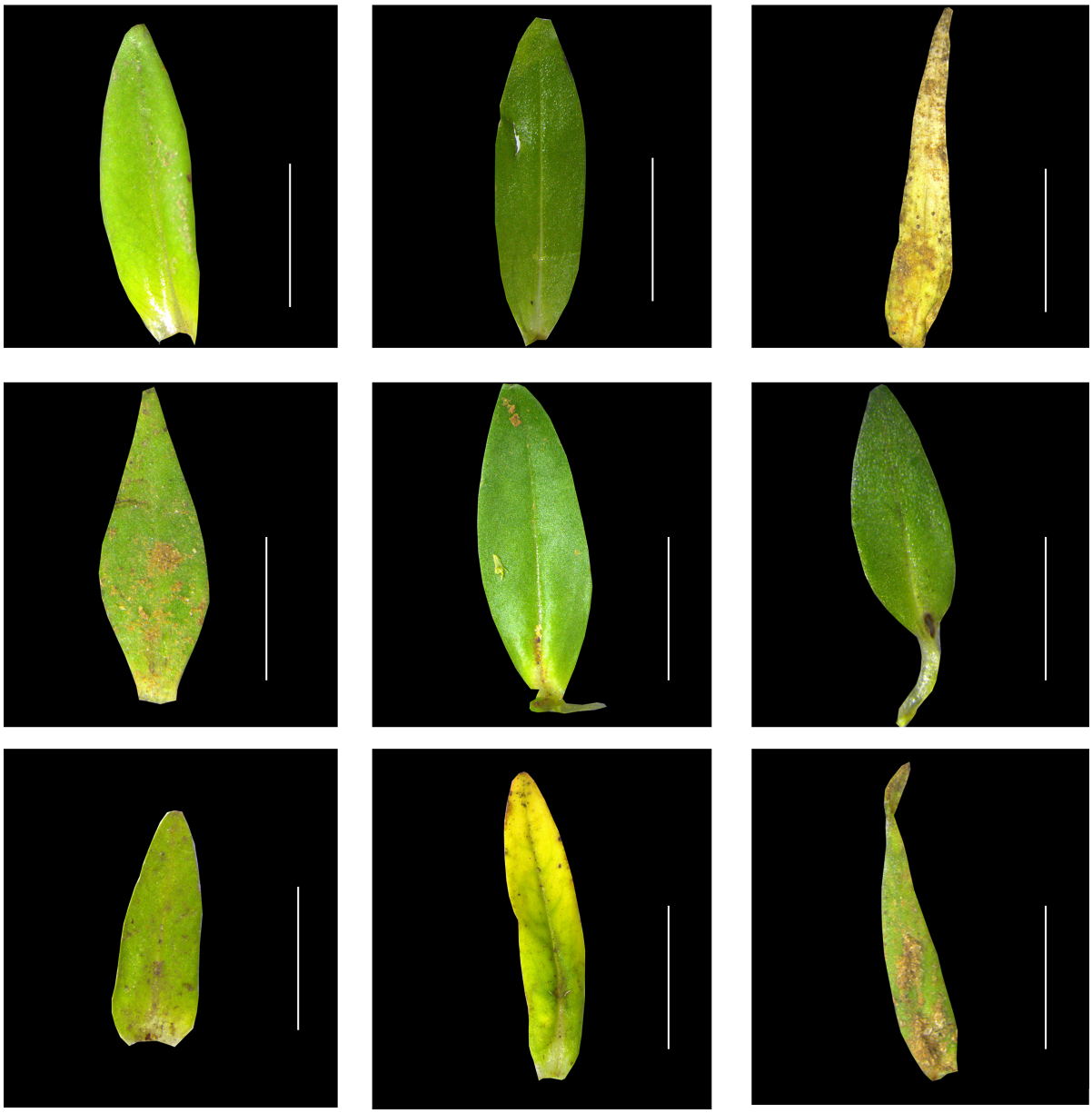

Figure 1. Leaf variations in *R. rosea* (Poir.) C.D.K Cook, scale bars = 5mm

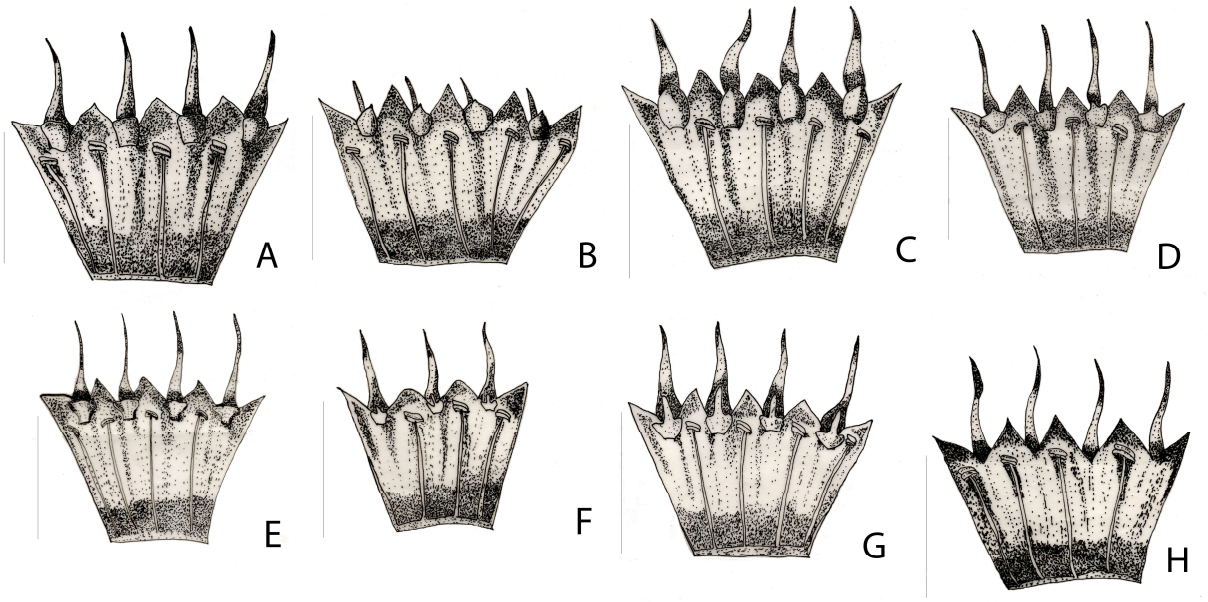

Figure 2. Petal variations, A-E *R. rosea,* F *R. khaleeliana*, G *R. dhaneshiana*, H *R. meenkulamensis.* Scale bars = 1mm

Table1. Morphological comparison of *R. rosea* with its allied species.

Character	*Rotala dhaneshiana*	*Rotala khaleeliana*	*Rotala meenkulamensis*	*Rotala rosea*
Habit	Erect marshy terrestrial annuals.	Erect aquatic to amphibious, annuals or perennials	Aquatic annual herbs.	Aquatic to amphibious, annuals.
Leaf	Simple, deccusate, sessile, reddish tinged when mature. Linear lanceolate to oblong. Base semicordate to semi amplexicaule. Margin entire. Apex acute. Midrib prominent below and canaliculated above.	Simple, deccusate, dimorphic, linear lanceolate, base cuneate to semi amplexicaule. Margin entire. Apex slightly truncate to bimucrunate. One nerved.	Simple deccusate, sessile, ovate lanceolate, base cuneate to attenuate, margin entire. Apex slightly bimucronate. One nerved and lateral veins are not distinct.	Simple, deccusate, sessile to subsessile. Ovate to linear lanceolate. Margin entire. Apex slightly truncate. Midrib prominent below and canaliculated above.
Inflorescence	Axillary soliraty cyme, foliage bracts	Axillary soliraty cyme, foliage bracts	Axillary soliraty cyme, foliage bracts	Axillary soliraty cyme, foliage bracts
Calyx	Calyx companulate, trimerous, sepals triangular, hyaline. Calyx appendages alternating with the calyx.	Calyx companulate, pentamerous, sepal triangular, hyaline. Calyx appendages alternating with the calyx.	Calyx companulate, pentamerous, sepal triangular, hyaline. Calyx appendages alternating with the calyx.	Calyx companulate, pentamerous, sepal triangular, hyaline. Calyx appendages alternating with the calyx.
Corolla	Three, attached alternating to sepals. Minute, hyaline, apiculate at apex.	Five, attached alternating to sepals. Minute, hyaline, truncate at apex.	Absent.	Five or four, attached alternating to sepals. Minute, hyaline pink, ovate, round at apex.

Androecium	Three, attached below middle of the calyx tube.	Five, attached below middle of the calyx tube.	Five, Attached at the base of calyx tube	Attached below middle of the calyx tube.
Gynoecium	Ovary Trilocular, syncarpous with many ovules. Style filiform and stigma capitate.	Ovary Trilocular, syncarpous with many ovules. Style filiform and stigma capitate.	Ovary Trilocular, syncarpous with many ovules. Style filiform and stigma capitate.	Ovary Trilocular, syncarpous with many ovules. Style filiform and stigma capitate.
Fruit	Capsule with three locules. Reddish when mature. Opening regularly with valves.	Capsule with three locules. Reddish when mature. Opening regularly with valves.	Capsule with three locules. Reddish when mature. Opening regularly with valves.	Capsule with three locules. Reddish when mature. Opening regularly with valves.
Seed	Numerous. Plano convex. Yellow, orange to red coloured.	Numerous. Plano convex. Green to yellow coloured.	Numerous. Plano convex. Straw coloured	Numerous. Plano convex. Yellow to orange red coloured.

Seed testa ornamentation is a distinguishing characteristic of *Rotala* species. The ornamentations consist of rectangular cells arranged end-to-end, with longer cells at the micropylar end and squarish cells at the funicular end (see Fig. 4; E-H). Pollen grains of *Rotala* species are monads, isopolar, and radially symmetrical (Mahmoodi *et al.,* 2022). Our study found that the pollen grains of *Rotala* are tricolporate with a single endopore located in the middle. The grains measure approximately 15 µm in polar diameter and 10 µm in equatorial diameter. Our findings indicate that the pollen grains of the four mentioned species are sub prolate. Unlike other *Rotala* species, these four share an identical exine sculpture that is rugulate with narrow grooves. Additionally, the sculpturing of the colpus membrane is characterized by widely spaced baculate structures, and the colpi lack opercula (see Fig. 4; A-D).

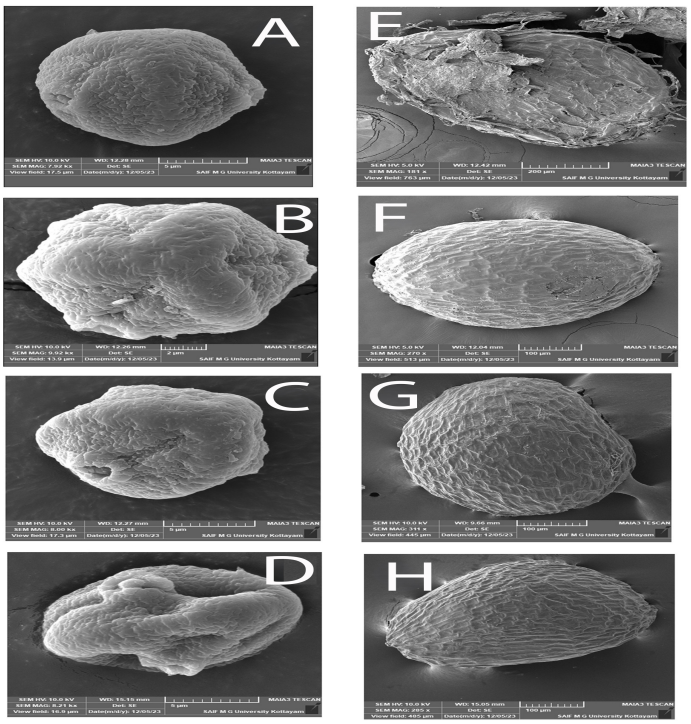

Figure 4. SEM images of seeds and Pollen grains, A-D pollen grains, E-H seed testa; A, E *R. dhaneshiana*, B, F *R. khaleeliana*, C, G *R. meenkulamensis*, D, H *R. rosea*

Anatomical observation: The anatomy of all four species shows a similar pattern of cellular distribution. All of them with cuticle and round to hexagonal parenchyma at dermal tissues. Cortex is with large aerenchyma in two to three layers. Stelar elements are surrounded by prominent endodermis and pericycle. Xylem with parenchyma and vessels. Phloem with parenchyma and sieve tube elements. Pith is very small and has round parenchyma. Cellular depositions of druses present in pith and dermal tissues (Fig. 3).

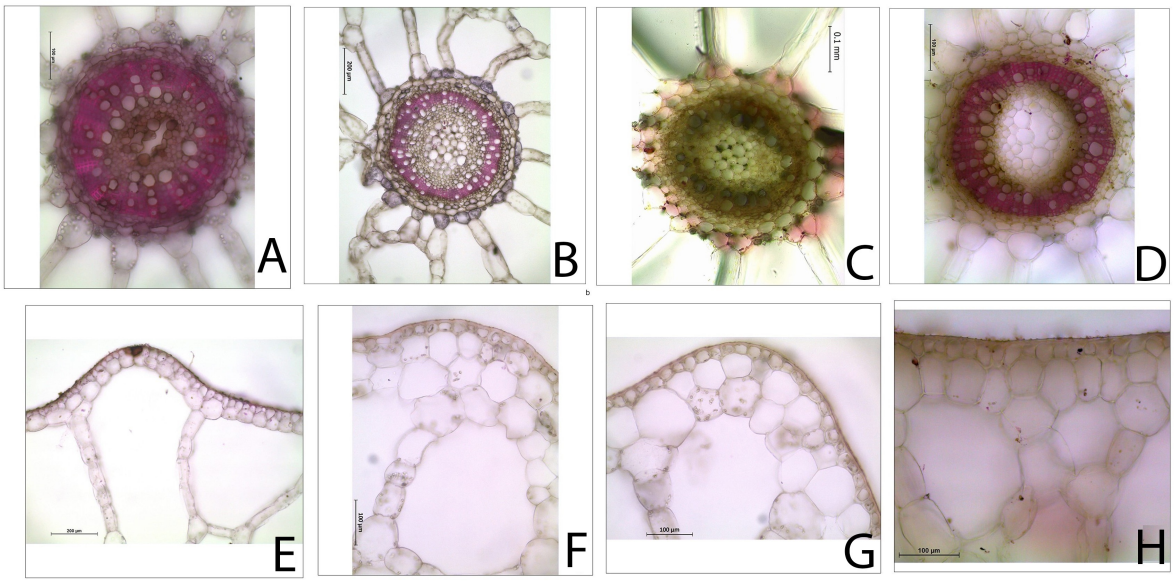

Figure 3. Cross section of stem, A-D Stelar region, E-H Epidermal region; A, E *R. dhaneshiana*, B, F *R. khaleeliana*, C, G *R. meenkulamensis*, D, H *R. rosea*

Concluding remarks:

The current investigation with modern taxonomic tools represents a pioneering attempt to delineate the specific boundaries of *Rotala rosea*, emphasizing a comprehensive comparison of palynological and morphological characteristics. Key morphological traits, including leaf shape, the number of lateral veins, floral morphology, and the number of valves in the seed capsule, are pivotal in classifying *Rotala* species. In addition to these morphological attributes, consistent pollen and seed characteristics are crucial in defining the species boundaries within the genus *Rotala*. Specifically, the ornamentation of the seed testa and the membrane sculpture of pollen grains are distinguishing features among *Rotala* species.

Notably, the morphological variations within the genus are continuous, which presents challenges, particularly with some recently described *Rotala* members. The delicate variations observed within species have often been utilized as distinguishing characters, complicating the classification process. A few recently described *Rotala* members are creating much confusion among taxonomists. Minor variations within species have been considered characters to distinguish between species. Classification and the delimitation of taxa will become a challenge for the botanists working on *Rotala* taxonomy.

1. Taxonomic treatment:

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

≡ *Ammannia rosea* Poiret in in Lamarck, Encycl. Méth. Bot. Suppl. 1: 329. 1810.

Type: Indes orientales, herb. *Desfontaines* (Holotype: FI).

Rotala dhaneshiana Sunil, Ratheesh & Sivad. **syn. nov.**

Type: India, Kerala, Wayanad district, wayanad wildlife Sanctuary, 18 September 2012 *Sunil* and *Ratheesh Narayanan 2211* (Holotype: CAL0000025344!)

Rotala khaleeliana Sunil, Ratheesh & Nandakumar **syn. nov.** Type not located

Rotala meenkulamensis Prasad & Ravindran **syn. nov.**

Type: India, Kerala, Kannur, Meenkulam, 2 September 2021*K. S. Prasad 03106* (Holotype: CAL0000033226!)

Description: Erect aquatic or amphibious annual or perennial herbs. Stem up to 30 cm tall, simple or branched; old stem often denuded of leaves, quadrangular, spongy with constricted nodes and slightly swollen internodes, striate and somewhat rough, reddish or dull green, 2–4 mm across; branches quadrangular, narrowly winged. Leaves sessile, decussate, dimorphic; submerged leaves 10–20 × 2–5 mm, linear-lanceolate, base cuneate to semi-amplexicaule, margins entire or slightly serrulate, apex long-acuminate, 1-nerved, thin and flaccid, pale green to pink, glabrous; aerial or upper leaves 5–14 2 ×–4 mm, linear, ovate-lanceolate, base cuneate to attenuate, semi-amplexicaule, margins entire or microscopically serrulate, apex truncate to slightly bi-mucronate or rarely obtuse or acute, green or reddish, slightly fleshy, lateral veins indistinct, glabrous. Flowers monomorphic, sessile, axillary, solitary; floral bracts like foliage leaves and decreasing in size towards apex, exceeding the flowers; bracteoles 2, 0.5–1.3 mm long, linear-subulate, acuminate, as long as or shorter than calyx tube; calyx tube 1–1.3 mm long, campanulate to urn-shaped; lobes 3–5, 0.15–0.2 mm long, triangular; calyx appendages alternating with calyx lobes, 0.1–0.6 mm long, linear- subulate, two times the size of calyx lobes, rarely rudimentary or absent; petals 3–5, minute, up to 0.2–0.5 mm long, narrowly obovate, trilobed, apiculate, often entire at apex, hyaline, persistent, sometimes absent; stamens 3–5; filaments inserted at the base of the calyx tube or below middle of calyx tube, 0.4–0.5 mm long; anthers level with the top of the calyx tube, globose-oblong, reddish. Nectar scale absent. Ovary 0.5–0.7 × 0.4–0.6 mm, subglobose, slightly trilobed; style short, ca. 0.2 mm long; stigma capitate, papillose. Capsules 1.8–2.2 × 1.6–2 mm, globose, obscurely trilobed, exceeding the calyx, red, and 3 valved. Seeds many, 0.3–0.4 × 0.2–0.3 mm, plano-convex shape, slightly hairy when soaked in water, green to yellow, rarely slightly red.

Phenology: Flowering and fruiting: June-March

Distribution: India, Indonesia, Southeast Asia, New Guinea, Korea, South China, Japan

Specimen examined:

India, *Klein 3086* (M). Peninsula India Orientalis, 1867, *Wight s.n.* (M); *sine loc*., 1867, *Hance s.n.* (K). Andra Pradesh, Mohli tank, 6 November 1960, *M. P. Balakrishnan 11512* (MH); Sankarghat Hill, 21 September 1959, *K.M. Sebastine 8914* (MH). Chittoor district, Thirumala Hills, 7 July 1987, *D. Rangacharyulu 1595* (MH). Karnataka, Chikkaballapur district, Nandi Doorg Mysore, 26 December 1911, *Rudolph D Anstead 116* (MH). Bagalkot district, Sirur, 9 September 1970, *G. V. Subbarao 36585* (MH). Udupi district, Kollur, 28 September 1991, *U. Sreekala 5079* (CALI); *Ibid*., 28 October 1991, *P. Sreeja 3183* (CALI); *Ibid*., 27 August 1991, *T.S. Devi 1131* (CALI); *Ibid*., 28 October 1991, *C. Thulasi 5657* (CALI); *Ibid*. *A. V. Sreeja 4660* (CALI); Kundapur, 28 October 1991, *A.A. Pushpamani 2263* (CALI); *Ibid*., 28 October 1991, *Jaisy Immanual 1553* (CALI). Shimoga district,

Nagodi, 28 October 1991, *V.T. Smitha 3873* (CALI); Malgode, 4 December 1989, *V.N. Ushakumari 4935* (CALI); *Ibid*., 4 December 1989, *A. Mini 4035* (CALI); *Ibid*., 4 December 1989, *Betsy K Mathai 1648* (CALI). Chikkamangaluru district, Sringeri, 5 Dec 1989, *K. Rejitha 2044* (CALI). Dakshina Kannada district, Mangaluru 8 November 2015, *K.M. Lemiya 132957* (CALI). Kerala, Kannur district, Kannoth Reserve forest, 21 December 1979, *V.S. Ramachandran 60089* (MH); Thellissery, 17 August 1978, *V.S. Ramachandran 63980* (MH); Panur Elangad, 21 December 1979, *V.S. Ramachandran 65367* (MH); Madayippara, Thavarathadam, 6 Dec 2012, *C. Pramod 290350* (CALI); *Ibid.,* 6 December 2012, *C. Pramod 134017* (CALI); *Ibid*., 15 September 2023, *C. Murshida 727* (BAM); *Ibid*. 17 September 2023, *C. Murshida 729* (BAM); Chemperi 12 Dec 1991, *Sheena Jacob 3518* (CALI); Meenkulam 2 July 2012, *K.S. Prasad 03106* (MBGH). Wayanad district, Muthanga, 28 December 2023, *C. Murshida 903* (BAM). Kozhikode district, Calicut 12 February 1978, *Asha K Nair 24302* (CALI); Poovattuparamba, 10 September 1999, *P.M. Krishnan 786* (MBGH); Pokkunnu, 14 September 2009, *Ajay Kumar 642* (MBGH); *Ibid.*, 1 September 1999, *P. M. Krishnan 778* (MBGH); *Ibid.*, *M. Liji 13830* (MBGH); Malappuram district, Calicut University Campus, 8 October 1990, *P.K. Shalima 3336* (CALI) Calicut University Botanical Garden, 21 January 2014, *K.M. Lemiya 132964* (CALI); Olipram Kadavu 7 August 1989, *N. Latha 1133* (CALI); *Ibid*., 22 November 1989, *Solly George 3395* (CALI); *Ibid*., 4 February 1989, *Beena T Cheriyan 2749* (CALI); Parappanangadi, 6 August 1986, *A. Babu 34951* (CALI); Munderi, 17 February 2007, *K. K. Suresh* and *P.K. Jeris 03276* (MBGH). Moothampadam, 17 August 2005, *K.K. Krishnan* (MBGH); Mangalam, 28 October 2023, *C. Murshida 894* (BAM); Valakkulam, 29 February 2024, C. *Murshida 980* (BAM). Palakkad district, Olavakkode, 16 October 1963, *J. Joseph* 17709 (MH); Mezhukumpara, Mannarkkad 6 November 2013, *K.M. Lemiya 132953* (CALI); Thrithala, 15 November 2013, *K.M. Lemiya 132958* (CALI); *Ibid*., 24 October 2023, *C. Murshida 893* (BAM); Pattambi, 28 November 2023, *C. Murshida 900* (BAM); Chittoor, 29 November 2023, *C. Murshida 901* (BAM). Thrissur district, Guruvayur, 6 September 1976, *K. Ramamurthi 47653* (MH); Mayannur, 7 September 2023, *C. Murshida 724* (BAM). Idukki district, Anakkara, 24 Feb 2024, *A.J. Robi 978* (BAM); Thannikkudy 6 November 1996, *Jomy Augustine 17033* (CALI). Alappuzha district, Nedumudi, 11 October 2013, *K.M. Lemiya 132949* (CALI). Thiruvananthapuram district, Kallara, 30 November 1991, *K. C. Joseph 45827* (CALI); Chirayankeezhu, 14 September1989, *K.K. Suresh 04576* (MBGH). Tamil Nadu, Kappakkanam 28 May 1982, *C.N. Mohan 74058* (MH). Ramanathapuram district, Peripattanam, 29 December 1989, *V. Balasubrahmanyam 2350* (MH). Nilgiris district, Balmadies Estate, 18 December 1958, *K. Subrahmanyan 7539* (MH); Dindigul district, Kodaikanal 12 March 1982, *Santhi K Nair 2148* (CALI); Namakkal district, Kumarapalayam, *K.M. Lemiya 132971* (CALI). Coimbatore district, Coimbatore, December 1847, *Wight 1096*(K); Madras district, Marmelong River, Madras, *S.coll., s.n.* (K!); Puthukottai district, Narthamalai, 19 October 2023, *C. Murshida 891* (BAM). Indonesia, *Blume s.n.* (L); *Zollinger1880* (BR); Java, *Horsfield s.n.* (U). Japan, *Poiret 967* (L); *Buger s.n.* (L); Japonia, *Buger s.n.* (M). Sri Lanka, *Macrae James172* (BR); Japonica, *Seibold s.n.* (M). Bangladesh, *Wallich 2102B* (BR).

Acknowledgments

We thank The Principal, St. Thomas College Kozhencherry, Pathanamthitta, and Bishop Abraham Memorial College Thuruthicad, Pathanamthitta, for the facilities. The authors are thankful to the authorities of all cited herbaria for their help in herbarium consultation and for making the specimen available digitally. We are great full to SAIF, Mahatma Gandhi University for the facilities provided. MC is thankful to University Grants Commission for the financial assistance (515/CSIRUGCJUNE2019).

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DOI:10.22192/ijarbs.2025.12.10.003	

How to cite this article:
Murshida Chakkamattil & Aloor Jose Robi. (2025). Re-visiting the taxonomy of *Rotala rosea* (Lythraceae) in South India. Int. J. Adv. Res. Biol. Sci. 12(10): 24-33.
DOI: http://dx.doi.org/10.22192/ijarbs.2025.12.10.003