

Morpho-molecular analysis and extended distribution of endemic *Jasminum* species (Oleaceae)

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Short Report

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Abstract

Jasmine is widely associated with aromatic applications, pharmaceuticals, phytochemicals and cosmetic industries. Application and proper identification of wild plants are becoming increasingly common globally. A detailed morphological description, domatia characters, foliar epidermal characteristics (crystal, epidermal cell wall pattern, trichome), venation types of three wild, strong aromatic Jasmines with their extended distribution are given here and also provided taxonomic key for differentiating them from related species. The morpho-taxonomic details with some additional key features and molecular characterization were performed here for proper identification. The molecular phylogeny of studied species and their closest taxa is detailed here based on the Internal Transcribed Spacer (ITS) consensus sequence of nuclear-ribosomal DNA and highlights species specific nucleotide variations. *Jasminum azoricum* L. and *J. malabaricum* Wight are documented to have extended distribution beyond Western Ghats (India), while *J. adenophyllum* Wall. ex C.B. Clarke is reported here outside of Assam to Peninsula Malaysia. Critically endangered species *J. azoricum* is resemblance with *J. flexile* Vahl, but can be distinguished by the total number of flowers (8–19) per inflorescence; absence of leaf articulation; particular secondary venation type; calyx teeth length; number of petals, sepals. The fruits of *J. malabaricum*, a species native to the Western Ghat, are consumed by local tribals of Burdwan (India).

Introduction

Jasminum L., the largest genus of Oleaceae Hoffmanns. & Link, has 201 accepted species worldwide (POWO 2024). India is a home of few cultivated Jasmines (*J. auriculatum* Vahl, *J. grandiflorum* L., *J. sambac* (L.) Aiton) due to their aroma, floral beauty, phytoconstituents and high medicinal values but a large number of wild *Jasminum* still present here but remaining unexplored. During revising of Oleaceae in India, authors located several *Jasminum* species with intriguing taxonomic identities and phytogeography.

Clarke (1882) reported *J. adenophyllum* from Khasi and Jaintia hills of Meghalaya (India). Later, Srivastava & Kapoor (1984) stated this plant as rare and endemic to Meghalaya only and detailed in depth based on existent literature and a sheet of ASSAM herbarium collected by G. Panigrahi in 1956 from Garampani, Jaintia hills of Meghalaya.

J. azoricum L., is a critically endangered plant native to Madeira Islands, Portugal species (Sequiera et al. 2010; Fernandes 2013; Kumar & Sabeena 2013; IUCN 2024). Linnaeus (1753) inadvertently mentioned its habitat in India, but subsequent researchers have not reported it. Despite its distribution being documented after a long period in Arunachal Pradesh, Kerala, Meghalaya, Tamil Nadu of India, (Manilal & Sivarajan 1982; Haridasan & Rao 1987; Sasidharan 2004; Giri et al. 2008) but these literatures (except Kumar & Sabeena 2013) were matched with *J. flexile* Vahl.

The taxonomic confusion between *J. azoricum* and *J. flexile* began with Matthew & Rani (1983) and some later workers (Mohan 1985, Haridasan & Rao 1987, Sasidharan 2004, Giri et al. 2008) referring *J.*

azoricum as accepted alongside its synonyms either *J. flexile* Vahl or *J. flexile* var. *ovatum* Wallich ex C. B. Clarke. *J. flexile* Vahl is now a completely distinct species (Kumar & Sabeena 2013; IPNI 2024; POWO 2024; WFO 2024) and *J. flexile* var. *ovatum* being a synonym of *J. flexile* Vahl. and unrelated to *J. azoricum*. Simultaneously, *J. azoricum* var. *travancorensis* (Gamble) M.Mohanan is a synonym of *J. flexile* Vahl.

Kumar & Sabeena (2013) reported *J. azoricum* from Kerala, a recent Indian distribution with distinguishing it from *J. flexile*, by shorter leaves without domatia; calyx lobes 0.5–1 mm long; 7–8 lobed corolla and shorter corolla tube (10–15 mm). *J. azoricum* flower, leaf is valuable for pharmaceutical purposes and contains good amounts of alkanes, amines, esters and aromatic compounds (Hari et al. 2018).

J. malabaricum, an endemic plant of Western Ghat (India), is known for its antioxidant, antitumor, antibacterial, and blood purifying capabilities, but high demand leads to reduction of its natural community (Hurakadle et al. 2012; Gadkar et al. 2014).

So, a thorough review reveals that till date *J. azoricum* and *J. malabaricum* are not validly recorded outside of Western Ghats, India and *J. adenophyllum* is also undocumented outside of Assam, India. The present work attempts to explore their new distribution, morphological characterization and potential local application if any. Now days, molecular study is increasingly used with traditional taxonomic parameters to remove any taxonomic ambiguities. Hence, morpho-molecular characterization was performed for proper identification and finding relationship with closest taxa.

Materials and methods

Sample collection, morphological observations

J. azoricum and *J. adenophyllum*, *J. malabaricum* were collected from Jalpaiguri and Paschim Burdwan (West Bengal) districts respectively from natural habitat (Fig. 1). Raw samples were analysed under stereo zoom microscope (Olympus, SZ2-ILST 8A11112), trinocular upright microscope (Magnus, 528013) and dry leaf samples under Scanning Electron Microscope (SEM) (JEOL JSM-IT100) followed by different authors Hickey 1973; Barthlott 1981; Modak & Chowdhury 2021). Plants were identified using various floras (Clarke 1882; Gamble 1967; Manilal & Sivarajan 1982; Matthew & Rani 1983; Haridasan 1987; Pradhan 2001; Sasidharan 2004; Giri et al. 2008), literatures (Green 2003; Kumar & Sabeena 2013) and herbarium images of BM, CAL, CALI, E, G, IVH, K, NY, P, RRLH (abbreviations after Thiers 2021).

DNA extraction, PCR amplification, sequence submission

Genomic DNA was extracted from fresh leaves using DNA Extraction Kit (Promega) with CTAB method (Doyle & Doyle 1990). 10.5 µL PCR mixture contained 2X PCR Master Mix (5µL), ddH₂O (4µL), DNA template (1µL) and each primer (0.25µL). ITS region (ITS1-5.8S-ITS2) (Fig. 2) was amplified using

ITS1/ITS4 (White et al. 1990) primers. PCR thermal cycles of denaturation for 30 s at 98 °C, followed by 40 cycles of 5s at 98 °C, 10 s at 58 °C and 15 s at 72 °C, elongation for 60 s at 72 °C and final extension at 4 °C until it is used for gel analysis and sequencing. PCR amplified and ethidium bromide-stained products were observed under 1% agarose electrophoresis gel. PCR products were sent to a commercial sequencing provider for purification and sequencing. The raw sequence and chromatograms were thoroughly checked and trimmed if required and created in FASTA format. BLAST search was conducted to find closest samples and submitted the sequence to NCBI.

Sequence alignment and phylogenetic analyses

The sequence data from these samples were matched with related species in GenBank (<https://blast.ncbi.nlm.nih.gov>) using nucleotide BLAST searches. Data were aligned using MEGA 11.0.13 (Tamura et al. 2021) and manually modified in BioEdit v. 7.0 (Hall 2004) as required. Maximum likelihood (ML) analysis was done using 1000 replicates under Tamura best fit substitution model with MUSCLE algorithm on MEGA, resulting in a phylogenetic tree that was modified in FigTree v1.4.0 (Rambaut 2012).

Results

Taxonomy

***Jasminum adenophyllum* Wall. ex C.B. Clarke**, Wall. Cat. 2876; Clarke in Hook.f., Fl. Brit. India 3: 597. 1882 (Fig. 2)

Type

INDIA. Meghalaya, Mt Silhet, Wallich 2876 (Holotype, K001118106!); MALAYSIA. Penang, 1831, King's Coll. 1736 (Isotype, CAL0000017753!).

Scandent shrub, wiry climber. Matured stem woody, glabrous, young stem puberulent. Leaves opposite, unifoliate, entire, dark green, glossy, elliptic-oblong, apex obtuse to acuminate, base cuneate, 50–88 × 24–50 mm, glabrous (except midrib); non-glandular, papillate, upright trichomes present along the midrib and base; petiole 4–11 mm glabrous or slightly pilose on abaxial surface at young stage; midrib and veins prominent on adaxial surface, 4–8 secondary veins on each side of midrib, secondary veins forms arching, free ending ultimate veins are 2–more branched; prominent tufted domatia at abaxial surface vein axils. Inflorescence axillary or terminal cyme, 1–5 flowered, glabrous, flowers white, peduncle 7–16 mm long; calyx tube 2–3 mm long, sepals subulate, glabrous or glabrescent (at young stage), 7–11 mm long; corolla tube 9–15 mm, petals 6–9, spreading and recurving, narrow, 10–17 mm long, very sweet fragrant; bracts linear, up to 1.5 mm. Stamens 2, anthers c. 3.5 mm. Pistil 7–10 mm long, glabrous, stigma bifid. Fruit single or paired berry, spherical, greenish to purplish-black, glossy (Fig. 2).

Distribution and ecology

Andaman, Bangladesh, India (Meghalaya and now from West Bengal), Laos, Peninsular Malaysia, Thailand, Vietnam (Fig. 1). 5–7 large populations were found in association with *Cleodendrum infortunatum* L., *Auxonopus* sp.

Phenology

May–July.

Specimen examined: INDIA. West Bengal: Burdwan, Durgapur, 23.659444N & 87.324722E, 26.05.2022, 20220526K (NBU).

Additional specimens examined: INDIA. Andaman & Nicobar Islands: Little Andaman, 30 November 2005, *L. Rasingam* 25864 (PBL0000012840); Little Andaman, 30 November 2005, *L. Rasingam* 25864 (PBL0000012841); Assam: Garampani, 35 miles east of Jowai, Khasi and Jaintia hills, 30 October 1956, *G. Panigrahi* 4206, (ASSAM0000049326); Shillong, 15 July 1970, N.P Balakrishnan, Sheet no.14931; LAOS. Namtha, June 1929, *F. Kingdon-Ward* 8977 (NY3146963); Xiangkhouang, 19° 36' 33.48" N & 103° 43' 44.112" E, 11 November 1920, E. Poilane 2332 (MNHN-P-P04492681); MALAY ARCHIPELAGO. 100ft, May 1881, *Dr. King's Collection* 1736 (CAL0000017753); THAILAND. Siam: Chiangmai, 1891, *A. F. G. Kerr* 3.7.11 (MNHN-P4046536); Trang: Pahin, 50m, 25 June 1930, *A. F. G. Kerr* 19099 (K000901455); Trang: Pahin, 50m, 25 April 1930, *A. F. G. Kerr* 19099 (MNHN-P-P00644249); VIETNAM. Cochinchine: Dinh Quan, 3 December 1932, *E. Poilane* 21578 (MNHN-P-P00267134).

***Jasminum azoricum* L., Sp. Pl. 7: 1753 (Fig. 3)**

Type: – INDIA. 1753, *G. Clifford s.n* 5 (lectotype, BM000557520!) designated by Wijnands 1983: 156); NETHERLANDS. *G. Clifford s.n* (Original, BM000557521!).

Scandent shrub, pendulous branching pattern. Stem glabrous, very minute white gland present in young stage, matured stem woody with greyish striations. Leaves opposite, trifoliate, entire, dark green, glabrous, petiolate, elliptic-ovate or deltoid-ovate, apex acute to acuminate, base acute or cuneate, asymmetrical sometimes, terminal leaflet 30–61 × 14–30 mm long, lateral leaflets 22–43 × 10–24 mm, shallowly impressed above; druse crystal present on abaxial surface; domatia present at 1st and 2nd vein axils at lamina base; secondary veins 4–7 in each side of midrib, free ending ultimate veins 2–more branched; shallowly impressed above and raised in abaxial surface; petiole 10–26 mm, glabrous; terminal leaflet petiolule 8–13 mm, lateral leaflet petiolule 3–8 mm. Inflorescence axillary or terminal cyme, glabrous to very minutely glabrescent; flowers white, sweet fragrant; pedicel 3–4 mm, glabrous or very minutely hairy; peduncle 5–18 mm; bract subulate, 2–3 mm, minutely hairy; bracteole 1–2 mm; calyx lobes 5, minute, 0.5–1 mm, calyx tube 2–4 mm, glabrous. Corolla tube 15–21 mm, lobes 6–8, 9–14 mm long, white, fragrant, salverform. Stamen 2, anther 3–5 mm, apex apiculate. Stigma very minutely bifid, style 12–20 mm, ovary 2 mm. Fruit ellipsoid, immature fruit length 2.5–5 mm (Fig. 3).

Notes

Domatia hairs are very small (0.15–0.3 mm), multicellular, eglandular, uniseriate, slender, surface papillate type and may not visible through naked eyes or under simple microscope.

Distribution and ecology

Portugal, India (Kerala and now from West Bengal). (Fig. 1). Single population was found in partially shaded, road side area in climbing condition with *Ficus religiosa* L.

Phenology

May–November

Specimen examined: INDIA. West Bengal: Jalpaiguri, Way of Jalpaiguri to Haldibari, 26.533611N, 88.658611E, 14.05.2022, 20220514K (NBU).

Additional specimens examined: ENGLAND. London: 1733, *Royal Society & H. Sloane 564* (BM000810172) (not natural condition); INDIA. Karnataka: Mysore, Mahadeswara hills, 7 April 1979, *S. B. Manohar, K.P. Sreenath KFP6635* (CAL0000212274); Kerala: Way to Vellerankuthu, 22 November 1991, *P. Bhargavan 98673* (MH 0012290); Kerala: Peerumede. Road side. 11 November 1988, *V. Geetha 1850* (CALI145518); Chandanathode-Theethundimala, 28 January 1987, *R.T.Balakrishnan 42751* (CALI162960); PORTUGAL. Madeira: 1948, *A.P. Lang*. (NY03146650); Madeira: 1000–1200 m, 1865–1866, *G. Moandon 172* (NY03146651); NE Atlantic Islands, 1891, *J. Ball 172* (E00747980).

Jasminum malabaricum Wight, Icon. Pl. Ind. Orient. 4(1): 13. 1848 (Fig. 4)

Type

– INDIA. Calicut, March 1846, *R. Wight s.n.* (Holotype, K000545646!).

Large, straggling, woody climbing shrub. Leaves simple, entire, opposite, petiolate, glabrous, broadly ovate, or elliptic-ovate to cordate, apex acuminate, base rounded to cordate, 40–120 × 22–73 mm; glandular, peltate trichome present on both surface; striated cuticle on abaxial surface; lateral veins 5–10 pairs in each side of midrib, free ending ultimate veins 1-branched; domatia absent; petiole 7–12 mm long, minutely puberulent. Inflorescence a trichotomous, terminal cyme, 3–many flowered; bud very pointed, greenish-white, bracteate 0.5–1 mm; pedicel 5–8 mm long; peduncle 15–35 mm long; calyx persistent, pubescent, sepals 5–7, calyx teeth 3–4 mm, corolla white, sweet fragrant, single whorled, petals 7–9, very acute, linear; 10–16 mm long; corolla tube 10–18 mm, greenish white to light brown; Ovary 1.5–2.5 mm, stigma bifid; anthers 3 mm, filaments 0.5–1 mm, Fruit a berry, ovoid, single, greenish to purplish black, glossy (Fig. 4).

Notes: *J. malabaricum* fruit is edible by local ‘Murmu’ tribe. Tiny hairs on calyx, petiole was observed under microscope. It may not be seen with naked eyes. Few microscopic reddish glands were observed on petiole.

Distribution and ecology

India (Andhra Pradesh, Goa, Gujrat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu and now from West Bengal) and Sri Lanka. (Fig. 1). Six individuals of *J. malabaricum* was observed in dry, grey and laterite soil areas of shaded deciduous forest of *Shorea robusta* Roth, *Madhuca longifolia* (J.Konig) J.F.Macbr., *Terminalia arjuna* (Roxb.) Wight & Arn. and it thrives sheltering bushes like *Lantana camara* L., *Chromolaena odorata* (L.) R.M.King & H.Rob.

Phenology

March–June.

Specimen examined: INDIA. West Bengal: Burdwan, Madhaiganj, Durgapur-Faridpur, 23.661865N & 87.320553E, 13.06.2022, 20220613K (NBU).

Additional specimens examined: INDIA. Karnataka: Uttara Kannada, Kumta, 28 April 1939, *N. L. Bor* 11377 (NY 3146740); Kerala: Calicut, 1846, *R. Wight s.n.* (K); Kerala, 15 June 2000, *M.K. Ratheesh Narayanan* 3401 (CALI 162956); Madayippara, 2 March 2011, *C. Pramod* 126656 (CALI128825); Madayippara, 2 March 2011, *C. Pramod* 126656 (CALI128824); Calicut, 27 February 1972, *E. Nabeesa* 31 (CALI145583); Kerala, Madayippara, climber zone, 14 December 2011, *C. Pramod* 126935 (CALI128821); Maharashtra: Mahabaleshwar, 6 April 1956, *G. S. Puri* 271 (CAL0000212275); Mahabaleshwar, 1310.6 m, 5 May 1937, *R.L. Badhwar* 4785 (RRLH 6643); Tamil Nadu: Madras, 1871; 1911, *R. Wight* (K); Salem, 10 May 1978, *A. Mohan* 13512 (CAL0000031070); Nagariyar Estate, 30 April 1991, *S. R. Srinivasan* 96767 (BSID0012310).

Analysis of ITS sequence of three Jasmine

DNA sequencing is an excellent approach for measuring genetic differences at the nucleotide level and finding SNPs in evolutionary conserved gene regions. Ribosomal RNA (rRNA) is a part of a multigene family, encodes the RNA component of the ribosome. In eukaryotes, nuclear copies of rRNA are arranged in tandem arrays, with each unit consisting of genes for small and large rRNA subunits (18S and 26S). The 5.8S nuclear rRNA is embedded between these genes but separated by ITS1 and ITS2. The ITS regions evolve faster and may vary at lower taxonomic levels than plastid loci (Mort et al. 2007, Song et al. 2012, Duan et al. 2019).

Collected plants were investigated with ITS primers which amplified both rRNA genes and ITS regions. A 546 bp sequence of *J. azoricum* containing ITS1, partial sequence; 5.8S rRNA gene, complete sequence and ITS2, partial sequence was submitted to NCBI (accession no. OR886094). 597 bp and 632 bp containing ITS1, partial sequence; 5.8S rRNA and ITS2, complete sequence and LSU, partial sequence of *J. malabaricum* and *J. adenophyllum* was submitted and accession no. OR921383 and PP064888 were obtained respectively.

The BLAST analysis of *J. adenophyllum* showed 93.93–94.47% similarity with *J. sambac* and *J. malabaricum* showed 94.17–94.33% similarity with *J. sambac* whereas, *J. azoricum* showed 97.07% similarity with submitted *J. azoricum* (accession no. MG727721), 96.07% similarity with *J. fluminense*, 96.34% similarity with *J. nummularifolium* and 94.59% with available *J. flexile* sequence in NCBI. *J. malabaricum* had the highest GC content (62.7%) among the studied plants (Fig. 6). Phylogenetic tree was prepared to relate these three *Jasminum* species with their closest relatives (Fig. 7).

Discussion

J. azoricum shares similarities with *J. fluminense*, *J. flexile* and *J. caudatum* due to presence of trifoliolate leaf, elliptic or deltoid-ovate leaflets; white petals. Whereas, *J. malabaricum* and *J. sambac* share a simple, unifoliolate, ovate leaf, inflorescence more or less compact with 3–more flowers and similar length of corolla tube (9–18 mm). *J. adenophyllum* is closely related to *J. sambac* as presence of domatia on abaxial surface; 3–more flowered cyme, prominent venation beneath, corolla lobes similar length to corolla tube. Taxonomic key was prepared for easy identification and foliar micro-characteristics of the studied species were compared in Table 1.

Table 1
Comparative account on foliar micro-characteristic variations of studied species

Characteristics	<i>J. adenophyllum</i>	<i>J. azoricum</i>	<i>J. malabaricum</i>
Domatia	Present	Present	Absent
Crystal	Multi-faceted Calcium oxalate druses	Absent	Absent
Epidermal cell	Straight anticlinal walls	Straight or slightly sinuous anticlinal walls	Straight or slightly sinuous anticlinal walls with highly striated cuticle
Venation types	1× pinnate, 2× weak-brochidodromous, 3× alternate percurrent, 4× regular polygonal-reticulate, 5× vein dichotomizing, veinlets 1-branched	1× pinnate, 2× weak-brochidodromous, 3× random reticulate, 4× regular polygonal-reticulate, 5× vein dichotomizing, veinlets 2-more branched	1× pinnate, 2× weak-brochidodromous, 3× random reticulate, 4× and 5× vein dichotomizing, veinlets 2-more branched

Table 2 ITS sequence polymorphic sites of studied species and their related members

ITS	ITS1														5.8S	ITS2																						
Sl. No of polymorphic site	1	2	3	4	5	6	7	8	9	10	1	12	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3			
Nucleotide position	8	1	1	1	2	2	3	5	9	10	1	14	1	1	1	1	1	2	2	3	3	3	3	3	3	4	4	4	4	4	4	4	5	5	5			
	6	7	8	0	7	6	6	3	1	1	3	5	6	7	7	7	4	4	1	1	4	6	7	7	8	1	2	5	5	6	8	8	9	9	1	2	3	
										2			1	2	2	4	9	1	6	1	8	2	0	4	9	3	9	5	3	7	5	6	7	5	9	1	1	3
<i>J. adenophyllum</i>	C	A	A	A	C	-	A	T	A	C	T	G	A	G	C	-	C	C	C	C	G	C	C	G	G	C	C	G	-	G	C	T	A	T	T	T	C	C
<i>J. azoricum</i>	T	G	G	T	C	C	A	C	G	T	C	T	A	A	C	A	C	C	C	C	G	C	C	C	G	G	C	T	T	A	C	T	A	T	T	C	T	C
<i>J. flexile</i>	C	G	G	A	C	-	A	T	A	C	T	T	G	G	C	-	C	C	C	C	G	C	C	C	G	C	C	G	-	G	C	C	C	C	T	C	C	T
<i>J. fluminense</i>	C	G	G	T	C	-	A	C	A	T	C	T	A	G	C	-	C	C	C	C	A	C	C	C	G	C	C	G	-	G	C	T	A	T	T	C	C	C
<i>J. malabaricum</i>	T	G	C	G	T	-	A	T	A	C	T	T	A	G	T	-	T	G	A	T	G	A	T	C	A	C	T	G	-	G	C	T	A	C	C	C	C	T
<i>J. nummularifolium</i>	C	G	G	T	C	-	G	C	A	T	C	T	A	G	C	-	C	C	C	C	G	C	C	C	G	C	C	G	-	G	C	T	A	T	T	C	C	C
<i>J. sambac</i>	T	G	C	G	T	-	A	T	A	C	T	T	A	G	C	-	C	C	C	C	G	C	C	A	G	C	C	G	-	G	T	T	A	T	T	C	C	C

Key to the studied species and their related species:

- 1a. Leaf trifoliate; terminal and lateral leaflets petiolulate.....2
- 1b. Leaf unifoliate; petiolate but lateral leaflets and petiolules absent.....5
- 2a. Young stem densely hairy, matured stem glabrous to glabrescent; leaf velvet hairy at both surface. *J. fluminense*
- 2b. Young and matured both stem glabrous; leaf not velvet hairy.....3
- 3a. Leaf recurved; secondary veins 6–8 at each side of midrib; petiole 10–19 mm..... *J. caudatum*
- 3b. Leaf not recurved; secondary veins 4–7 at each side of midrib; petiole 10–30 mm.....4
- 4a. 15–30 flowered inflorescence; terminal leaf auriculate, lateral leaflets not auriculate; venation distinct and camptodromous-brochidodromous with strong loops along the lamina margin; bract linear; calyx teeth length < 0.25 mm; corolla lobes 5–6..... *J. flexile*
- 4b. 8–19 flowered inflorescence; auriculation absent; venation not clearly distinct, secondary veins camptodromous-eucamptodromous without forming strong loops; bract subulate with minute hairs; calyx teeth length 0.5–1 mm; corolla lobes 6–8..... *J. azoricum*
- 5a. Leaf apex acuminate, presence of reddish glands on petiole; domatia absent; bud apex sharply pointed; calyx not setaceous..... *J. malabaricum*
- 5b. Leaf apex acute to obtuse, absence of reddish glands on petiole; domatia present; bud apex not sharply pointed; calyx linear filiform to setaceous.....6

6a. Leaf broadly ovate; corolla layer double than one layered, petal ovate-oblong to orbicular..... *J. sambac*

6b. Leaf elliptic-oblong; corolla single layered, petal elliptic-lanceolate.....*J. adenophyllum*

Phylogenetic analysis revealed overall four clades (Fig. 7) were present in *Jasminum*. Clade I contain all members with trifoliate, opposite, deltoid ovate to elliptic-lanceolate leaves; clade II with unifoliate, simple, ovate, opposite or opposite-decussate leaves; clade III contains either penta to heptafoliate or unifoliate, opposite leaves, white with pinkish/reddish shade flowers; clade IV contains either simple or reduced trifoliate, opposite decussate leaves, white flowers. *Fraxinus* and *Nyctanthes* were selected as out group. Three examined species namely *J. adenophyllum*, *J. malabaricum* and *J. azoricum* grouped in one clade (Gr-1). Further analysis of phylogenetics revealed that *J. nummularifolium*, *J. fluminense*, *J. azoricum* and *J. flexile* grouped in one clade and *J. adenophyllum* & *J. malabaricum* grouped with *J. sambac* in another one clade showing similar morphological characters. The GC contents are utilized for speciation in closely related angiosperms and also for thermostability testing (Yu et al. 2021, Liu et al. 2023). We found highest GC content in *J. malabaricum* (Fig. 6), which consists larger leaf than *J. adenophyllum* and *J. azoricum*, and grows comparatively warmer locations.

Conclusion

Here, morphological study clearly supported by molecular data. Related speciation can be done with morphologically as well as genetically with their GC content and nucleotide variations (Table 2). *J. azoricum* and *J. flexile* key characters are revised here and concluded that domatia is present in both the species but domatia of *J. azoricum* is really very small for characterisation. Furthermore, our research clearly indicates that not only South-Indian cultivated species, Eastern and Northern India also have natural *Jasminum* distribution, which only needs to be critically investigated and identified. These findings will be beneficial for future floristic and molecular works.

Declarations

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflict of interests Authors declare that there is no conflict of interests.

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Figures

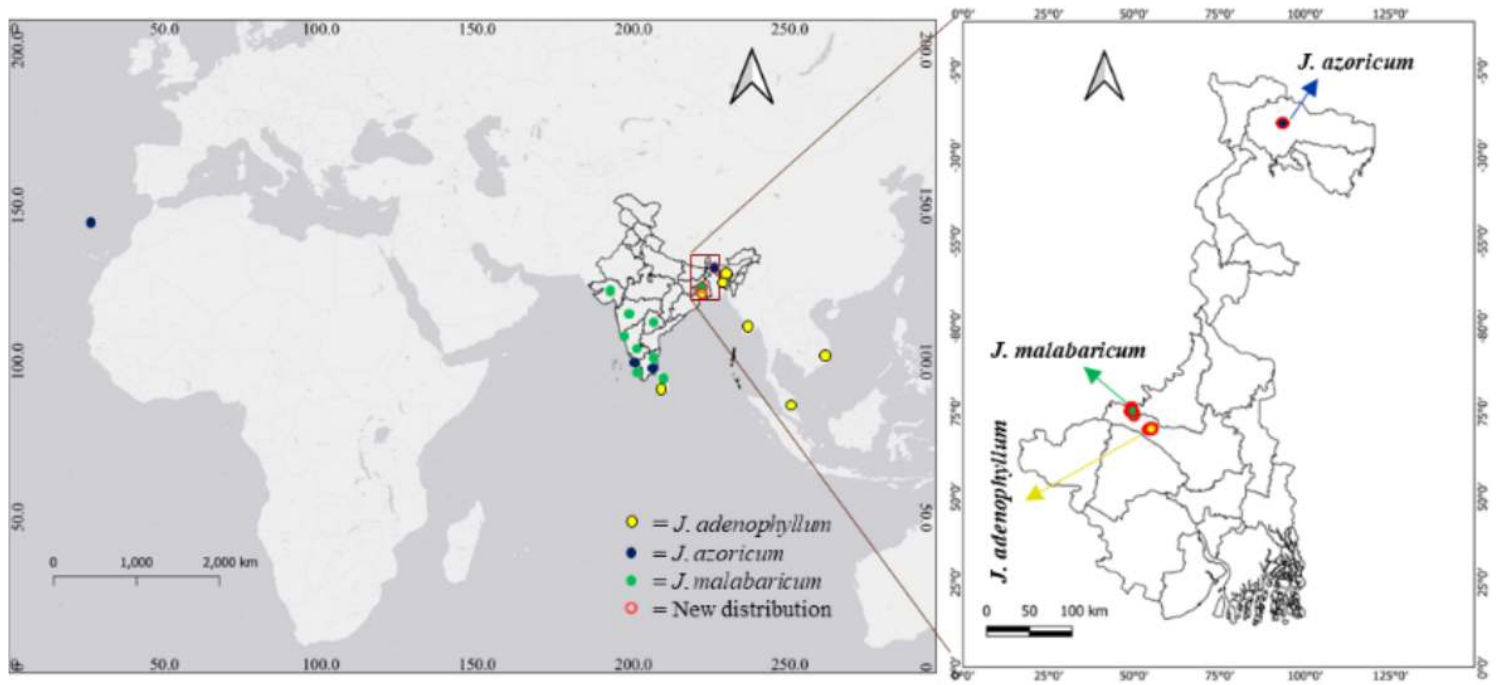


Figure 1

Distribution map of *J. adenophyllum*, *J. azoricum* & *J. malabaricum*

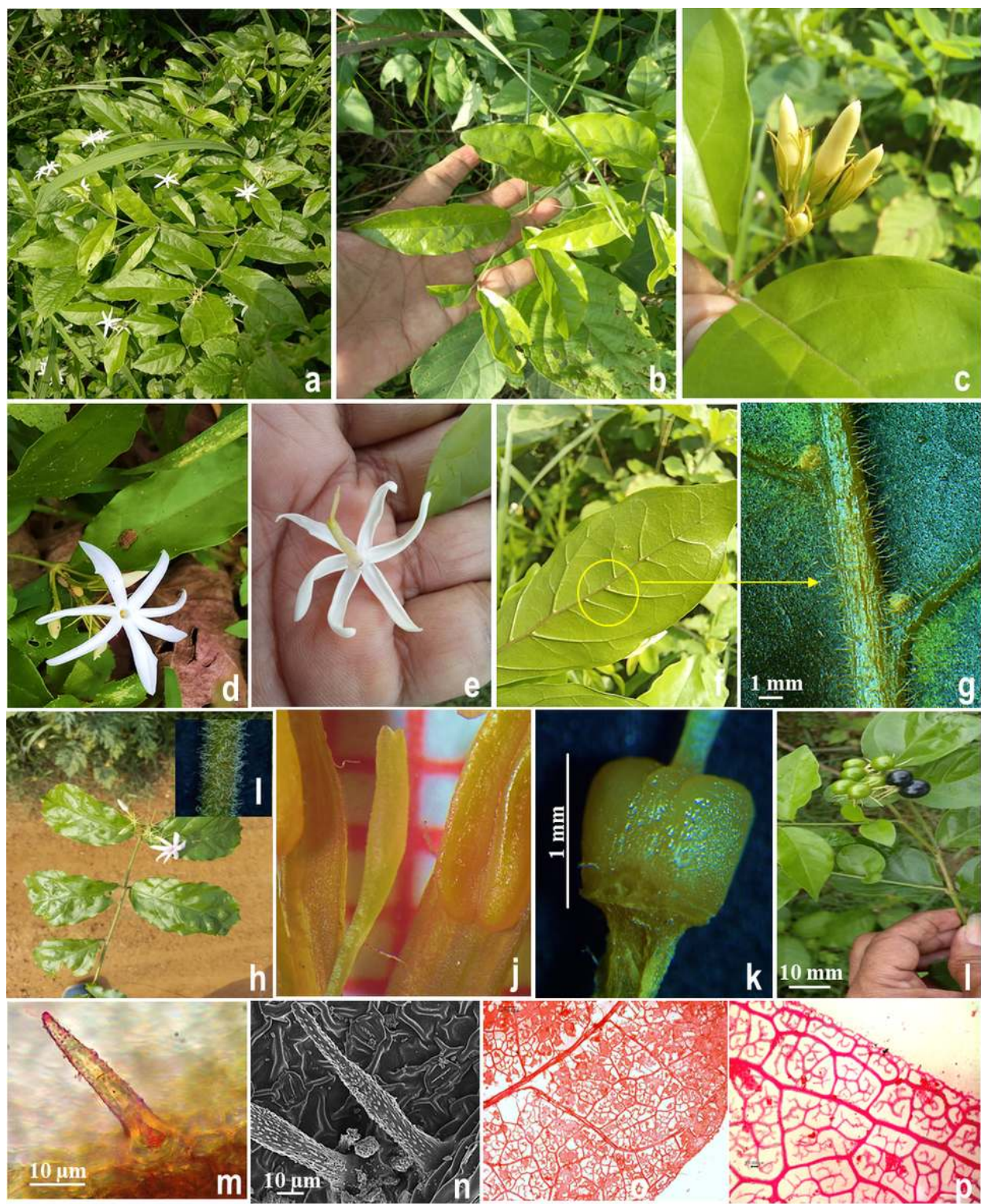


Figure 2

Jasminum adenophyllum Wall. ex C.B. Clarke. **a** Habit, **b** Adaxial, glossy lamina, **c** Bud, **d** Open flower, **e** Abaxial petal curling at tip, **f** Domatia, **g** Enlarge hairy midrib and domatia at abaxial surface, **h** Opposite leaves and terminal inflorescence, **i** Hairy young stem, **j** Bifid stigma, **k** Ovary, **l** Ripe and green fruits, **m** & **n** LM & SEM of upright, eglandular, papillate foliar trichome, **o** Partial view of reticulate venation, **p** Vein terminal

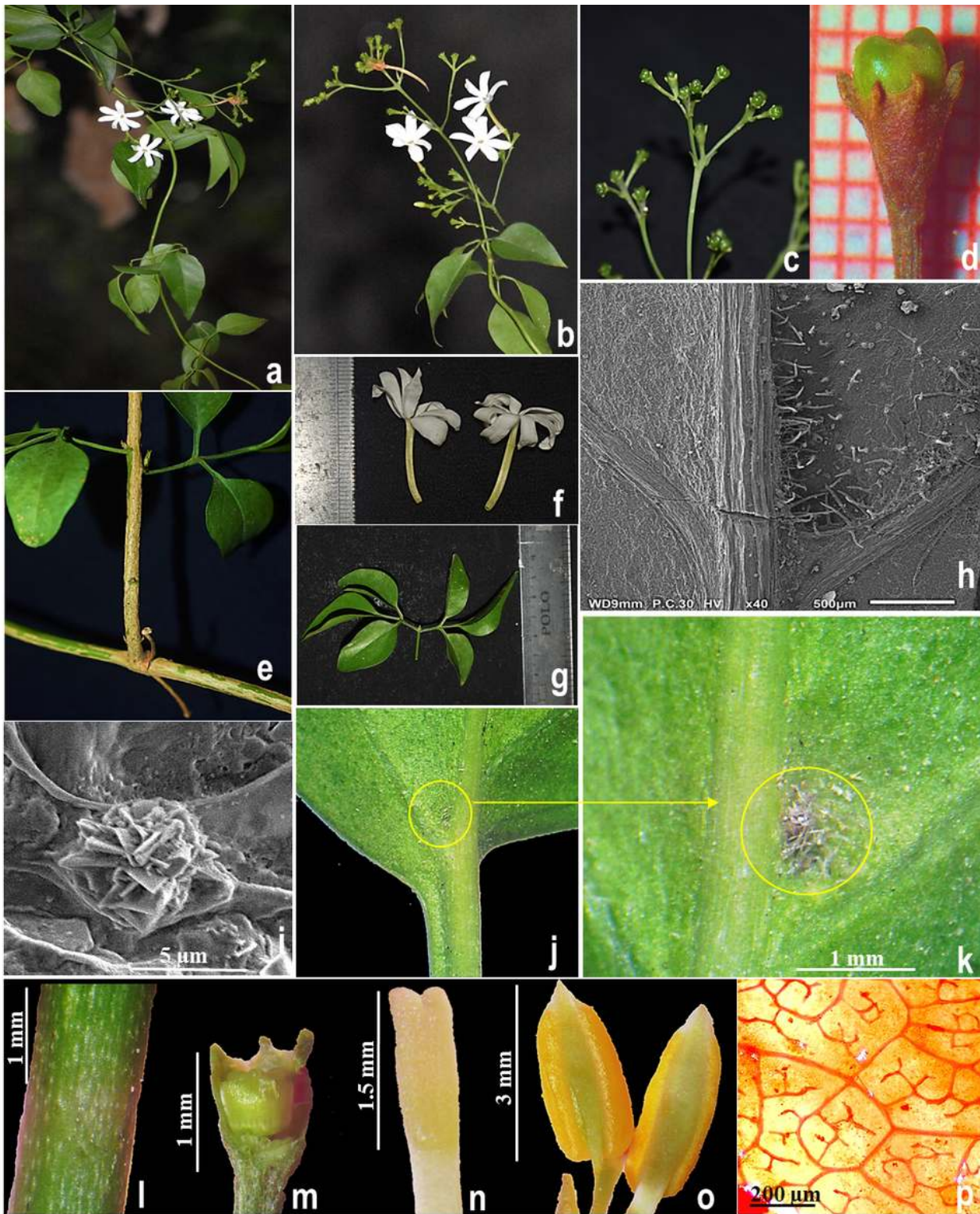


Figure 3

Jasminum azoricum L. **a** Habit, **b** Inflorescence, **c** Immature fruit, **d** Single immature fruit, **e** Matured stem, **f** Flowers, **g** Opposite trifoliate leaves, **h** & **i** SEM of domatia & druse crystal, **j** LM of domatia, **k** Enlarge domatia, **l** Young stem with white glands, **m** Ovary, **n** Bifid stigma, **o** Anthers, **p** Vein termination

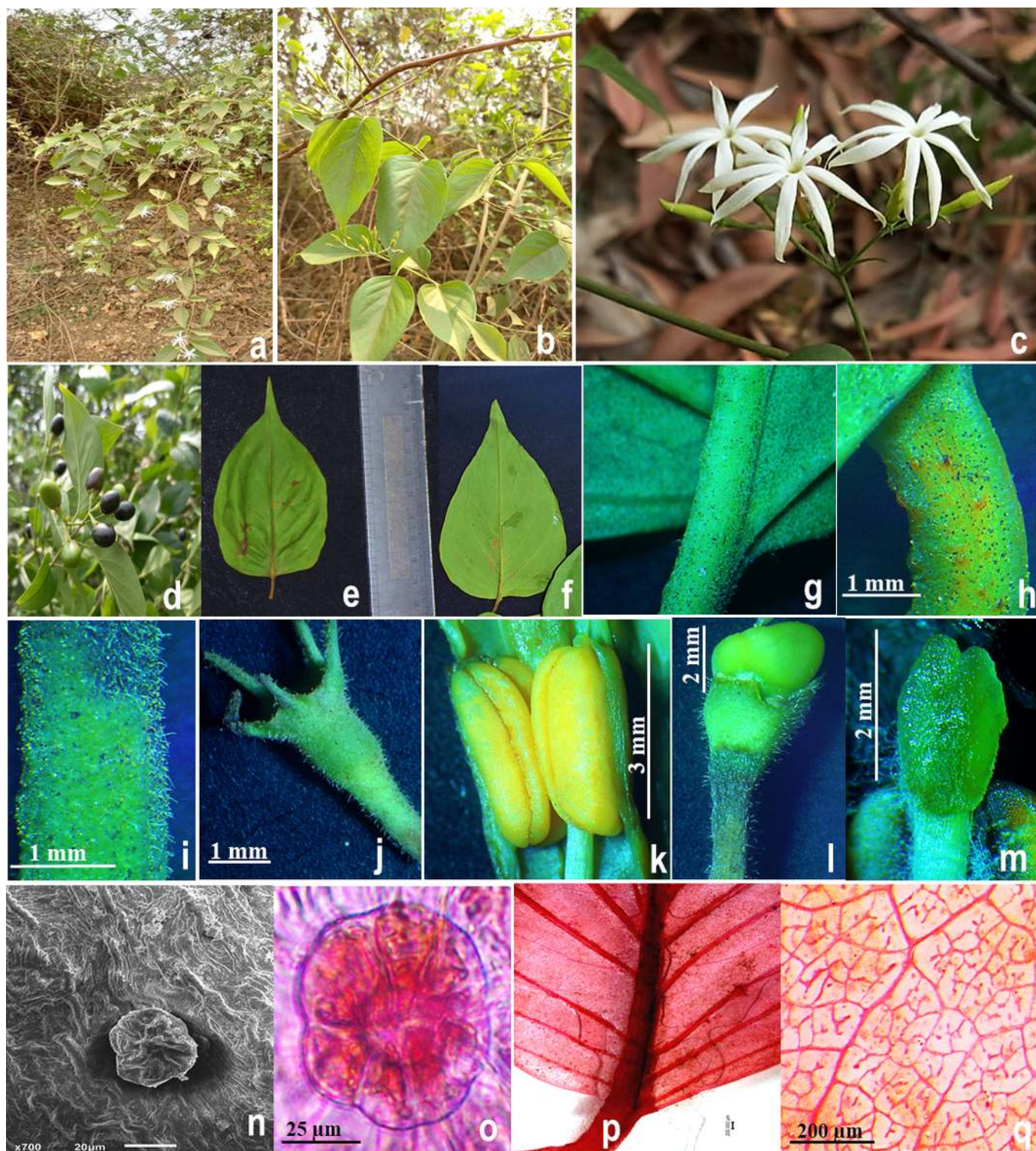


Figure 4

Jasminum malabaricum Wight. **a** Habit, **b** Enlarge view of leafy branch with buds, **c** Spread flowering twig showing lance shaped petals, **d** Ripe and green fruits, **e** & **f** Foliar adaxial & abaxial surface, **g** Asymmetrical leaf base, **h** Glandular reddish hairs on abaxial side of petiole, **i** Petiole hairs **j** Hairy calyx, **k** Anthers, **l** Ovary with pedicel, **m** Bifid stigma, **n** & **o** SEM & LM of glandular hair, **p** Foliar basal venation, **q** Vein termination

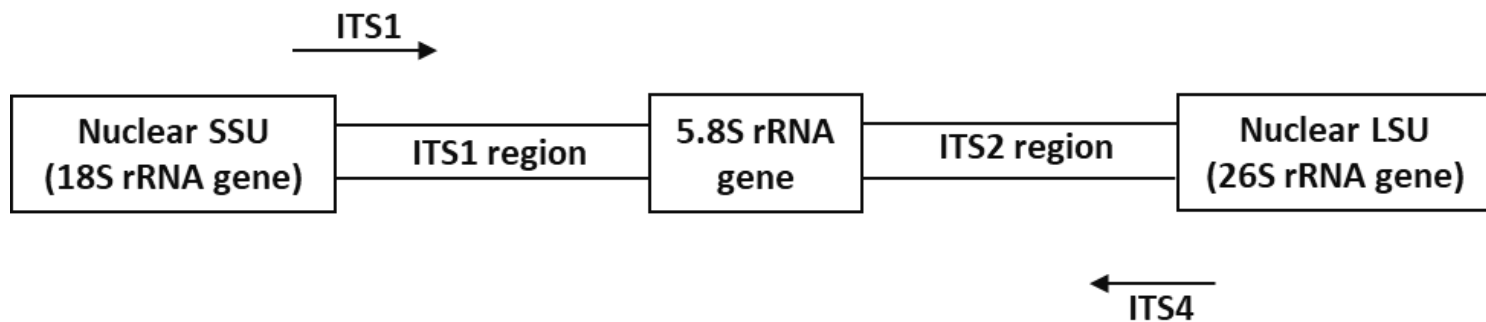


Figure 5

Nuclear ITS region and used primers attachment position.

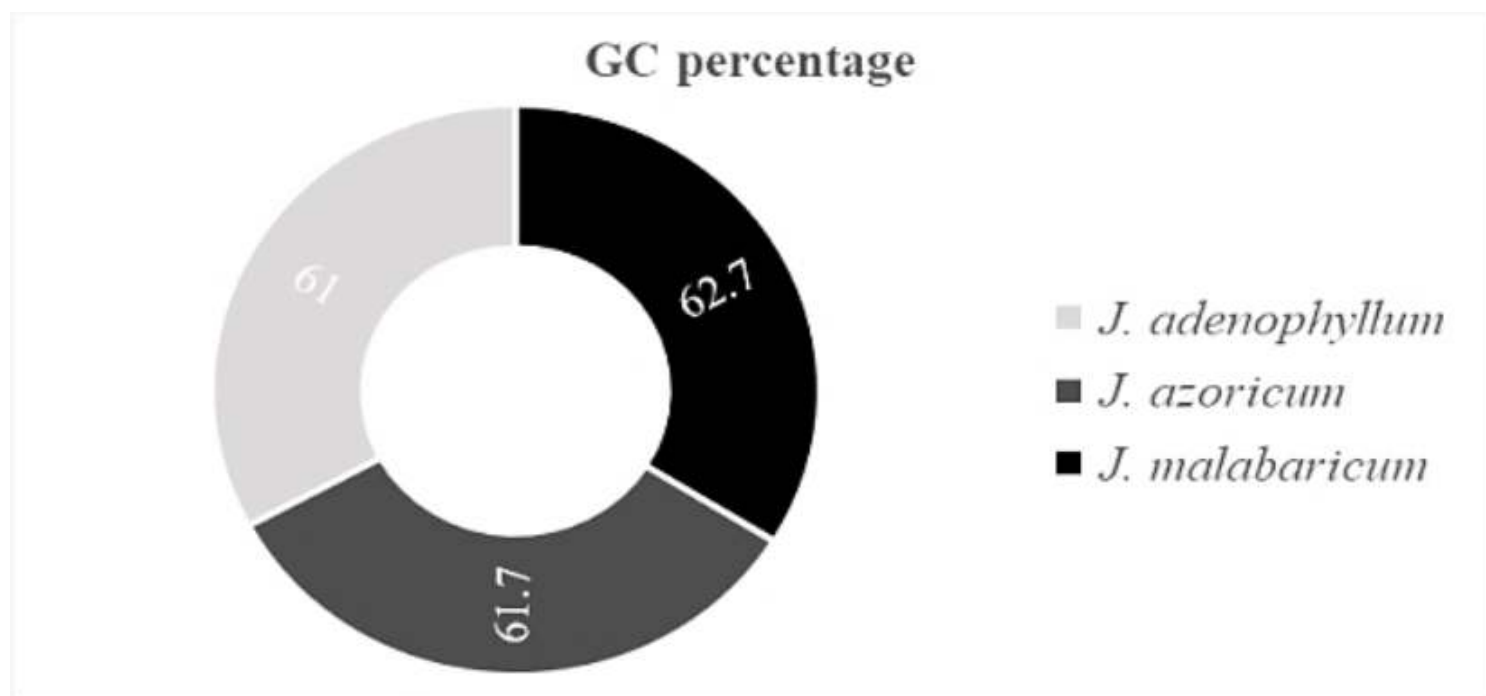


Figure 6

GC percentage of ITS sequence of studied plants

