RHEEDEA
Journal of the Indian Association for Angiosperm Taxonomy
RESEARCH ARTICLE
Vol. 33(2): 64–73 (2023)
ISSN: 0971-2313 (Print edition)
ISSN: 2582-2438 (Online edition)
https://dx.doi.org/10.22244/rheedea.2023.33.02.03

# Preliminary evaluation of the phylogenetic position of *Trigonella griffithii* (Fabaceae): a newly recorded species to the flora of India

Mittal K.[1], Dangi R.[1]*, Mallik A.H.[2], Ingle P.[3] & F.A. Shah[4]

[1]Bharati Vidyapeeth Deemed to be University Rajiv Gandhi Institute of Information Technology and Biotechnology, Pune-Satara Road, Katraj-Dhankawadi, Pune, Maharashtra – 411 046, India

[2]Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir, Srinagar, Jammu & Kashmir – 190 006, India

[3]Botanical Survey of India, Western Regional Centre, 7, Koregaon Park Road, Western Regional Centre Sangamvadi, Pune, Maharashtra – 411 001, India

[4]Govt. Degree College for Women, Anantnag, Jammu & Kashmir – 192 101, India

*E-mail: rakhee.dangi@bharatividyapeeth.edu

**Abstract:** *Trigonella griffithii* Boiss. from Jammu and Kashmir (India) is described here as an addition to the flora of India. The species shows morphological resemblances with *T. cachemiriana* Camb., *T. emodi* Benth., *T. emodi* Benth. var. *fimbriata* (Royle ex Benth.) Širj., and *T. gracilis* Benth. of *Trigonella* sect. *Ellipticae* but differs in key morphological characters related to legumes and stipules. Along with morphological characters the species is supported as a member of sect. *Ellipticae* in a preliminary molecular phylogenetic analysis based on four out of *c.* 60 species of the section included, using the nuclear ribosomal DNA internal transcribed spacers (ITS) and plastid *trn*L-*trn*F + *psb*E-*pet*L regions. Several morphological characters not indicated in the protologue along with a correction of the inaccurately given number of flowers in inflorescences are also discussed.

**Keywords**: *Ellipticae,* Northwest Himalayas, Perennial, Plant record, *Trigonella.*

## Introduction

The genus *Trigonella* L. (Fabaceae: Faboideae: Trifolieae) with *c.* 106 species contains small shrubs and herbs with most annual species found around the Mediterranean region, while all perennial species are endemic to central and west Asia (Mabberley, 2017; Andrella *et al*., 2022). In India, the genus is represented by 15 taxa belonging to sect. *Falcatulae* Boiss., sect. *Foenum-graecum* Širj. and sect. *Ellipticae* Boiss. (Širjaev, 1928–1932; Balodi & Rao, 1991; Sanjappa, 1992; Mittal *et al*., 2020). Species belonging to sect. *Ellipticae* are perennial xerophytes preferring mountainous regions with dry and windy conditions and do not form dense populations (Širjaev, 1928–1932; Ranjbar *et al*., 2014; Sharghi *et al*., 2021). This section includes *c.* 60 species and in India is represented by five taxa (*T. emodi* Benth, *T. cachemiriana* Camb., *T. emodi* Benth. var. *fimbriata* (Royal ex Benth.) Širj., *T. podperae* (Širj.) Vass. and *T. gracilis* Benth.) largely distributed in the northwest Himalayan region (Balodi & Rao, 1991; Sanjappa, 1992; Aghaahmadi *et al*., 2015; Chen *et al.,* 2021).

During our revision of *Trigonella* sect. *Ellipticae* from India, the authors located a population of *Trigonella* growing at approximately 1500 m asl., near the Manasbal lake area in Ganderbal district of Jammu and Kashmir. A critical morphological examination revealed that the specimen collected belonged to sect. *Ellipticae* (Širjaev, 1928–1932; Ranjbar *et al*., 2012). A detailed morphological comparison with all species of sect. *Ellipticae*, study of protologues, type specimens, and herbarium specimens housed at AHMA, BSA, BSD, BSI, CAL, DD, KASH, and several online herbaria (https://jstor.org.) identified it as *T. griffithii* Boiss. Sanjappa (1992) did not include *T. griffithii* in his checklist of legumes of India. Prior to the present collection, *T. griffithii* was collected by Bhattacharryya from Himachal Pradesh (Losar) in 1972 (CAL0000214436!). However, the specimen was poorly preserved, with immature fruits, without official documentation and further evidence of its survival, until it was rediscovered by the authors in 2018. In the present work, *T. griffithii* is reported new to the Indian flora and its preliminary phylogenetic affinities with allied species of sect. *Ellipticae* in India are discussed.

*Received*: 19.04.2022; *Revised & Accepted*: 22.06.2023
*Published Online*: 30.06.2023

## Materials and Methods

*Materials and morphology*: Field trips for the collection of *Trigonella* species belonging to sect. *Ellipticae* from the northwest Himalayan region of India were conducted from 2018 to 2022. During these field visits specimens of *T. cachemiriana* (one accession from Ladakh, BSI141562 and two accessions, BSI141702 and BSI141703, from Jammu and Kashmir respectively), *T. emodi* (one accession BSI141603 from Jammu and Kashmir), *T. emodi* var. *fimbriata* (one accession BSI141563 from Himachal Pradesh) and *T. gracilis* (one accession BSI141605 from Jammu and Kashmir) along with *T. griffithii* (one accession BSI141599 from Jammu and Kashmir) were collected from their natural habitats. For all sampled taxa, species identification was carried out by consulting the protologues, the relevant taxonomic literature (Royle, 1839; Cambessèdes, 1844; Boissier, 1872; Baker, 1879; Širjaev, 1928–1932; Ali, 1967; Stewart, 1972; Balodi & Rao, 1991) and digital images of specimens housed at BSI, CAL, K and P. Voucher specimens of all collected taxa were deposited in the Herbarium of the Botanical Survey of India (BSI) Western Circle, Pune, India (Table 1). Despite repeated efforts, *T. podperae* could not be sampled. Observation and measurements of floral and vegetative characters of *T. cachemiriana*, *T. emodi*, *T. emodi* var. *fimbriata*, *T. gracilis* and *T. griffithii* were performed using a SZ61 stereo zoom microscope (Olympus, Tokyo, Japan). Photographs were taken using a DP27 camera (Olympus, Tokyo, Japan) with macro settings. Photo plates were prepared using Adobe Photoshop (Adobe. Inc., San Jose, California, USA).

*Ingroup and outgroup sampling for phylogenetic study*: For phylogenetic analyses, in addition to *T. cachemiriana*, *T. emodi*, *T. emodi* var. *fimbriata*, *T. gracilis* and *T. griffithii* sampled from the Indian floristic regions, germplasm of 18 *Trigonella* species were procured from the United States Department of Agriculture, Agricultural Research Service, The Western Regional Plant Introduction Station, South Australian Research and Development Institute's (SARDI) Australian Medicago Genetic Resource Centre (AMGRC) South Australia and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany through the National Bureau of Plant Genetic Resources (NBPGR) New Delhi, India (Table 1). The ingroup sampling included almost 25% of species in *Trigonella* with representatives from all 12 sections described in the genus (Širjaev, 1928–1932; Small, 1987). Based on a previous phylogenetic analysis (Choi *et al*., 2022), four *Medicago* L. and two *Trifolium* L. species were used as outgroups and the phylogenetic trees were rooted on *Trifolium* species. Of the four *Medicago* species used, the germplasms of *M. plicata* (Boiss.) Širj. and *M. brachycarpa* Fisch. ex M. Bieb. were procured from SARDI (Table 1).

*DNA extraction, PCR amplification and amplicon sequencing*: For *T. cachemiriana* (three accessions), *T. emodi*, *T. emodi* var. *fimbriata, T. gracilis* and *T. griffithii*, DNA extraction was done from field-collected and silica gel preserved leaf tissue. The germplasms (seeds) of the other ingroup and two outgroup species were grown in pots to obtain fresh young leaves for DNA extraction. For all included taxa (25 taxa from *Trigonella* and two species from *Medicago*) DNA extraction was performed as described previously (Dangi, 2013).

PCR amplification of the entire nuclear ribosomal internal transcribed spacer region (ITS, including ITS1, 5.8S nrDNA gene and ITS2) was performed as described by Dangi (2013) for *T. cachemiriana* (three accessions), *T. corniculata* L., *T. emodi*, *T. emodi* var. *fimbriata, T. gracilis*, *T. griffithii*, *T. rechingeri* Širj. and *T. spinosum* L. PCR amplification for the plastid *trn*L-*trn*F region was done for *T. cachemiriana* (three accessions), *T. corniculata*., *T. emodi*, *T. emodi* var. *fimbriata, T. gracilis*, *T. griffithii*, and *T. rechingeri* as described previously (Dangi, 2013). For 25 taxa from *Trigonella* and two species from *Medicago*, PCR amplification of the *psb*E-*pet*L intergenic spacer was performed using the primers *pet*L (5′ AGTAGAAAACCGAAATAACTAGTTA3′) and *psb*E(5′TATCGAATACTGGTAATAATATCAGC 3′) of Shaw *et al.* (2007). The thermal cycling conditions for PCR amplification of the *psb*E-*pet*L region were as follows: initial denaturation of 3 min at 94° C, 35 cycles of 30 s denaturation (94° C), 45 s annealing (58° C), 1 min 30 s elongation (72° C) followed by final extension of 5 min at 72° C.

PCR products were purified with the BioEra's gel purification kit according to the manufacturer's protocol and sequenced at 1st Base Labs., Seri Kembangan 43300, Selangor, Malaysia using Sanger ABI technology. Chromatograms for forward and reverse sequences were assembled and

edited using Chromas Lite v.2.6.6. (Technelysium, 1998–2018). A total of 46 new sequences were generated and submitted to GenBank (Table 1). For the remaining ingroup and outgroup taxa ITS (for 15 *Trigonella,* two *Trifolium* and four *Medicago* species) and *trn*L-*trn*F sequences (for 14 *Trigonella*, two *Trifolium* and four *Medicago* species) were sourced from GenBank (Table 1). Complete chloroplast genomes of *Medicago sativa* L.*, M. lupulina* L., *Trifolium repens* L. and *T. pratense* L. were downloaded from GenBank to retrieve the *trn*L-*trn*F and/or *psb*E-*pet*L intron/intergenic spacer sequences (Table 1).

*Phylogenetic analyses*: Sequences were aligned using MUSCLE implemented in MEGA X v.10.1.8 (Kumar *et al*., 2018) and adjusted manually. The best-fitting partitioning scheme and models for evolution of nuclear, chloroplast as well as concatenated (ITS + *trn*L-*trn*F + *psb*E-*pet*L) sequences were selected using PartitionFinder2 (Lanfear *et al*., 2017). For the ITS region, the data were partitioned into ITS1, 5.8S and ITS2 and for *trn*L-*trn*F + *psb*E-*pet*L into coding region, intron and intergenic spacer. Maximum likelihood (ML) analyses were performed on separate nuclear, plastid and concatenated nuclear and plastid sequences using the bootstrap option and models determined by Partitionfinder2 in RAxMLGUI 2.0 with 1000 bootstrap replicates for branch support estimation (Edler *et al*., 2021). Bayesian Inference (BI) under the respective best-fit models were conducted on individual and concatenated nuclear and plastid datasets using MrBayes v.3.2.7 (Ronquist *et al*., 2012). The best-fit models of sequence evolution for the Bayesian inference analyses were chosen using the Akaike information criterion (AIC), calculated with Modeltest 3.7 (Posada & Crandall, 1998). These models (for ITS TrNef+G, for *trn*L-*trn*F + *psb*E-*pet*L K81uf+G, for ITS + *trn*L-*trn*F + *psb*E-*pet*L GTR+I+G) were applied to their respective partitions in separate and combined analyses. BI analyses consisted of two independent analyses of two parallel runs and four chains for 30,00,000 generations by sampling every 500 generations until convergence. Convergence was determined when the standard deviation of split frequency was below 0.01. The first 25% of sampled trees was discarded as burn-in, and the rest were used to calculate posterior probabilities (PP). The output parameters were inspected in Tracer v.1.6 (Rambaut, *et al*., 2018) to assess convergence and that all ESS values were above 1000.

## Results

*Sequence alignments*: For this study, 10 ITS, 9 *trn*L-*trn*F, and 27 *psb*E-*pet*L sequences were newly generated (Table 1). For the ITS region, the length varied from 707 to 730 bp (including the outgroups) with a mean GC content of 49.08%. The *trn*L-*trn*F region with a mean GC content of 34.56% varied in length from 604 to 804 bp while the *psb*E-*pet*L region with a mean GC content of 30.61% varied in length from 661 to 1058 bp. With 31 taxa, the combined nuclear and plastid matrix (ITS + *trn*L-*trn*F + *psb*E-*pet*L) had a total length of 2881 bp of which 2245 were constant, 370 were variable but parsimony uninformative and 266 were parsimony informative.

*Phylogenetic analyses*: The topology of the maximum likelihood (ML) and Bayesian Inference (BI) trees of combined ITS + *trn*L-*trn*F + *psb*E-*pet*L datasets were identical and the tree generated with RAxMLGUI is presented as Fig. 1. The tree indicated that all species of *Trigonella* included in the present study originated from a common ancestor and diverged into two major clades representing all 12 sections within the genus. *Trigonella griffithii* was placed with other allied species of sect. *Ellipticae* (*T. cachemiriana*, *T. emodi, T. emodi* var. *fimbriata* and *T. gracilis*; BP=100%, PP=1) in the phylogeny (Fig. 1). The ML and BI trees generated by separate ITS and *trn*L-*trn*F + *psb*E-*pet*L datasets supported this placement (data not shown). In sect. *Ellipticae*, the three accessions of *T. cachemiriana* formed a strongly supported clade (BP=96%, PP=1) sister to the clade containing *T. emodi, T. emodi* var. *fimbriata* and *T. gracilis* (BP=90%, PP=1) with *T. griffithii* on the first diverging branch (Fig. 1). Species of this clade (sect. *Ellipticae*) share key morphological characters such as an erect to ascending shrubby habit, considerably hardened, thicker, sparsely appressed hairy stem, glabrous leaves, yellow flowers, transversely veined short straight or slightly bent pods and smooth seeds. In addition to these characters, *T. griffithii* is similar to *T. cachemiriana* in its floral features (keel petal longer than wing petal). It differs from *T. cachemiriana* in legume apex (acute *vs.* obtuse) and

**Table 1.** Ingroup (*Trigonella*) and outgroup (*Medicago* and *Trifolium*) samples, accession/voucher numbers and GenBank numbers for ITS, *trn*L–*trn*F and *psb*E–*pet*L sequences

| Species | [a]EC/[b]VN | GenBank accession numbers | | |
|---|---|---|---|---|
| | | ITS | *trn*L–F | *psb*E–*pet*L |
| *Trigonella balansae* Boiss. et Reut. | 546586 | JX274132 | JX274188 | ON228107 |
| *T. cachemiriana* Cambess. | BSI141562 | ON053318 | ON131009 | ON228122 |
| *T. cachemiriana* Cambess. | BSI141702 | ON053221 | ON131013 | ON228126 |
| *T. cachemiriana* Cambess. | BSI141703 | ON053223 | ON131015 | ON228128 |
| *T. caelesyriaca* Boiss. | 583565 | JX274200 | JX274144 | ON228086 |
| *T. caerulea* (L) Ser. | 583568 | JX274202 | JX274146 | ON228087 |
| *T. calliceras* Fisch ex M.Bieb. | 583570 | JX274204 | JX274148 | ON228088 |
| *T. coerulescens* (M.Bieb.) Halácsy | 583573 | JX274205 | JX274149 | ON228089 |
| *T. corniculata* L. | 829785 | MW194886 | MW216675 | ON228108 |
| *T. cretica* (L.) Boiss. | 583576 | JX274208 | JX274152 | ON228090 |
| *T. cylindracea* Desv. | 583578 | JX274210 | JX274154 | ON228091 |
| *T. emodi* Benth. | BSI141603 | ON053315 | ON131006 | ON228118 |
| *T. emodi* var. *fimbriata* Širj. | BSI141563 | MW194883 | MW216672 | ON228121 |
| *T. filipes* Boiss. | 583584 | JX274214 | JX274158 | ON228092 |
| *T. foenum-graecum* L. | 583590 | JX274217 | JX274161 | ON228093 |
| *T. gladiata* Steven ex M.Beib. | 583593 | JX274107 | JX274164 | ON228094 |
| *T. gracilis* Benth. | BSI141605 | ON053316 | ON131007 | ON228119 |
| *T. grandiflora* Bunge | 583595 | JX274109 | JX274166 | ON228095 |
| *T. griffithii* Boiss. | BSI141599 | ON053325 | ON131017 | ON228130 |
| *T. kotschyi* Boiss. | 583598 | JX274111 | JX274168 | ON228097 |
| *T. mesopotamica* Hub.-Mor. | 583605 | JX274116 | JX274173 | ON228100 |
| *T. rechingeri* Širj. | 971951 | MW194888 | MW216677 | ON228115 |
| *T. spicata* Sm. | 583616 | JX274125 | JX274182 | ON228102 |
| *T. spinosum* L. | 583620 | ON053308 | JX274183 | ON228103 |
| *T. strangulata* Boiss. | 583622 | JX274128 | JX274185 | ON228105 |
| *Medicago sativa* L. | na | AF053142 | NC042841 | NC042841 |
| *M. lupulina* L. | na | DQ311980 | GQ488612 | NC042847 |
| *M. brachycarpa* Fisch. ex M.Bieb. | 583556 | JX274196 | JX274140 | ON228084 |
| *M. plicata* (Boiss.) Širj. | 583608 | JX274119 | JX274176 | ON228101 |
| *Trifolium pratense* L. | na | DQ312138 | NC047412 | NC047412 |
| *Trifolium repens* L. | na | KY968960 | MT120812 | MT120812 |

[a] EC: Accession identity number – National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India.

[b] VN: voucher number BSI – Herbarium submitted at Botanical Survey of India, Western Regional Centre. Pune, India.

na – not applicable

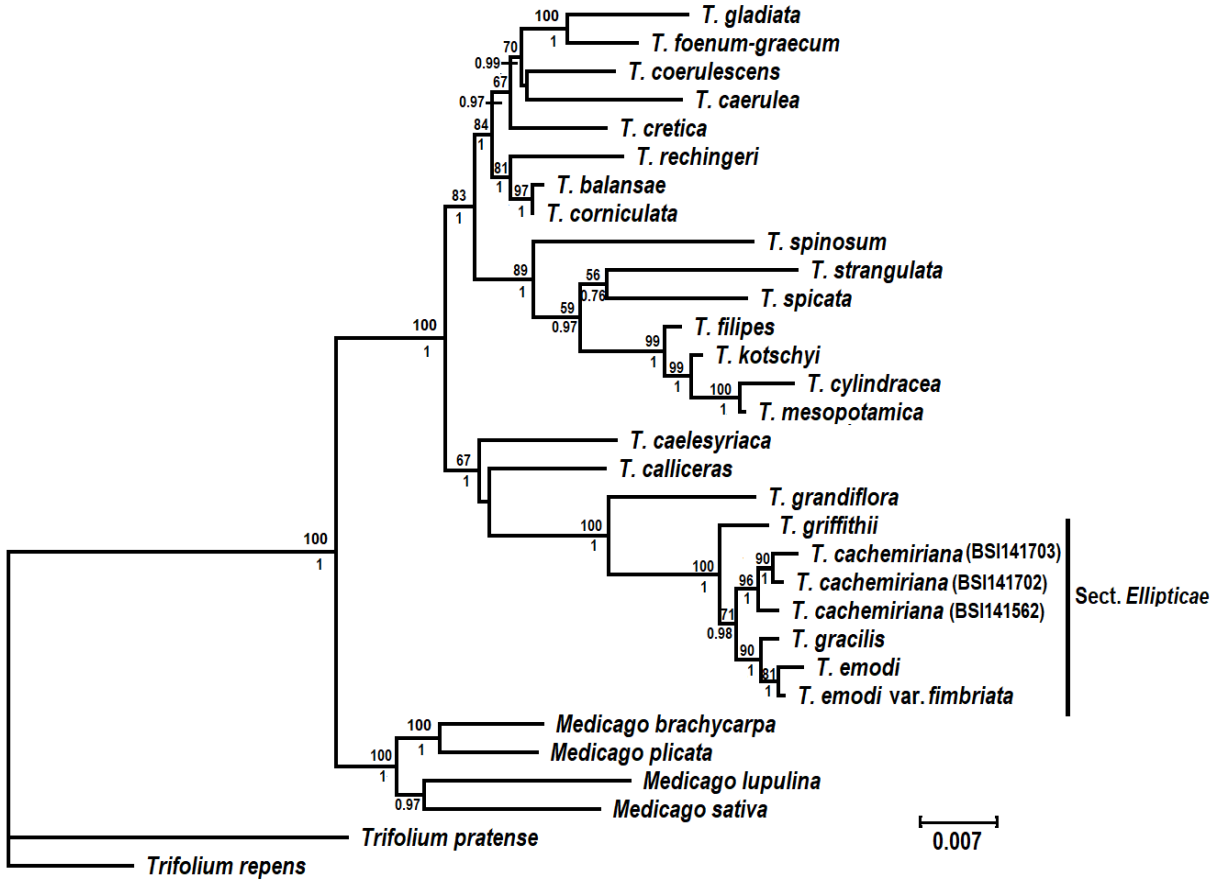


**Fig. 1.** Maximum Likelihood tree retrieved after analysing the combined ITS + *trn*L-*trn*F + *psb*E-*pet*L sequence data for 25 accessions of 23 *Trigonella* L. species. The tree is rooted on *Trifolium* L. samples. Numbers above the branches indicate bootstrap percentages above 50 % (BP). Numbers below the branches indicate Bayesian posterior probabilities (PP). *Trigonella* sect. *Ellipticae* Boiss. is indicated to the right of the tree.

stipules (base more serrate *vs.* incised–dentate and apiculate Fig. 2, Table 2). *Trigonella griffithii* differs from *T. emodi, T. emodi* var. *fimbriata* and *T. gracilis* in floral features (keel petal longer than wing petal *vs.* keel petal shorter than wing petal) in addition to legume apex and stipule characteristics (Fig. 2, Table 2).

## Taxonomic Treatment

**Trigonella griffithii** Boiss. Fl. Orient. 2: 88. 1872. *Type*: AFGHANISTAN, *s.d.*, *W. Griffith* 1134 (holo G[G00330462] digital image!; iso CAL [CAL0000024620] digital image!) **Fig. 2**

Perennial herbs, up to 20–30 cm high. Caudex branched, erect, appressed pilose. Stipules semi-sagittate, 4–5 mm long, adnate to petiole, base dentate, upper part entire, sparsely pubescent. Petiole 4–7 mm long. Leaves trifoliolate, terminal leaflet 8–10 × 2–5 mm, lateral leaflets 7–9 × 3–4 mm, sub-sessile, obovate with a narrow base, margin distinctly crenulate in upper part, apex apiculate, abaxially sparsely appressed trichomes, adaxially glabrous. Inflorescence 20–25 mm long; pedicel 2–3 mm long; peduncle 20–30 mm long, longer than subtending leaf, pubescent, 7–8-flowered. Calyx 5-lobed, 3–4 mm long, persistent, campanulate; calyx teeth triangular, 1.5–2 mm long, calyx tube 2.5–3 mm long, pilose on outer surface. Corolla yellow. Standard petal 6–8 mm long, obovate, apex round, distinctly clawed, glabrous. Keel petals 6–8 mm long, apex acute, pocket absent, ± as long as standard, glabrous. Wing petals 5–6 mm long, apex rounded, shorter than keel petal, glabrous. Stamens 10, diadelphous, filament persistent. Ovary linear oblong; style shorter than the ovary, filiform, glabrous. Pods lanceolate, 10–12 × 2–4 mm, apex acute, stipitate, exocarp nervation parallel, oblique to longitudinal axis, upper suture

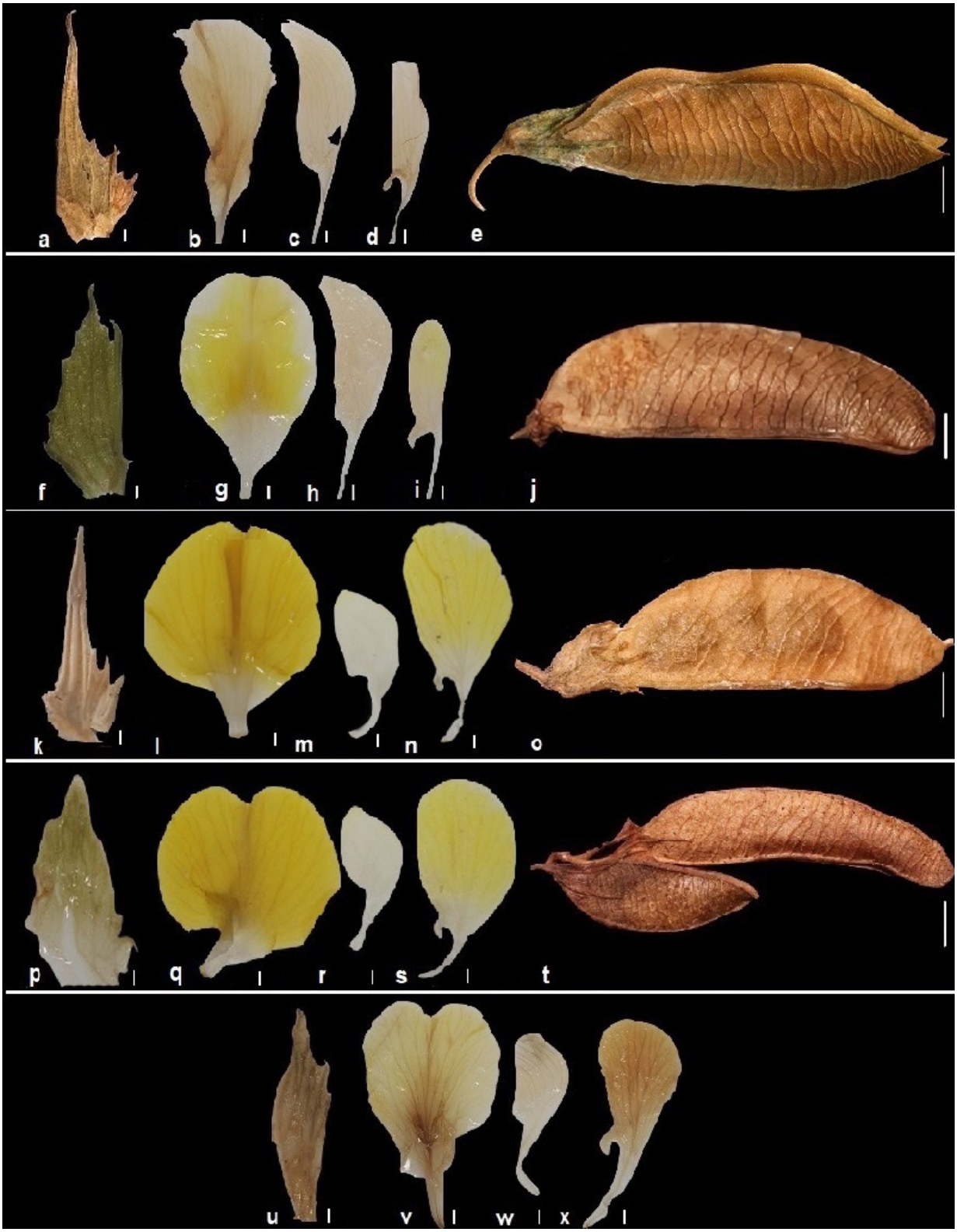


**Fig. 2. a–e.** *Trigonella griffithii* Boiss.: **a.** Stipule; **b.** Standard; **c.** Keel; **d.** Wing; **e.** Pod. **f–j**. *T. cachemiriana* Camb.: **f.** Stipule; **g.** Standard; **h.** Keel; **i.** Wing; **j.** Pod. **k–o**. *T. emodi Benth.*: **k.** Stipule; **l.** Standard; **m.** Keel; **n.** Wing; **o.** Pod. **p–t.** *T. gracilis* Benth.: **p.** Stipule; **q.** Standard; **r.** Keel; **s.** Wing; **t.** Pod**. u–x**. *T. emodi* Benth. var. *fimbriata* (Royal ex Benth.) Širj. : **u.** Stipule; **v.** Standard; **w.** Keel; **x.** Wing. Scale bars a-x = 200 µm. (**a–e** from *Dangi* 141599; **f–j** from *Dangi & Malik* 141702; **k–o** from *Dangi & Malik* 141603; **p–t** from *Dangi* 141605; **u–x** from *Dangi & Ingle* 141563; photos by K. Mittal)

**Table 2.** Morphological differences between *T. griffithii*, *T. cachemiriana*, *T. emodi* and *T. gracilis* based on sampled specimens and Širjaev (1928–1932).

| Characters | *T. griffithii* Boiss. | *T. cachemiriana* Camb. | *T. emodi* Benth. | *T. gracilis* Benth. |
|---|---|---|---|---|
| Plant indumentum | Appressed hairy | Appressed hairy | Appressed hairy | Glabrous |
| Stipule | Semi-sagittate, base serrate, upper part entire | Semi-sagittate, incised–dentate and apiculate | Semi-sagittate, base serrate, rarely entire | Lanceolate and dentate |
| Standard petal length | 6–8 mm | 6–8 mm | 6–7 mm | 5–5.5 mm |
| Keel petal length | 6–8 mm | 6–8 mm | 4.5–5 mm | 3.5–4 mm |
| Wing petal length | 5–6 mm | 5–6 mm | 6–7 mm | 5–5.5 mm |
| Legume apex | Acute | Obtuse | Obtuse | Obtuse |
| Seeds | Ovoid | Ovoid | Oblong–elliptic | Round–elliptic |

carinate, 2–4 seeded. Seeds ovoid, 2–3 mm long, reddish brown.

*Flowering & fruiting*: Flowering from May to July and fruiting from July to August.

*Habitat*: Found growing on dry and stony areas.

*Distribution*: Native to Afghanistan, Kirgizstan, Tadzhikistan, Uzbekistan and now India.

*Specimens examined*: AFGHANISTAN, **Daulatshah**, *s.loc*., 31.05.1937, *W. Koelz* 11650 (E00336655). **Ghor**, Hauz-i-Mahiha, 17.07.1948, *Köie & Mogens Engell* 2498 (E00336656). **Sirotai**, 19.06.1937, *W. Koelz* 11963 (E00336653). INDIA, **Himachal Pradesh**, Losar, 26.07.1972, *U.C. Bhattacharryya* 49012 (CAL0000214436). **Jammu and Kashmir**, Manasbal Lake, N 34°14' 49", E 74°40'18", 1583 m, 10.09.2018, *Dangi* 141599 (BSI). PAKISTAN, **Baltistan**, 01.07.1892, *J.F. Duthie* 11733 (CAL0000214435). *Other specimens examined: T. cachemiriana*: INDIA, **Jammu and Kashmir**, Bandipora district, Gurez valley, N 34°31'31.08", E 75°17'20.4", 2864 m, 09.08.2021, *Dangi* 141703 (BSI); Budgam district, Doodhpathri, N 33°51'24", E 74°33' 48", 2750 m, 30.08.2021, *Dangi & Malik* 141702 (BSI). **Ladakh**, Leh-Kargil road, N 34°23'48", E 76°18'34", 3200 m, 27.07.2019, *Dangi & Malik* 141562 (BSI). *T. gracilis*: INDIA, **Jammu and Kashmir**, Shri Hama River glacier, N 33°41' 00", E 74°43'00", 05.08.2020, *Dangi* 141605 (BSI). *T. emodi* var. *fimbriata*: INDIA, **Himachal Pradesh**, Kullu, Rohtang viewpoint, N 32°21'54", E 77°14'30", 2050 m, 15.08.2019, *Dangi & Ingle* 141563 (BSI) *T. emodi*: Jammu and Kashmir, Shopian district, Hirpora Wildlife Sanctuary, 12 km from Shopian, N 33°41'00", E 74°43'00", 2800 m, 18.07.2020, *Dangi & Malik* 141603 (BSI).

*Conservation status*: According to the IUCN Red List Categories version 14 (IUCN, 2022) this taxon has been provisionally assessed here as Data Deficient (DD). Despite exhaustive and consecutive effort to various locations from 2018 to 2020, only a single population of *T. griffithii* was located.

## Discussion

The genus *Trigonella* contains *c.* 106 species occurring mainly from the Mediterranean Sea to the Himalayan Mountain range (Andrella *et al*., 2022). On the basis of morphological characters, the genus is divided into 12 sections, of which sect. *Ellipticae* with *c*. 60 species is the largest (Širjaev, 1928–1932; Aghaahmadi *et al*., 2015). Sanjappa (1992) listed 5 species of sect. *Ellipticae* in the legume flora of India. The present study is the first report on the documentation of *T. griffithii* in the Indian flora. The placement of the species in sect. *Ellipticae* was supported by a preliminary phylogenetic analysis with approximately 25%

of species within the genus representing all 12 sections and four out of the *c*. 60 species (*c*. 7%) in the section included.

*Trigonella griffithii* was first described by Boissier (1872) and later by Širjaev (1928–1932) and Köie & Rechinger (1957) as a species of *Trigonella* sect. *Ellipticae*, morphologically very similar to the sympatric *T. cachemiriana* differing from it only in the legume apex and stipules (Fig. 2). It differs from other co-occurring members of sect. *Ellipticae* in India (*T. emodi, T. emodi* var. *fimbriata* and *T. gracilis*) majorly in its floral features (Fig. 2, Table 2).

The original description of *T. griffithii* mentions 1–5-flowered inflorescences. However, the second author noticed 7–8-flowered inflorescences in the sampled specimens of *T. griffithii*. A detailed study of high-resolution digital images of herbarium specimens of *T. griffithii* from CAL (CAL0000214435, CAL0000214436) and E (E00336653, E00336655, E00336656) along with the present collection revealed that the number of flowers in the inflorescence was inaccurately mentioned in the protologue. Moreover, several important morphological characters such as length of inflorescence, peduncle, pedicel, calyx tube, calyx teeth, ovary indumentum, exocarp nervation and seed morphology, documented in the present study were not fully described in the protologue.

Despite extensive searching, we were not able to locate and make new collections of another morphologically allied *T. podperae* for inclusion in our phylogenetic analysis. Nonetheless, detailed comparisons between *T. griffithii* and *T. podperae* based on high-resolution digital herbarium images (K000998739, CAL000214439) and taxonomic descriptions in the relevant literature (Širjaev, 1928-1932; Ali, 1967; Stewart, 1972; Balodi & Rao, 1991) revealed significant differences in the sizes of the legumes (10–22 × 2–4 mm *vs.* 10–15 × 6–7 mm), legume apex (acute *vs.* obtuse) and plant indumentum (glabrous *vs.* hairy). Therefore, the addition of *T. griffithii* to India's *Trigonella* genetic resources is supported by its morphological and phylogenetic characteristics.

## Acknowledgements

The first and second authors are thankful to the Department of Biotechnology, Government of India for providing the funding. The Director, Botanical Survey of India, Kolkata, West Bengal is thanked for providing herbarium facilities and to carry out morphological work at Western Regional Centre, Pune, MH. The second author is also thankful to the curators of the United States Department of Agriculture, Agricultural Research Service, The Western Regional Plant Introduction Station (USDA-ARS-WRPIS), the South Australian Research and Development Institute's (SARDI) Australian Medicago Genetic Resource Centre (AMGRC) South Australia, and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany, for the supply of the germplasm used in the present study.

## Literature Cited

ALI S.I. 1967. Revision of genus *Trigonella* Linn. from W. Pakistan. *Pakistan Journal of Forestry* 17: 255–266.

AGHAAHMADI M., NEGARESH K., SAEIDI H., & M. ASSADI. 2015. Typification of two species names of *Trigonella* sect. *Ellipticae* (Leguminosae-Papilionoideae) from Afghanistan. Phytotaxa 238(1): 97–100. http://dx.doi.org/10.11646/phytotaxa.238.1.6

ANDRELLA G.C., ATAHUACHI BURGOS M., BAGNATORI SARTORI Â.L., BALAN A., BANDYOPADHYAY S., BARBOSA PINTO R., BARRETT R., BOATWRIGHT, J.S., BORGES L. M., BROICH S.L., BRULLO S., BRUNEAU A., CARDINAL-MCTEAGUE W., CARDOSO D., CASTRO SILVA I.C., CERVANTES A., CHOO L.M., CLARKE R., *et al.* 2022. The World Checklist of Vascular Plants (WCVP) Fabaceae. Facilitated by The Royal Botanic Gardens, Kew. Available at: http://sftp.kew.org/pub/data_collaborations/Fabaceae/DwCA/ (Accessed on 21.05.2023).

BAKER J.G. 1879. *Trigonella. In:* HOOKER J.D. (ed.), *The flora of British India.* Volume 2. L. Reeve & Co. Ltd., London. pp 87–89.

BALODI B. & R.R. RAO 1991. The genus *Trigonella* L. (Fabaceae) in the Northwest Himalaya. *Journal of Economic and Taxonomic Botany* 15: 185–194.

BOISSIER P.E. 1872. *Trigonella. In*: BOISSIER P.E. (ed.), *Flora Orientalis.* Volume 2. Geneva Basileae & Lugundi. pp. 64–91.

CAMBESSÈDES J. 1844. Botanique. *In:* JACQUEMONT V. (ed.), *Voyage dans l'Inde.* Volume 4. Firmin Didot frères, Paris. p. 36

CHEN J., WU G., SHRESTHA N., WU S., GUO W., YIN M., LI A., LIU J. & G. REN 2021.

Phylogeny and species delimitation of Chinese *Medicago* (Leguminosae) and its relatives based on molecular and morphological evidence. *Frontiers in Plant Science* 11:619799. https://doi.org/10.3389/fpls.2020.619799

CHOI I-S., CARDOSO D., DE QUEIROZ L.P., DE LIMA H.C., LEE C., RUHLMAN T.A., JANSEN R.K. & M.F. WOJCIECHOWSKI 2022. Highly resolved papilionoid legume phylogeny based on plastid phylogenomics. *Frontiers in Plant Science* 13: 823190. https://doi.org/10.3389/fpls.2022.823190

DANGI R. 2013. *Diversity in genus Trigonella: molecular and chemical characterization.* Ph.D. Thesis, Savitribai Phule Pune University, Pune. http://hdl.handle.net/10603/198985

EDLER D., KLEIN J., ANTONELLI A. & D. SILVESTRO 2021. raxmlGUI 2.0 beta: a graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12: 373–377. https://doi.org/10.1111/2041-210X.13512

IUCN 2022. Guidelines for using the IUCN Red list Categories and Criteria. Version 15. Available at: http://www. iucnredlist.org/documents/RedListGuidelines.pdf (Accessed on 25.06. 2022).

KÖIE M. & K.H. RECHINGER 1957. Enumeration and descriptions of the plants collected by L. Edelberg and M. Koeie on "The 3rd Danish expedition to central Asia" and by G. Kerstan, W. Koelz, H.F. Heubauer and O.H. Volk, and others in Afghanistan. *Biologiske Skrifter udgivet af Det Kongelige Danske Videnskabernes Selskab* 9(3): 1–18.

KUMAR S., STECHER G., LI M., KNYAZ C. & K. TAMURA 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

LANFEAR R., FRANDSEN P.B., WRIGHT A.M., SENFELD T. & B. CALCOTT 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34(3): 772–773. https://doi.org/10.1093/molbev/msw260

MABBERLEY D.J. 2017. *Mabberley's plant-book: a portable dictionary of vascular plants, their classification and uses*. Fourth edition. Cambridge University Press, Cambridge.

MITTAL K., INGLE P. & R. DANGI 2020. *Trigonella glabra* (Leguminosae): a new addition to *Trigonella* genetic resources in India. *Journal of Economic and Taxonomic Botany* 44: 5–7.

POSADA D. & K.A. CRANDALL 1998. ModelTest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

RAMBAUT A., DRUMMOND A.J., XIE D., BAELE G., & M.A. SUCHARD 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

RANJBAR M., HAJMORADI Z. & R. KARAMIAN 2014. Novelty in *Trigonella* sect. *Ellipticae* (Fabaceae) from Iran. *Novon* 23: 209–216. http://dx.doi.org/10.3417/2011024

RANJBAR M., KARAMIAN R., HAJMORADI Z. & M.R. JOHARCHI 2012. A revision of *Trigonella* sect. *Ellipticae* (Fabaceae) in Iran. *Nordic Journal of Botany* 30(1): 17–35. https://doi.org/10.1111/j.1756-1051.2009.00171.x

RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D.L., DARLING A., HÖHNA S., LARGET B., LIU L., SUCHARD M. & J.P. HUELSENBECK 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

ROYLE J.F. 1839. *Illustrations of the Botany and other branches of the Natural History of the Himalayan Mountains and of the Flora of Cashmere.* Volume 1. Wm. H. Allen & Co., Leadenhall Street, London, p. 197.

SANJAPPA M. 1992. *Legumes of India.* Bishen Singh Mahendra Pal Singh, Dehra Dun.

SHARGHI H.R., AZIZI M., MOAZZENI H. & M.R. JOHARCHI 2021. Notes on the taxonomy of some perennial species of *Trigonella* sect. *Ellipticae* (Fabaceae) in Iran. *Nordic Journal of Botany* 39(2): 1–10. https://doi.org/10.1111/njb.02819

SHAW J., LICKEY E.B., SCHILLING E.E. & R.L. SMALL 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94 (3): 275–288. https://doi.org/10.3732/ajb.94.3.275

ŠIRJAEV 1928–1932. Generis *Trigonella* L. Revisio Critica I. *Spisyvydavane´ Prirodovedecka´ Fakultou Masarykovy University* 102: 1–57.

SMALL E. 1987. Polythetic generic separation in the tribe Trifolieae subtribe Trigonellinae (Leguminosae). *Canadian Journal of Botany* 67(5): 1480–1492. https://doi.org/10.1139/b89-197

STEWART R.R. 1972. An annotated catalogue of the vascular plants of West Pakistan and Kashmir. *In*: NASIR E. & S.I. ALI (eds.), *Flora of West Pakistan*. Fakhri Printing Press, Karachi. p. 423.

TECHNELYSIUM PTY LTD. 1998–2018. Chromas (Version 2.6.6). Available at: http://technelysium.com.au/wp/chromas/ (Accessed on 20.01.2021).