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RESEARCH ARTICLE

A New Subspecies of *Momordica sahyadrica* (Cucurbitaceae) from Southern Western Ghats of Kerala, India

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Momordica sahyadrica subsp. *anamalayana* KJ John, K Pradheep *et* Krishnaraj, a narrow endemic taxon, is described and illustrated. This new subspecies differs from *Momordica sahyadrica* subsp. *sahyadrica* in having a slender stem, subsessile male flower pedicel, narrow male sepal, corolla base cupular with acuminate apex, stigma greenish-yellow and fruits small (14-18 g) with snout-like rostration. It has a delicate growth habit with niche-specificity, occupying the evergreen forest floor in the mid elevation ranges in the Anamalai Range of Western Ghats in Kerala, India. Sequence analysis of the candidate gene *matK* in *M. sahyadrica* subsp. *anamalayana*, along with the sequences of *M. sahyadrica* subsp. *sahyadrica*, and other available species in India, indicated that *M. sahyadrica* subsp. *anamalayana* to be genetically close to *M. sahyadrica* subsp. *sahyadrica* but distinct from other species. An identification key for Indian species of *Momordica* is also provided.

Key Words: Crop wild relatives, Endemism, *Momordica sahyadrica*, Western Ghats

Introduction

The genus *Momordica* L. comprises about 60 species (Schaefer and Renner, 2011) distributed in the warm Tropics, chiefly in Africa and with about 10 species in South East Asia (de Wilde and Duyfjes, 2002). Six species occur in India including the recently described *M. sahyadrica* KJ John & VT Antony (Joseph John, 2005; Joseph John and Antony, 2007; Joseph John and Antony, 2010; Bharathi and Joseph John, 2013).

Van Rheede describes four entities of *Momordica* from Kerala in his monumental treatise 'Hortus Malabaricus'. The entity '*bempaval*' (8: 34-35) is referred to as *M. denudata* (Thwaites) CB Clarke by Nicolson *et al*. (1988) and (Manilal, 2003), and the study site is spread over the erstwhile Cochin state, matching with that of our present collection site. Interestingly, the original specimen cited by Chakravarty (1959, 1982) was examined (*Meebold 12241*) at CAL and was found to be *M. dioica* Roxb. ex Willd. None of the Kerala specimens matches with the type specimen *Thwaites CP 1615* (Sri Lanka) of *M. denudata*. Moreover, an extensive field study in Kerala failed to locate even a single plant with 'branched male inflorescence' characteristic of *M. denudata.* The 'Bempaval' drawing in *Hortus Malabaricus* probably appeared to be an artefact; tuber of *M. dioica* and aerial part of *Gymnopetalum tubiflorum* (Wight & Arn.) Cogn. However, in the Sholayar forest in Anamalai Range at a 40 km radius in the evergreen forests, and again in Nelliampathy Hills, Palakkad district, the authors came across a few populations of day-flowering dioecious *Momordica* allied to *M. sahyadrica* but with a fragile, delicate habit, short pedicel of male flowers, narrow male sepals, cupular corolla base with acuminate apex, greenish-yellow stigma, small fruits with snout-like rostration, and the same is described here as an infraspecific taxon under *M. sahyadrica* KJ John *et* VT Antony.

Herbarium specimens were collected and prepared by using the standard methods (Jain and Rao, 1977). Data on fruit and seed characters was recorded in the natural population itself. Morphological observations were substantiated by molecular characterisation of typical subspecies

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and variant populations through candidate gene analysis. Chloroplast gene *matK* is an established candidate DNA barcoding locus in cucurbits (Girme, 2014). Three accessions each of the new entity of *M. sahyadrica* and typical *M. sahyadrica* and one accession each of *M. charantia* L., *M. subangulata* Blume subsp. *renigera* (Wall. ex G.Don) WJ de Wilde, *M. dioica, M. balsamina* L., *M. cochinchinensis* (Lour.) Spreng. and *M. cymbalaria* Fenzl ex Naudin were used in the study. Total genomic DNA was isolated and the *matK* locus was amplified through thermal cycling. The forward and reverse primers used in the thermal cycling were

5'-ATGTCACCACAAACAGAAACTAAAGCAAGT-3' and 5'-CTTCACAAGCACCAGCTAGTTCAGGACTCC-3', respectively. Thermal cycling was performed with initial denaturation at 95°C for 6 min. followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 58°C for 45 s and primer extension at 72°C for 1 min., followed by final extension at 72°C for 10 min. The products were electrophoresed on 1.2 % agarose gel, bands eluted and paired-end sequencing of *matK* locus was performed on Sanger platform. Sequences were aligned and annotated using standard software such as MAFFT, Clustal Omega, BLASTn and ExPASy.

Momordica sahyadrica KJ John subsp. ***anamalayana*** KJ John, K Pradheep *et* Krishnaraj *subsp. nov*. (Fig. 1)

Diagnosis

The new subspecies is allied to typical *Momordica sahyadrica* subsp. *sahyadrica* but differs from it in having a slender stem, short (1 mm long) or subsessile male flower pedicel, narrow (1 mm wide) male sepal, corolla base cupular with acuminate apex; stigma greenish-yellow, small fruits (14-18 g) with snout-like rostration.

Typus: India, Kerala, Thrissur District, Sholayar forest, Pathadippalam, Joseph John K, 29 October 1999 JJK/99-587 (male), (holotypus CAL; Isotypi, NHCP, MH).

Description

Herbaceous, tendrillar climber, dioecious, vines up to 6 m long. Tap root perennial, tuberous, fusiform when young, subglobose or irregularly swollen when mature, warty, 7-10 × 6-8 cm, outer skin brownish, inner flesh creamy. Stem slender, angular, glabrous, internodes 9-12 cm long. Tendrils unbranched, glabrous, 5-9 cm. Leaves simple, petiolate; lamina triangular-ovate, lobes not prominent, subchartaceous, ciliate, subglabrous, palmately veined, 6-12 × 4-7.5 cm, cordate at base, serrate at margins, acuminate at apex, acumen mucronate; petiole slender, light green, 4-6 cm long, canaliculate, ciliate. Male flowers axillary, solitary or in a loose fascicle of 5-7 flowers; peduncle up to 7 cm long, light green, glabrous; flowers subsessile, pedicels light green, short, up to 1 mm long, puberulous, subtended and covered by a bract; bract cordate or reniform, cucullate, entire, base cordate, apex mucronate, with seven longitudinal veins, 0.8-1.1 × 1.1-1.6 cm. Calyx base funneliform, c. 0.5 cm long and 1 cm across, light green at bud stage turning black on anthesis; lobes linear, striate, shortly recurved at apex, puberulous on both surfaces, purple pigmented, margin entire, c. 0.8 × 0.1 cm. Corolla base cupular, bright yellow, c. 5 cm in diam.; petals elliptic-oblong, puberulous, mucronate at apex, 2.5-2.9 ×1.0-1.5 cm. Stamens yellowish; filament glabrous, c. 2 mm long; anthers c. 3.5 × 2 mm, extrorse, thecae dull black. Female flowers axillary, solitary, peduncle 4 cm long, glabrous; pedicel slender, glabrous, c. 0.7 cm long, subtended by a minute bract. Sepals light green, with a black blotch at base, 1 cm long, striate, puberulous, margin entire, shortly recurved at apex. Corolla same as in male flowers. Staminodes 3, bright yellow, alternating the sepals. Ovary ovoid, light green, base truncate, c. 9 × 7 mm, soft papillose; papillae acute at the tip; style light green, glabrous, c. 4 mm long; stigma greenish yellow, narrowly 'V' shaped, apex acute, c. 3 mm long. Fruits dark green, turning bright orange on ripening, ovoid, base rounded, papillae broadly deltoid, 4-5 × 2.5-3 cm, 14-18g, rostrate at apex; rostrum c. 1 cm long, straight; stalk green, slender, glabrous, accrescent up to 9 cm long, with a persistent minute bract. Seeds black, shining, losing lustre on drying, round or faintly cogwheel shaped, warty, margin undulate, 6.5-7.6 × 6-6.5 × 4.0-4.5 mm, sculptured on faces with irregular furrows and ridges; seed coat hard, brittle; cotyledons oily.

Key to the dioecious species of Momordica in India

1a. Anthesis in the evening, flowers small, pale yellow, intensely musk-scented, male calyx whitish yellow, sepals of male flower narrow acute
---------- ***M. dioica***

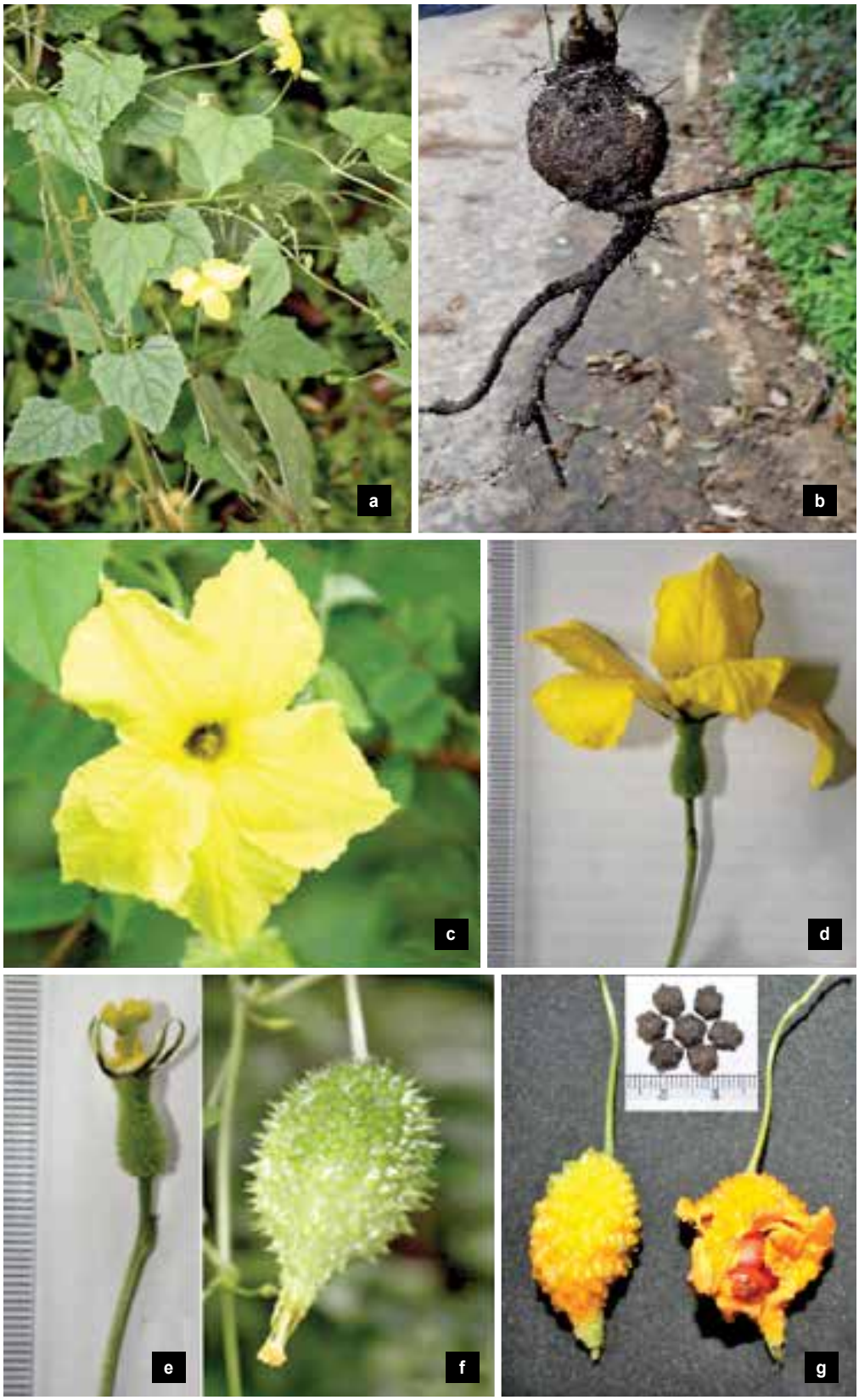

Fig. 1. ***Momordica sahyadrica*** **subsp.** ***anamalayana*** **a. Habit; b. Taproot tuber; c. Male flower; d. Female flower; e. Female flower without petals; f. Fruit; g. Ripe fruits (inset: seeds)**

1b. Anthesis in the early morning, flowers showy, bright yellow, feebly scented, male calyx blackish purple, sepals of male flower broad, tip oval, round or scarious ---------- 2

2b. Perennial with adventitious root tubers or woody taproot, petals (3 inner) with black purple blotch, male calyx- hypanthium saucer shaped. ----------4

2a. Perennial with tuberous taproot, petals without purple blotch, male calyx-hypanthium cup-shaped ----------3

3a. Stem stout; male pedicel 10 mm long; flowers 6.5-8 cm across; male and female petals not overlapping at base; male sepal c. 6 mm wide; corolla saucer shaped, mucronate at apex; stigma bright yellow; papillae on the ovary with a hyaline apex; fruit with blunt rostration, 35-50g ---------- ***M. sahyadrica*** subsp. ***sahyadrica***

3b. Stem slender; male pedicel c.1 mm long; flowers c. 5 cm across; male and female petals overlapping at base; male sepal c. 1 mm wide; corolla cupular, acuminate at apex; stigma greenish yellow; papillae on the ovary without a hyaline apex; fruit with snout-like rostration, 14-18g ---------- ***M. sahyadrica*** subsp. ***anamalayana***

4a. Adventitious root tubers many, leaf cordate, unlobed, margins dentate, petiole eglandular, male calyx blackish purple, broad, tip round-oval, fruits faintly or prominently ridged, softly echinate or muricate, seeds medium sized, pitted on surface, rectangularly cog-wheel shaped ---------- 5

4b. Adventitious root tubers absent, leaf unlobed or shallowly 3-lobed, margins undulate, petiole with bead like umbilical glands, male calyx green, broad, tip triangular, fruits with abundant short conical projections, seeds large, flat and smooth on surface, hexa-octagonal, subtridentate on ends ---------- 6

5a. Plants delicate, fruits 5-8 cm long, irregularly ridged or longitudinally alate, non-spiny on surface ---------- ***M. subangulata*** **subsp.** ***subangulata***

5b. Plants stout, fruits 5-10 cm long, densely soft spiny, rarely with remnant ridges -------- ***M. subangulata*** **subsp.** ***renigera***

6a. Flowers up to 9 cm wide, creamish, male flower corolla not reflexed, staminodes with a black tip, fruit dark green, elliptic ---------- ***M. cochinchinensis*** **subsp.** ***cochinchinensis***

6b. Flowers up to 6 cm wide, orange-yellow, male flower corolla reflexed at middle, staminodes without a black tip, fruit light green, ovate ---------- ***M. cochinchinensis*** **subsp.** ***andamanica***

M. sahyadrica subsp. *anamalayana* differs from *M. dioica* in its flowers being non-scented, anthesis during the day, black - purple pigmented calyx, unlobed to shallowly lobed membranous leaves and fragile habit. The prominent morphological differences with *M. sahyadrica* subsp. *sahyadrica* are highlighted in Table 1 and Fig. 1 and 2.

The sequences of *matK* locus from two accessions of *M. sahyadrica* and one accession each of *M. charantia*, *M. subangulata* subsp. *renigera, M. dioica, M. balsamina*, *M. cochinchinensis* and *M. cymbalaria* were annotated and deposited in GenBank (accession numbers KT004665, KT004664, KP696795, KP895557, KP997313, KT984124, KT984125 and KP696796, respectively). The annotated sequences of three accessions of *M. sahyadrica* subsp. *anamalayana* and one accession of *M. sahyadrica* are submitted to GenBank (accession numbers awaited). The accessions used in the study have represented the available species in India. The phylogram (Fig. 3) generated through the phylogenetic analyses had yielded three distinct clusters. First cluster included the accessions of *M. sahyadrica* along with the accessions of *M. sahyadrica* subsp. *anamalayana* and an accession of *M. dioica*. The accessions of *M. sahyadrica* subsp. *anamalayana* were found to cluster within one sub-cluster. Second cluster has accommodated the accessions from *M. subangulata* subsp. *renigera* and *M. cochinchinensis* whereas, cluster three had accessions from *M. cymbalaria*, *M. charantia* and *M. balsamina*. Thus, the results of the *matK* barcode locus analysis had shown that morphologically unique populations of *M. sahyadrica* from Anamalai region of southern Western Ghats deserves a sub-specific status under *M. sahyadrica* as it is distinct from other taxa.

M. sahyadrica or mountain spine gourd is an endemic species of Western Ghats running across

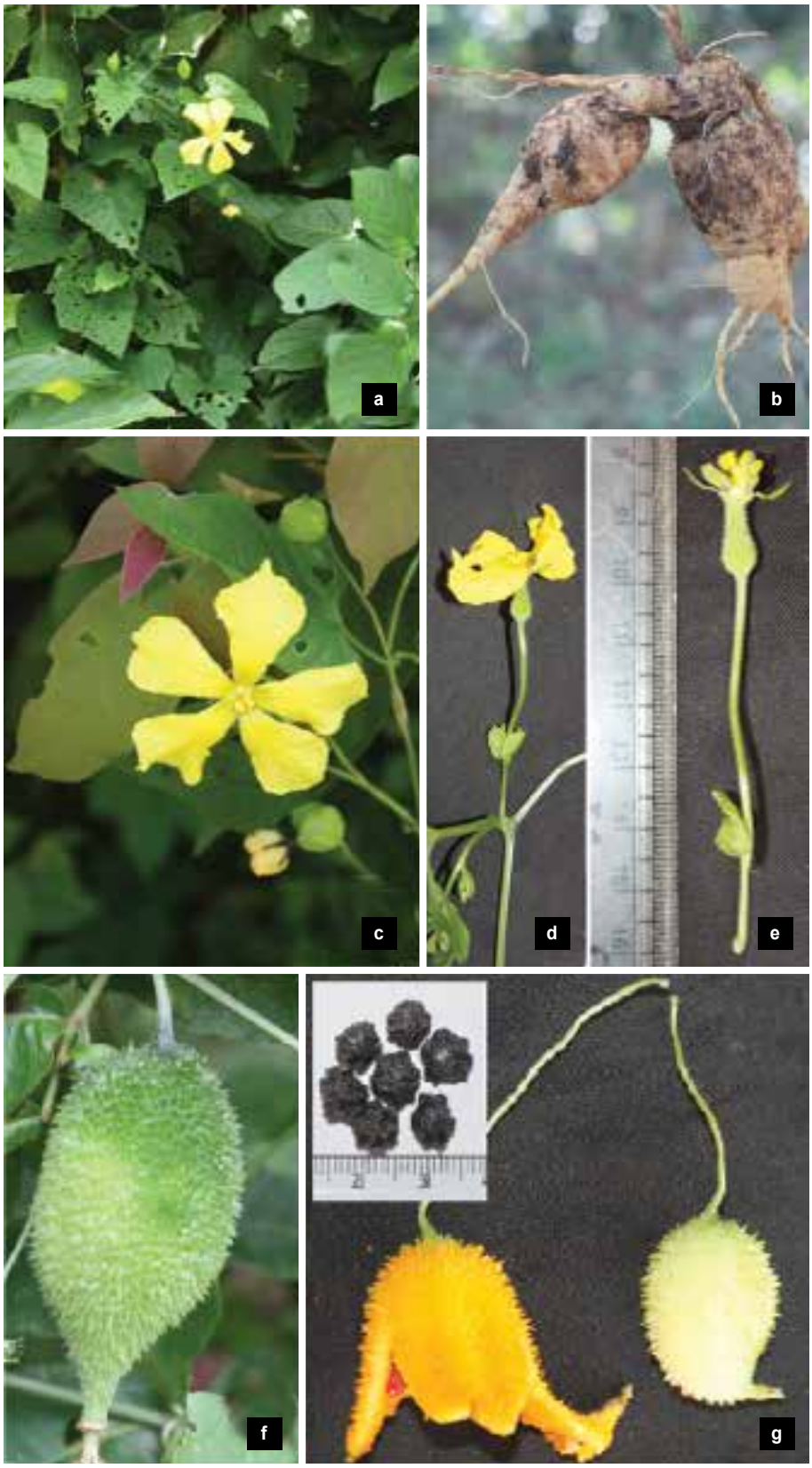

Fig. 2. ***Momordica sahyadrica*** **subsp.** ***sahyadrica*** **a. Habit; b. Taproot tuber; c. Male flower; d. Female flower; e. Female flower without petals; f. Fruit; g. Mature and ripe fruits (inset: seeds)**

Table 1. Comparative morphological characters of *Momordica sahyadrica* subsp. *sahyadrica* and *M. sahyadrica* subsp. *anamalayana*

Character	*M. sahyadrica* subsp. *sahyadrica*	*M. sahyadrica* subsp. *anamalayana*
Stem	Stout	Slender-delicate
Leaves	Thick, lobes prominent, dark green	Sub-chartaceous, lobes not prominent, pale green
Male flower – peduncle length (cm)	Up to 5	Up to 7
Male flower – pedicel length (mm)	10	1.0
Male flower – sepal	Linear, 6 mm broad, margin undulate	Linear, 1 mm broad, margin entire
Female flower – sepal	c. 7 mm long, margin undulate	c. 10 mm long, margin entire
Corolla (male & female)	Up to 8 cm in diam., base saucer shaped, lemon yellow	Up to 5 cm in diam., base cupular, bright yellow
Petals (male & female)	Obovate, acute or acuminate	elliptic-oblong, mucronate at apex
Stigma	Bright yellow, broadly 'V' shaped, apex obtuse	Greenish yellow, narrowly 'V' shaped, apex acute
Ovary	Hypanthium c. 8 mm long, papillae with hyaline apex	Hypanthium c. 4 mm long, papillae without hyaline apex
Fruit	Ovate- ellipsoid, with c. 2 mm long rostrum with blunt end	Ovate-round, with c. 10 mm long snout like rostrum
Single fruit weight (g)	35-50	14-18
Seed size (length × width × thickness) (mm)	6.2-7.7 × 5.4-6.9 × 3.5-4.0	6.5-7.6 × 6-6.5 × 4.0-4.5
No. of seeds/ fruit	35-50	25-32

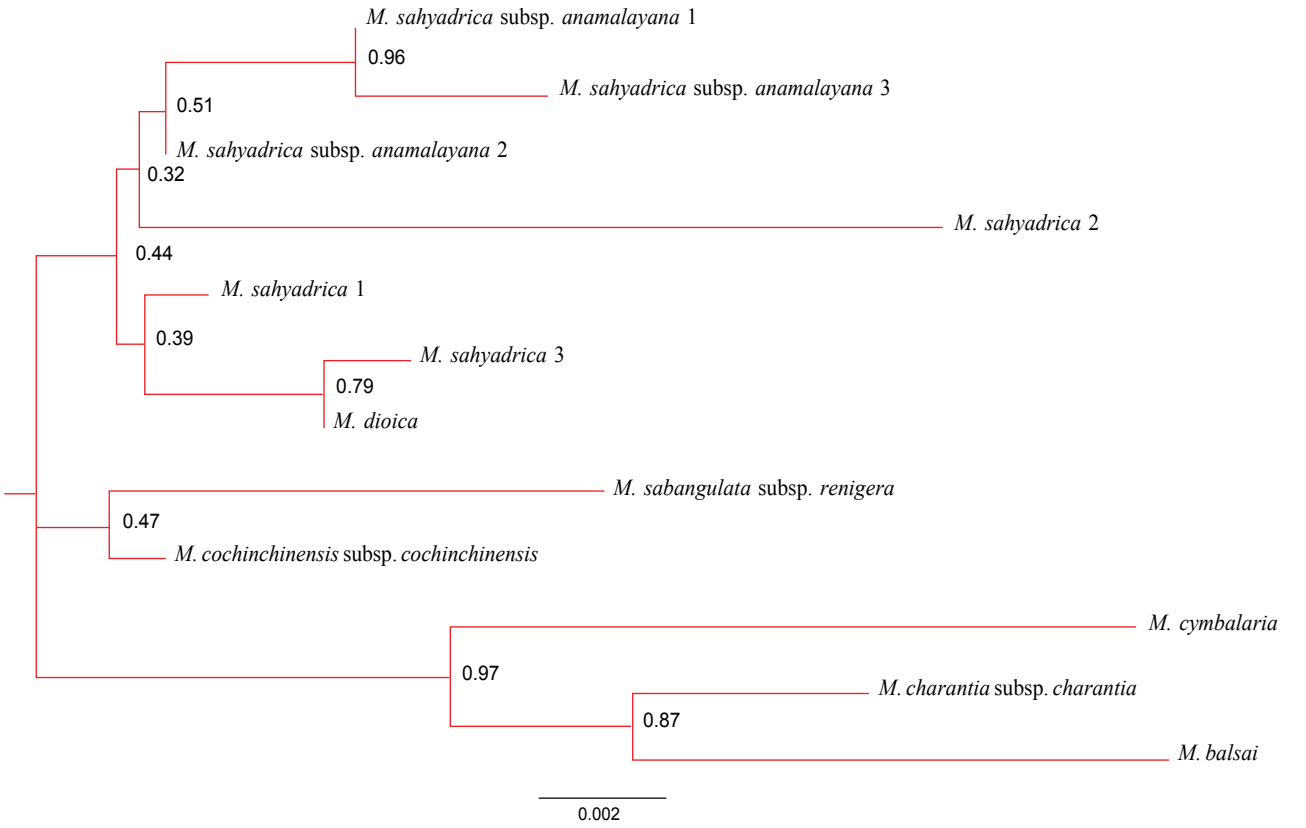

Fig. 3. Neighbour joining tree showing the genetic relatedness among different species of *Momordica*, based on the sequences of *matK* chloroplast locus

the states of Kerala, Tamil Nadu, Karnataka, Goa and Maharashtra. It is used extensively as a wild gathered vegetable and consumed by forest dwelling tribes in high ranges of Kerala and elsewhere in the Western Ghats of Karnataka, Goa, Tamil Nadu and Konkan region of Maharashtra. It is cultivated occasionally in home gardens in high ranges of Karnataka and Kerala for tender fruits and leaves used as vegetables. The newly described subspecies is a niche-specific and narrow endemic taxon restricted to a small stretch of evergreen forest at c. 700 m amsl in the Sholayar forest adjoining Parambikulam Wildlife Sanctuary and Nelliampathy Hills in Palakkad district of Kerala. Humus-accumulated rocky crevices in the evergreen forest openings are its ideal habitat. Some of the associated vegetation observed are *Ochlandra travancorica* (Bedd.) Benth. ex Gamble, *O. rheedei* (Kunth) Benth. & Hook f. ex Gamble, *Cullenia excelsa* Wight, *Nothapodytes nimmoniana* (Graham) Mabb., *Cucumis sativus* L. var. *hardwickii* (Royle) W.J. de Wilde & Duyfjes, *Piper barberi* Gamble, *P. nigrum* L., *Dioscorea oppositifolia* L., *Zingiber zerumbet* (L.) Sm., *Vigna bourneae* Gamble, *Musa acuminata* Colla, *Musa kattuvazhana* K.C. Jacob and *Abelmoschus angulosus* Wight & Arn. The habitat is characterized by evergreen vegetation and high rainfall (3000 mm) spread over the entire growth period. The above-ground parts dry up on attaining senescence by the end of November and fresh sprouts emerge from the underground tuber with pre-monsoon rains in April. Even though its habitat comes under reserve forests, extensive spread of the alien exotic weed, *Mikania micrantha* Kunth., poses a serious threat to its survival by impeding the emergence of juvenile plants. It is used as a wild gathered vegetable by Mannan and Muthuvan tribes in Sholayar forest range, Kerala.

Phenology: Flowering from mid-June to early October and fruit ripening from mid-July to early November.

Eponymy

The new subspecies is named after its type locality; Anamalai Hills in the Sahyadri Hill Ranges which comes under Southern Western Ghats, one of the hotspots of endemism.

Additional specimens examined:

1. India, Kerala, Thrissur district, Sholayar, Patha-dippalam, 16.11.2014, Joseph John K & K Pradheep JP/14-61 (NHCP)
2. India, Kerala, Palakkad district, Nelliampathy, Kaikatty, 6.10.2016, Joseph John K. JB/16-16 (NHCP)
3. India, Kerala, Thrissur district, Charpa- Vazhachal, 5.10.2016, Joseph John K. JB/16-13 (NHCP) (Lat. 10.18.136, Long. 076.42.304)
4. India, Kerala, Thrissur district, Malakkappara, 5.10.2016, Joseph John K. JB/16-12 (NHCP) (Lat. 10.08.054, Long. 076.45.061)
5. India, Kerala, Thrissur district, Athirappally, 29.10.1999, Joseph John K 99-585 (IC 256223) (NHCP)
6. India, Kerala, Thrissur district, Sholayar, Patha-dippalam, alt. 900m., 29.10.1999, Joseph John K 99-590 (IC-256228) (NHCP)
7. India, Kerala, Thrissur district, Malakkappara Tea estate, alt. 900m., 29.10.1999, Joseph John K 99-593 (IC-256231) (NHCP).

Observations under ex-situ conditions

Fresh seeds do not germinate readily as they have a dormancy period of about six months. Seeds germinated in sand beds were used to raise plants *ex situ*. Seedlings come to flower within 60-65 days and fruits take 28-30 days to reach dead-ripe stage. Pollination is by native bees like *Trigona* sp. and *Chrysomya* sp. Seed dispersal is through frugivorous birds. It is prolific bearing in the cool climate of mid elevation ranges of Western Ghats evergreen landscapes and may be maintained on-farm in tribal hamlets as a wild gathered vegetable. It sets fruits and viable seeds on crossing with *M. dioica* pollen. It can be a valuable genetic resource for improvement of spine gourd, teasel gourd and sweet gourd group of vegetables. Four populations are maintained in the field gene bank and medium term seed storage facility at ICAR-NBPGR, Thrissur, Kerala.

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References

Bharathi LK and K Joseph John (2013) *Momordica Genus in Asia – An Overview*. Springer Verlag, New Delhi, 147 p.

Chakravarty HL (1959) Monograph of Indian *Cucurbitaceae* (Taxonomy and distribution). *Rec. Bot. Surv. India* **17:** 1-234.

Chakravarty HL (1982) *Fascicles of Flora of India*, 11. *Cucurbitaceae*. Botanical Survey of India, Calcutta, 136 p.

de Wilde WJJO and BEE Duyfjes (2002) Synopsis of *Momordica* (Cucubitaceae) in South East Asia and Malesia. *Bot. Zhurn.* **87(3):** 132-148.

Girme AR (2014) *DNA Barcoding in Momordica spp*., M.Sc. Dissertation. Kerala Agricultural University, Thrissur, India.

Jain SK and RR Rao (1977) *A Handbook of Field and Herbarium Methods*. Today and Tomorrow Printers and Publishers, New Delhi, 157 p.

Joseph John K (2005) *Studies on Ecogeography and Genetic Diversity of the Genus Momordica L. in India*. Dissertation submitted to the Mahatma Gandhi University, Kottayam, Kerala. Unpublished Ph.D. Thesis.

Joseph John K and VT Antony (2007) *Momordica sahyadrica* sp. Nov. (Cucurbitaceae), an endemic species of Western Ghats of India. *Nord. J. Bot.* **24:** 539-542.

Joseph John K and VT Antony (2010) A taxonomic revision of the genus *Momordica* L. (Cucurbitaceae) in India. *Indian J. Plant Genet. Resour*. **23(2):** 122-184.

Manilal KS (2003) *Van Rheede's Hortus Malabaricus*, *English Edition, with Annotations and Modern Botanical Nomenclature*, *12 Vols*. University of Kerala, Trivandrum, 3200 p.

Nicolson DH, CR Suresh and KS Manilal (1988) An interpretation of van Rheede's *Hortus Malabaricus*. Koeltz Scientific Books, D-6240, Konigstein, Germany.

Schaefer H and SS Renner (2011) Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxon* **60(1):** 122-138.