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MICROMORPHOLOGY, KARYOLOGY AND PHYTOCHEMICAL STUDIES ON THE *CARALLUMA ADSCENDENS* (ASCLEPIADACEAE) COMPLEX IN TAMIL NADU, INDIA

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ABSTRACT

Caralluma adscendens (Roxb.) R. Br. (Apocynaceae) is a wild growing variable succulent herb distributed frequently in drier regions of Peninsular India especially more diversified in Tamilnadu. Seven varieties under the taxa recognized and they have given detail morphological and distributional notes for these varieties. Local Floras are not treating these varieties properly and still exhibits controversy in the identification on these taxa. The present study emphasizes on the floral micro-morphology, chromosome numbers and amount of flavonoid compounds in each variety, resulted that clear differences showed between each varieties. Specifically rounded angle varieties namely var. *adscendens, attenuate, bicolor* and *fimbriata* are possessed uniform chromosome number (2n = 22), whereas acute angled varieties such as var. *carinata* and var. *gracilis* are having tetraploid (autoploid 2n = 44). Var. *geniculata* is reported only 2n = 22 chromosomes but it has acute angled with phytogeographically segregated in nature when compared to all other varieties. The present study provided separate manual keys for *Caralluma adcendens* and their varieties based on the stem morphology, corolla epidermal micromorphology, karyology and chemical compounds present in them. This study would be supported that the elevation of var. *geniculata* into species level as *Caralluma geniculata* mentioned by Meve and Leide (2002).

Keywords: *Caralluma adscendens,* Micromorphology, Karyology Phytochemical studies**.**

1. INTRODUCTION

Caralluma was first described in 1810 by Robert Brown to include an Indian species with very characteristic elongated flowering stems. A number of other related genera were consequently described: *Desmidorchis* (1829), *Boucerosia* and *Hutchinia* (1834), *Aptheranthes* (1835), *Sarcodon* (1878), *Orbeobsis* and *Pachycymbium* (1978), *Quaqua* (1879), *Tridentea* (1980), *Pectinaria* (1981), *Urmalcala* and *Rhytidocaulon* (1990), *Australluma, Borealluma, Caudanthera, Crenulluma, Cryptolluma, Cylindrilluma, Monolluma, Sanguilluma, Saurolluma, Somalluma, Spiralluma* and *Sulcolluma* (1995). Indian *Caralluma*, with over 14 species and perhaps 4 genera, have been much less studied and are more diverse than is generally realized (Karuppusamy *et al*., 2013). Many new species have been described since Gravely and Mayuranathan (1931), Kumari and Subba Rao (1976), Lavranos and Frandsen (1978), and Sarkaria (1980). However, only very tentative primitive steps have been taken towards untangling the problems of overall species relationships and generic delimitation. After getting revision of the genus *Caralluma* by Gilbert (1990) and Plowes (1995), the genus is attracting the botanists in many countries. But a still vast lacuna in the taxonomy of Indian *Caralluma* is great controversy.

Caralluma adscendens (Roxb.) R.Br. (Syn. *Stapelia adscendens* Roxb.) is originally described from coast coromandal area of Peninsular India without indicate precise locality by Roxburgh. Gravely and Mayuranathan (1931) recognized a total of six varieties within this species. Recently one more new species *Caralluma bicolor* described from Tamil Nadu and which is now consider under the variety of *Caralluma adscedens* (Karuppusamy *et al*., 2013). Mainly these varieties can be divided into 2 major groups based on whether the stems have rounded or acute angles. But Bruyns (1984) has drawn attention to the very great similarity between one of these variety *fimbriata*, and *Caralluma subulata* from Arabia and *Caralluma dalzielii* from West Africa. This could be taken to indicate that the two groups such as round and acute angles varieties merit formal recognition at least as subspecies. The status of the varieties within *C. adscendens* seems more questionable as Gravely and Mayuranathan indicated that intermediates are apparently common (Gilbert, 1990).

The earlier accounts indicates that *Caralluma adscendens* is variable and widely distributed species

at least seven distinct morphoforms have recognized. In the three varieties, viz. var. *adscendens s.str*., *attenuata* and *fimbriata*, that have long been known and have hitherto been regarded as distinct species (Gamble, 1919). The stem is squarish in section with the sides usually slightly hollowed and the angles always rounded. Another three varieties are acute angles i.e. var. *carinata, gracilis* and *geniculata* which are distributed in narrow ranges in Tamilnadu. Gravely and Mayuranathan (1931) have suggested to use classification of three varieties partly in the flower morphology and partly in the stem morphology. But these characters unable to hold in herbarium specimens of these groups, because of these are succulent genera. There is considerable floral (but also vegetative) variability in *C. adscendens*, quite often even between plants of single populations, but almost always between different populations. These circumstances have stimulated many taxonomists and regional florists to describe morphotypes, ecotypes or varieties or forms. The diversity of *C. adscendens* in Tamilnadu is indeed higher than other states of Peninsular India. Even though in many places, overlapping populations are exhibiting among these varieties. Bruyns (1987) suggested that variable forms of African *Caralluma* complex taxonomically still needs to be answered because of this complex has never been revised as a whole. Egyptian *Caralluma europaea* complex have been revised based on the morphology and karyology studies by Meve and Heneidak (2005). Meve and Liede (2002) tried molecular taxonomy of this group, they treated on variety under *C. adscendens* var. *geniculata*, is raised to species level (*Caralluma geniculata*) based on their molecular data.

With special reference to micromorphological, karyological and chemical data, this study is the first attempt to present a detailed picture of the infraspecific differentiation within the *Caralluma adscendens* complex.

2. MATERIALS AND METHODS

2.1. Plant specimens

The materials used for morphological, karyological and chemical investigations are summarized in Table 1.

2.2. Morphological study

Floral parts viz. corolla epidermis, hairs, corona, pollinaria have been investigated under the stereo microscope.

2.3. Chromosome counts

Chromosome numbers were established from adventitious root tip squash preparations. The root tips were pretreated in 0.02 M hydroxyquinoline for 4 h at 20^0C (Meve and Heneidak, 2005), fixed in Carnoy's solution for 24 h at 20^0C and stained with carmine for 24 h at 60^0C (Snow, 1963).

2.4. Chemicals analysis

For extraction of flavonoids 1 g of crushed fresh stem material was extracted in 3ml absolute ethyl alcohol, boiled for about 2 min, cooled and left for 24 h. Samples were filtered, dried and rehydrated im 3 ml of 70% ethyl alcohol. About 20µl were used for analytical High Performance Liquid Chromatography (HPLC) analysis. They were identified by means of UV spectroscopy using shift reagents (Markham, 1982), acid hydrolysis and analysis of aglycone and sugar moieties (Harbone, 1998), cochromatography with authentic standards (FLC, 2006) were compared for the amount and types of flavonoid subgroups.

Table 1. Voucher specimens collected and used in this study.

Name of the variety	Place of collection	Voucher number
Caralluma adscendens var. *adscendens*	Madurai (Madurai district)	S. Karuppusamy, 118
var. *adscendens*	Nagamalai (Madurai district)	S. Karuppusamy, 265
var. *attenuata*	Oddanchatram (Dindigul district)	S. Karuppusamy, 259
var. *attenuata*	Palani (Dindigul district)	S. Karuppusamy, 75
var. *attenuata*	Tirumayam (Tanjaore district)	S. Karuppusamy, 752
var. *attenuata*	Madhukkarai (Coimbatore district)	S. Karuppusamy, 824
var. *fimbriata*	Barigam (Dharmapuri district)	S. Karuppusamy, 53
var. *fimbriata*	Bhavani (Erode district)	S. Karuppusamy, 325
var. *fimbriata*	Kolli hills (Namakkal district)	S. Karuppusamy, 68
var. *carinata*	Nagamalai (Madurai district)	S. Karuppusamy, 329
var. *carinata*	Sirumalai (Dindigul district)	S. Karuppusamy, 783
var. *geniculata*	Maruthuvamalai (Kanyakumari dt.)	S. Karuppusamy, 589
var. *gracilis*	Varathamanathi (Dindigul district)	S. Karuppusamy, 673
var. *gracilis*	Vaguthumalai (Madurai district)	S. Karuppusamy, 89
var. *gracilis*	Pudukottai (Pudukottai district)	S. Karuppusamy, 435

3. RESULTS AND DISCUSSION

3.1. Habit and habitat

As is typical for most Carallumas, *Caralluma adscendens* prefers shaded stands such as the base of thorny bushes (Jonkers and Walker, 1993). The individuals nevertheless vary considerably in their general habit depending on the edaphic situation. In deep, sandy soil there is a strong tendency to form diffuse stem base especially variety *attenuata*. On rocks the plants grows usually from compact clumps of rather thick stems (var. *geniculata*). Typically, the species grows on rocky slopes among rocks under stunted bushes in Salem, Dharmapuri and Dindigul districts. Var. *carinata* attains more than a meter height in Nagamalai in Madurai district and Sirumalai hills in Dindigul districts (Fig.2a).

3.2. Vegetative morphology

Stem surfaces and angels are variable in all six varieties. The colour varies from uniform bright green (var. *gracilis*) to dark blue green (var. *geniculata*). Varieties *adscendens, attenuata* and *fimbriata* are rounded angles and varieties *carinata, gracilis* and *geniculata* are acute angled stems.

3.3. Floral morphology

Variability in flower morphology of *C. adsendens* is higher than typically found in widespread stapeliads, and has therefore hampered a sound taxonomic treatment. The increasing availability of plant material has demonstrated infraspecific variability, where almost every population shows its own features (the many illustration published by Gravely and Mayuranathan, 1931). Especially with regard to frequently occurring differences in corolla structure, size and ciliation, a wealth of taxa has been described (see floral key). However, several distinct tendencies that support infraspecific differentiation have to be considered. Inner coloration of the corolla surface is predominantly purple, but creamy yellow developed in var. *gracilis*. Dense purple-red transversal stripes or streaks are found in all the six varieties of *C. adscendens* but each variety it has differentiated by amount and patterns of striation (Fig 1&2). Flower size is small in var. *gracilis* but rather larger in var. *carinata.* Amount of hairiness of the petals are also varied in all six varieties (Fig. 1& 2).

Microscopic studies of the corolla epidermis of samples (each voucher from Table 1) were conducted that revealed a characteristic separation between the verities and its populations. In the varieties *attenuata, fimbriata, adscendens* showed homogeneously isodiametric with slightly convex to dome-shaped papillate (bottle-neck like) cells (Fig.2e). But in the varieties of *gracilis, geniculata* and *carinata* have observed heterogeneous cells mingled with papillate-apiculate cells on the corolla epidermis. The very smooth and tiny periclinals lacks a pleated cuticle but they are topped by mighty papilla-like structure showing a verrucose to prickly surfaces. Corona structures and their coloration are rather similar over all the varieties. The corona is normally purplish; however varieties *fimbriata* and *attenuata* is occasionally yellowish-brown. Shape and size of pollinaria are also greatly differing in all six varieties.

3.4. Chromosome numbers

All 14 samples of six varieties investigated (Table 2) possess basic somatic 2n = 22 chromosomes in the varieties *adscendens, attenuata, fimbriata* and *geniculata* and the chromosome number tetraploid (2n = 44) in the varieties *carinata* and *gracilis*. This is the standard situation in Asclepiadaceae, where ca 96% of the investigated taxa have a basic chromosome number of n = 11, and over 90% are euploid diploids (Albers and Meve, 2001). Karyotypic analysis revealed a homogeneous gemone of predominantly meta- to sub-metacentric chromosomes for *C. adscendens*. One pair of chromosome shows secondary constrictions with satellites in all the varieties. This feature is already mentioned by Albers and Meve (2001).

3.5. Chemical analysis

The flavonoid profiles of all the six varieties were investigated (Table 3). The HPLC retention time and UV spectral maxima of the flavonoid glycoside compounds analyzed and given. The overall flavonoid compounds rich in the variety *carinata* when compared with all other fivevarieties.

Table 2. Chromosome number of *Caralluma adscendens* complex.

Variety	Voucher used	Chromosome number	Karyo type
var.*adscendens*	S. Karuppusamy, 165	22	28.23
var.*attenuata*	S. Karuppusamy, 75	22	27.31
var.*bicolor*	S. Karuppusamy 1026	22	27.58
var.*fimbriata*	S. Karuppusamy, 68	22	28.12
var.*carinata*	S. Karuppusamy, 329	44	28.51
var.*gracilis*	S. Karuppusamy, 673	44	27.92
var.*geniculata*	S. Karuppusamy, 589	22	22.15

Morphologically, there are quantitative differences between all the six varieties. Typically the corolla epidermis of those samples is more strongly sculptured with markedly convex outer epidermal wall specifically in each variety. The amount of papillate sculpture serves as demarcation line between the varieties. The present study is

comparable with *Caralluma europaea* complex (Meve and Heneidak, 2005).

Table 3. HPLC retention times and UV spectral maxima and positive flavonoid compounds found in all six varieties of *C. adscendens* studied.

Variety	HPLC		Possible flavonoid compounds in percentage (%)
	Rt (min)	UV λmax (nm)	
Var. *adscendens*	13.55	274, 329	2.92
Var. *attenuata*	12.59	270, 331	2.96
Var. *bicolor*	13.22	272, 253	4.85
Var. *carinata*	14.07	274, 329	14.79
Var. *fimbriata*	13.52	271, 329	12.62
Var. *geniculata*	13.28	271, 328	5.28
Var. *gracilis*	13.92	254, 356	7.51

Taxonomically significant variation in chromosome sizes in stapeliads have been reported by Albers and Meve (2001) at the tribe level, Meve and Liede (2001) at genus and species level and Meve and Heneidak (2005) at infraspecific level. Even within species, however, chromosome length high varies around 0-2-%. The ca. 10% deviation found between the two varieties *C. adsecendens* is then not significant at al, and falls within the variation range which can be expected on species level.

All six varieties of *C. adscendens* investigated and collected in different places in Tamilnadu only in the amount of their flavonoid compounds. Flavonoids, so far known, generally are of limited occurrence and diversity in Apocynaceae-Asclepiadaceae (Meve and Heneidak, 2005). Mostly, aglycone and glycosides occur in limited diversity and quantity in comparison to many angiosperms. Especially from the *Caralluma*, glycosides are mostly known as pregnane glycosides (Halim and Khalil, 1996). This study were restricted to the easily investigated aglycone parts the flavonoid glycosides. In the presence of Luteolin-4'-O-neohesperidoside was already reported from *Caralluma adscendens* var. *attenuata* (Ramesh et al, 1999). All other varieties are also possessed the same compounds in different amount in their plant parts. The present study indicated the presence of the higher amount of flavonoid compounds in the var. *carinata* when compared to other varieties tested. Possibly this flavonoid is characteristic for this group and which is having more taxonomic significance.

Plowes (1995) suggested that the *C. adscendens* complex in India may segregate species level. This has not been questioned by succeeding *Caralluma* investigators. Considering the high vegetative, floral and chemical variability, the diploidy and tetraploidy, and the narrow and nearly discontinuously distribution, the treatment of the complex as variable species. The acute angled varieties (var. *carinata* and *gracilis*) is having tetraploid (autoploid) chromosomes, which is taxonomically significant but these populations are not overlapping anywhere in natural condition. Another acute angled variety *gneiculata* might have originated independently with its habitats due to geographical isolation. Hence, this variety may be considered as separate species as supported by Meve and Liede (2002). All other rounded angle varieties might be considered as ecotypes which are exhibiting overlapping populations sometimes. The differences between the varieties are really pronounced between rounded angled and sharp angled. However, the fairly high number of characters concerned point to a considerable degree of separation.

3.6. Taxonomy

3.6.1. Caralluma adscendens (Roxb.) R.Br.

Stems fleshy, quadrangular; internodes four-angled, 6–18 mm long and 2–6 mm across, slender above, glabrous. Latex watery. Leaves present only on young branches, reduced to scales, opposite, decussate, sessile, 1–2 mm long, subulate, tip acute, glabrous.

Flowers terminal or subterminal, solitary or paired, axillary; pedicel terete, 3–5 mm long and ca 1.0 mm in diam., brown, glabrous. Calyx 5-lobed, divided up to base, lobes ca 2.5 × 1.0 mm, ovate, apex acuminate, margin thin, glabrous. Corolla campanulate, ca 8 mm long; corolla tube ca 1.5 mm long; lobes 5, 6–7 × 2.5 mm, lanceolate-oblong, apex apiculate, hairy or glabrous. Corona staminal, biseriate; outer annualar, with two filiform filaments, ca 1.5 mm long, alternating with anther-lobes, glabrous; inner 5-lobed, flap-like, overlapping anther-lobes, ca 1.0 × 0.4 mm long, apex truncate or crenate. Stamens 5, ca 1.6 mm long; pollinia 5, pollen masses solitary in each anther cell, yellow, waxy, caudicles light brown, corpuscle dark brown. Follicles usually solitary, 5.5 × 2.3 cm long and 3.5–4.5 mm in diam., cylindrical, peaked with curved tip, base acute, glabrous; seeds many, ca 9.5 × 4.5 mm, oblong to obovate, base rounded, dark brown with light brown margin ca 1.0 mm wide; coma silky white, 2.0–3.0 mm long.

Gravely and Mayuranathan (1931) classified these varieties based on their stem and flower characters. The distribution of these varieties occupied different degree of frequency in Peninsular India. But most dominant and older varieties are var. *attenuata* and var. *fimbriata*, other varieties are

evolved from degree of reduction in hairiness of flower and roundness of stem.

Key to the varieties of *Caralluma adscendens* based on stem and floral morphology:

1. Corolla-lobes hairy 2

1. Corolla-lobes glabrous ……var. *adscendens*

2. Stems rounded towards the bas.....3

2. Stems acute towards the bas..... 5

3. Distal portion of the stem attenuate; 25–50 cm high ..... var. *attenuata*

3. Distal portion of the stem not attenuate; 12–20 cm high 4

4. Flowers large, deep purple hairs along the margin; pedicel pendulous …var. *fimbriata*

4. Flowers small, greenish purple; hairs not developed; pedicel erect.....var. *gracilis*

5. Pedicel abruptly bent; hairs on corolla not strongly developed; stems not attenuate …… var. *geniculata*

5. Pedicel never bent; hairs strongly developed; stems obviously attenuate....var. *carinata*

Key to the varieties of *C. adscendens* based on corolla epidermal morphology:

1. Corolla epidermal cells homogeneous …… (2)

1. Corolla epidermal cells heterogeneous …… (3)

2. Epidermal cells with distinct apiculate or papillate growth on the top..... (4)

2. Epidermal cells without apiculate process on the surface ..... var. *fimbriata*

3. Papillary growth sharply mucronate, cells intermingled with long hairs … var. *gracilis*

3. Papillary growth with blunt tip, cells without intermingled hairs.....(5)

4. Apiculate process distinctly bottle-neck shaped var. *attenuata*

4. Apiculate process simply elongate, not constricted below the papilla .. var. *adscendens*

5. Papilla uniformly elongated, cells hexagonal …….. var. *carinata*

5. Papilla differently elongated, cells polygonal …….. var. *geniculata*

3.6.2. Caralluma adscendens var. *adscendens*

Stems often with reddish streaks, not attenuate, but slightly tapering in distal end, their angles rounded, acute; leaf-scars raised on more or less distinct especially in older stems and outwardly directed tubercles; flowers hairless, more or less pendulous; pale base of inner side of petals finely spotted with purple spots or striped transversely, only on basal half of inner side.

Flowers**:** March–February; Fruits: July–September

Gravely and Mayuranathan noted that this variety occurs from northeast of the Godavari to south end of Attapady valley of Malabar. Dense populations of this variety occur in Coimbatore, Salem, Pudukottai and Madurai districts of Tamilnadu. Present study specimens were collected from Madurai and Pudukottai districts of Tamilnadu, but a few populations have been observed in Nagamalai hills near Madurai, where they have flowers with short hairs on the margins of the petals and are prominently striped purple on the inner side of basal part. In the same district some populations do not have these hairs, and petals are glabrous with a greenish yellow background. All of these populations grow close to cultivated land and exposed dry scrub forests of the foothills. In dry areas they grow about 75 cm tall, but in fertile areas it can reach a height of 1 m.

Distribution**:** Discontinuous population in Peninsular India. Endemic.

3.6.3. Caralluma adscendens var. *attenuata* (Wight) Gravely & Myuranathan

Stems bushy growth, strongly attenuate, usually much branched distally, angles streaked reddish, rounded; flowers hairy, pendulous, dark purple, flowering throughout the year.

This is the most widely distributed of the varieties of *C. adscendens*. The present study found it at Coimbatore, Dindigul, Madurai, Salem and Pudukottai districts of Tamilnadu. It was first described from the foothills of Nilgiris, and Hooker quotes Cochin as another locality, but at that time *Caralluma stalagmifera* unknown and seems often to have been confused with *C. attenuata*. This variety has more hybridizable potency with neighboring *Caralluma* varieties, hybridized forms having been recorded by Gravely and Mayuranathan (1931) from the Madurai and Pudukottai districts of Tamilnadu.

Distribution**:** Distributed all over the Deccan Peninsula.

Figure 1

a. *Caralluma adscendens* var. *adscendens* – habit from Nagamalai hills of Madurai.
b. *Caralluma adscendens* var. *adscendens* – Flower enlarged.
c. *Caralluma adscendens* var. *attenuata* – habit from Kuniyamuthur near Coimbatore.
d. *Caralluma adscendens* var. *attenuata* - Pollinarium

3.6.4.Caralluma adscendens var. *carinata* Sarkaria

Stems acute angled, green, distally strongly attenuate, rarely branched; flowers purplish, hairy, usually pendulous and campanulate but sometimes semi-erect and rotate; pedicels not bent. Maximum height a little over 1m.

Distribution**:** Very few populations known in Tamilnadu (endemic). This variety has been observed only in the Nagamalai and Azhargar hills of the Madurai district and Sirumalai hills of Dindigul district of Tamilnadu. This variety is threatened due to habitat modification.

3.6.5. Caralluma adscendens var. *geniculata* Gravely & Mayuranathan

Stems acute angled, green, slightly attenuate and unbranched distally, but more slender secondary stems frequently branched distally; flowers hairy but less conspicuously so than the other varieties, widely opened, facing upwards, dark chestnut brown markings, pedicel bent at an angle just below the flower; flowering throughout the year.

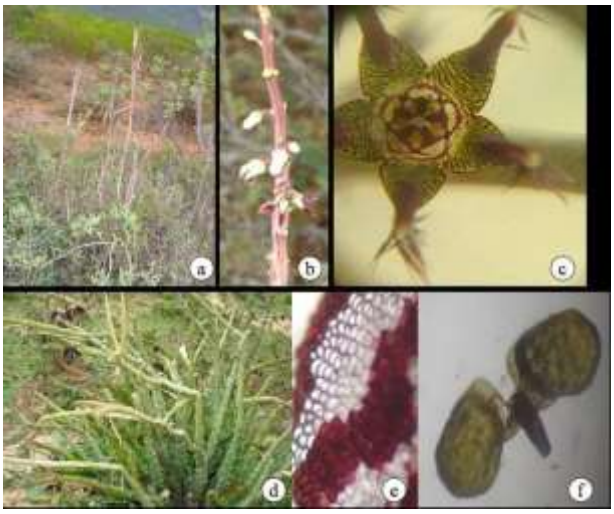
This variety is endemic to Tamilnadu. The present study recorded from Marthuvamalai hills of Kanyakumari district in Tamilnadu.

Distribution**:** A small population located in Tamilnadu.

3.6.6. Caralluma adscendens var. *fimbriata* Wallich

Stems small, not definitely attenuate, equal thickness throughout, creamy reddish distally, not streaked, angles rounded; flowers dark purple, densely hairy along the margin of petals, usually pendulous, flowering throughout the year.

Figure – 2



a. *Caralluma adscendens* var. *carinata* – natural habit growing in Sirumalai foot hills.
b. *Caralluma adscendens* var. *carinata* – flowering stem apex.
c. *Caralluma adscendens* var. *carinata* – Flower enlarged.
d. *Caralluma adscendens* var. *fimbriata* – natural habit.
e. *Caralluma adscendens* var. *fimbriata* – corolla epidermal surface view.
f. *Caralluma adscendens* var. *fimbriata* - Pollinarium

Distribution**:** This variety is described originally from Myanmar, but it has since been recorded from India and Sri Lanka. It exists in discontinuous populations in drier regions of peninsular India. Large numbers of natural populations have suddenly declined due to the increased demand in pharmaceutical market for its glycosides. It is not currently cultivated for this purpose, markets relying on wild collected material.

3.6.7. Caralluma adscendens var. *gracilis* Gravely & Mayuranathan

Stems greenish, purple streaks absent on stem, acute angled, slightly attenuate distally; flowers hairy but less than previous varieties, small, axillary paired below the distal apex, pedicel 1.5 cm; corolla chestnut brown. Maximum height of stems at least 75 cm; flowering throughout the year.

Gravely and Mayuranathan (1931) originally collected and described this variety from the town of Pudukottai forests of Tamilnadu. It is distributed throughout Tamilnadu and occurs in dense populations in Pudukottai forests. In the present investigation specimens were collected from the

forests of Palni hills. This variety also hybridizes with *C. stalagmifera* and other neighboring varieties presenting intermediate forms (Gravely & Mayuranathan, 1931).

Distribution**:** South Tamilnadu to north of Palni hills to Pudukottai. This variety is endemic to Tamilnadu.

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