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Typification and cytology of 'Arabian Wax Cissus' (*Cissus rotundifolia* Lam.)

Pooja Bhagure[1] | Nilesh Madhav[2] | Ashwini Mohite[3] | Anubhab Mondal[1] | Kumar Vinod Gosavi[2] | Jagdish Dalavi[1] | Mayur Kamble[1]

[1]Botanical Survey of India, Western Regional Centre, Pune, 411001, Maharashtra, India
[2]Department of Botany, HPT Arts & RYK Science College, Nashik, 422005, Maharashtra, India
[3]Department of Botany, Yashvantrao Chavan Institute of Science, Satara, 415001, Maharashtra, India
E-mail:
pshivrekha.77@gmail.com, madhavnilesh1@gmail.com, ashwinisu10@gmail.com, anubhabmondal37@gmail.com, kumarvinodgosavi@gmail.com, jagdish.taxonomy@gmail.com, mayurkamble1@rediffmail.com
ORCID-ID: 0009-0005-6213-9221 (PB), 000-0002-6581-4721 (NM), 0000-0002-6676-4965 (AsM), 0000-0003-4355-8189 (AnM), 0000-0001-6681-2253 (KVG), 0000-0002-0620-4835 (JD), 0000-0001-5290-0789 (MK)

Author for correspondence:
Jagdish Dalavi
Botanical Survey of India, Western Regional Centre, Pune, 411001, Maharashtra, India
E-mail:
jagdish.taxonomy@gmail.com

Abstract

Cissus rotundifolia Lam. (Vitaceae), a member of the grape family commonly called 'Arabian Wax Cissus', has recently been identified as a naturalizing exotic plant in the flora of Maharashtra and Karnataka. The plant has gained importance in India for its ornamental value and edible fruits. While conducting field studies in peninsular India, were collected four populations and were studied mitotic chromosomes. All the populations showed the somatic chromosome number of $2n = 24$. It was also found that the binomial *C. rotundifolia* needs to be typified. Therefore, in the present study, we discuss nomenclature and cytology of *C. rotundifolia.*

Keywords: Grape family, Karyology, Multipotent, *Saelanthus,* Taxonomy

Introduction

The genus *Cissus* L. (Vitaceae) comprises 280 species worldwide (Anonymous, 2024). In India, it is represented by 18 species and 2 varieties (Mao & Dash, 2020), of which 12 are reported from Maharashtra (Singh & Karthikeyan, 2001). During recent floristic exploration, authors collected *C. rotundifolia* Lam. from Kolhapur, Ratnagiri, and Pune districts of Maharashtra state and the Bagalkot district of Karnataka state. Some floras (Anonymous, 2019; Mao & Dash, 2020) report the species as cultivated; however, the species is widely growing in natural habitats (Dalavi et al., 2021; Sarvalingam et al., 2013). After critical examination and perusal of the literature, it is found that *C. rotundifolia* lacks type specimens, which is proposed based on its basionym, and the name is lectotypified according to ICN (Turland et al., 2018). Furthermore, there is no data on the karyotype of the species. There are only a few reports on the somatic chromosome number (Rice et al., 2015). Chu et al. (2018) reported a somatic count of $2n$=24. Four different populations of the species from India were collected and studied for their cytology. For the first time, karyotypic analysis of *C. rotundifolia* was reported. In the present study, we discuss the nomenclature and karyology of this potential ornamental plant.

Material and methods

Taxonomy and distribution

Fresh specimens for the new records were collected from their natural habitat and pressed following the proper herbarium preparation protocol. The specimens were identified using the Flora of India, Flora of Maharashtra, and Flora of Aegyptica-Arabia (Forsskal, 1775). The photographs were taken with OnePlus 10R and a Vivo 21e camera.

Nomenclature and typification

We checked the availability of the original material in the herbaria of B, BM, C, DS, LD, LINN, P, PC, P-JU, S, SBT, and UPS. Type specimens of *Cissus* species from Yemen and neighbouring countries were examined using high-resolution images from the Global Biodiversity Information Facility (GBIF, https://plants.jstor.org/, and www.gbif.org). The nomenclatural history of *C. rotundifolia* was reviewed by examining the original descriptions and subsequent taxonomic literature. The International Code of Nomenclature for algae, fungi, and plants (ICN) guidelines were strictly followed to determine the validity and priority of names (Turland et al., 2018).

Cytology

The mature fruits of *C. rotundifolia* were collected from the Pune, Kolhapur, and Ratnagiri districts of Maharashtra state and Jamkhandi (Bagalkot district) of Karnataka state. The voucher specimen (BSI211666) is housed in the herbarium at the Botanical Survey of India, Western Regional Centre, India (BSI). Mitosis from each population was studied using the root tips of germinated seeds. Root tips ranging 4–8 mm in length were pre-treated with 2 mM 8-hydroxyquinoline at 10± 1°C for 3–4 hrs. These root tips were then squashed in 2% propionic orcein. The well-spread chromosome plates were photographed using a Carl Zeiss Axio Imager A2, and ten ideal cells were used for karyotype analysis and graphing of ideogram. Types of chromosomes were ascertained using the centromeric index (i) as per Levan et

al. (1964). Karyotype morphometric characters were evaluated by calculating haploid complement length together with intrachromosomal asymmetry index (A1) and inter-chromosomal asymmetry index (A2) as per Zarco (1986).

Results and discussion

Distribution

In India's flora, *Cissus rotundifolia* is mentioned as cultivated (Mao & Dash, 2020). However, the species grows extensively in open areas and forest lands, invading natural habitats (Dalavi et al., 2021). Sarvalingam et al. (2013) reported the same species from the Maruthamalai foothills, Coimbatore district, Tamil Nadu. Dalavi et al. (2021) reported this plant in the wilder area of Jamkhandi (Bagalkot) of Karnataka state. The species has spread throughout the country because of the extensive flowering and fruiting period, rapid growth rate, high stress tolerance, edible fruits, and ornamental potential. Probably by droppings of birds and animals, the species is slowly naturalizing in waste places and forest lands. The plant appears similar to unrelated taxa such as *Anredera cordifolia* (Ten.) Steenis and members of Basellaceae when it grows in vegetative stages. However, it differs in the inflorescence structure, with tetramerous flowers with caducous perianth and crenate to dentate margin of leaves. A detailed plate is provided for easy identification (Fig. 2).

Typification

Cissus rotundifolia Lam. in Tab. Encyclo. 1: 331. 1792 (Fig. 2).

Basionym: *Saelanthus rotundifolius* Forssk. in Fl. Aegypt.-Arab.: 35. 1775. *nom. inval.*

Type: ARABIA (present-day YEMEN): Wadi Sordud, February 1763, 100, P. Forsskal [Lectotype designated here C (C10002922) digital image (Fig. 1a); Isolectotype C (C10002923) digital image!] Lamarck (1792) in his book Tableau Encyclopédique et Méthodique, proposed a new combination *Cissus rotundifolia* based on *Saelanthus rotundifolius* Forssk. and cited the illustration published in Icones rerum naturalium: quas in itinere orientali depingi by Forsskal & Niebuhr (1777). Later, Vahl (1794) made the same combination based on the same species, i.e., *Saelanthus rotundifolia* Forssk. as *C. rotundifolia* Vahl, which became an isonym. *S. rotundifolius* was first published by Forsskal (1775) in *Flora Aegyptica-Arabia* based on the specimens he collected from Arabia (present-day Yemen). It has been considered an invalid publication because the novel genus he had described, i.e., *Saelanthus*, lacks a proper diagnostic key for its distinction from all the allied genera. Later Scopoli (1777) in *Introductio ad Historiam Naturalem Sistens Genera Lapidum, Plantarum et Animalium Hactenus Detecta, Caracteribus Essentialibus Donata, in Tribus Divisa, Subinde ad Leges Naturae* validated the genus by giving a diagnostic key. However, it became a heterotypic synonym of *Cissus* L., described by Linnaeus (1753). Subsequently, the species in genus *Saelanthus* have been transferred to *Cissus.*

During our study, we checked the availability of the original material of *S. rotundifolius* in B, BM, C, DS, LD, LINN, P, PC, P-JU, S, SBT, and UPS, where Forsskal's collections are known to be preserved (Stafleu & Cowan, 1976). We could locate two herbarium sheets of the original materials at C (Herbarium of the University of Copenhagen, Denmark). The

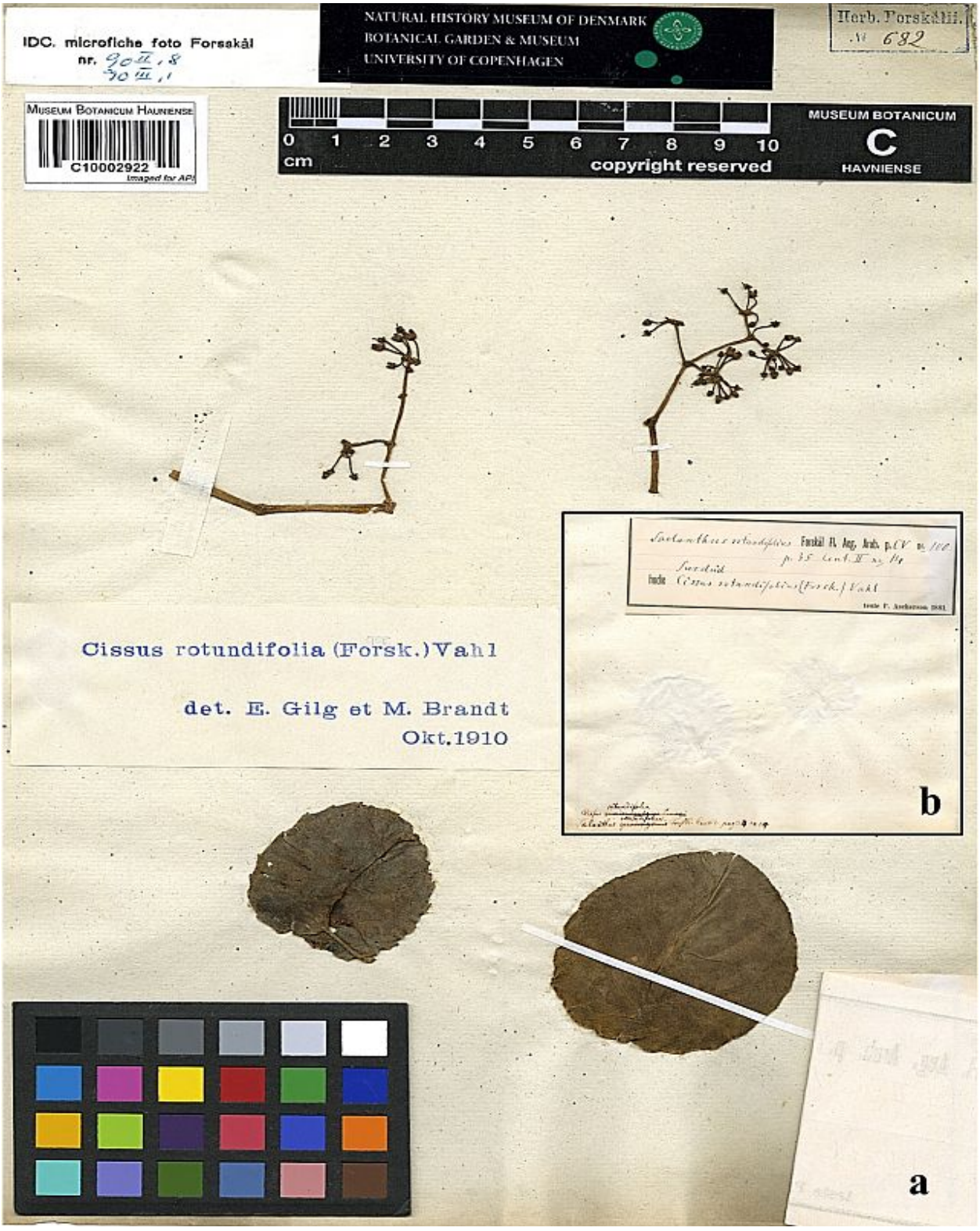

Fig. 1. a: Lectotype of *Cissus rotundifolia* Lam. (Vitaceae). C10002922 [© Board of Trustees of the Natural History Museum of Denmark]; b: Annotations on species cover by Forsskal at bottom and tag of Flora Aegyptiaco-Arabica by Ascherson

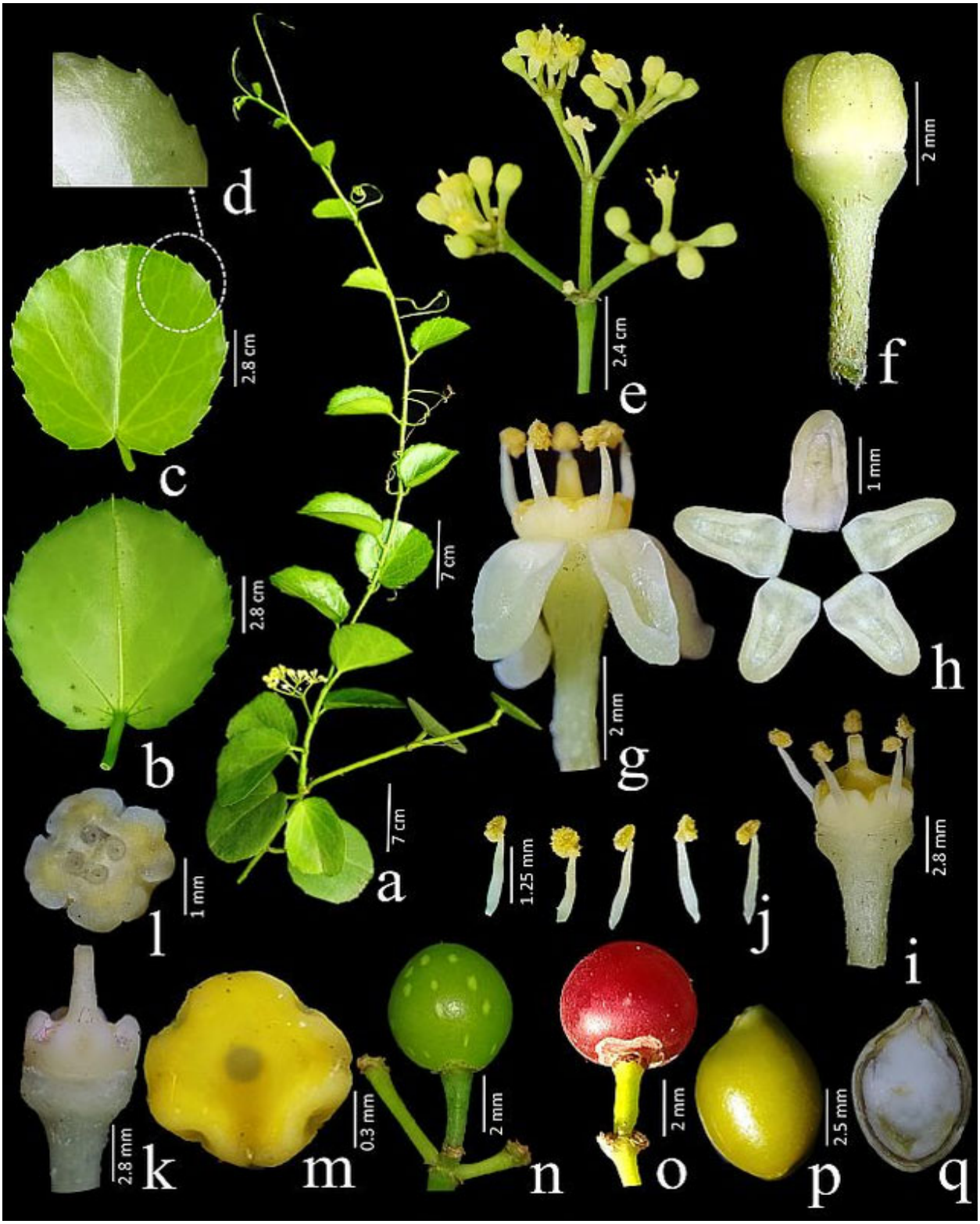

Fig. 2. a: Habit; b: Abaxial side of leaf; c: Adaxial side of leaf; d: Serrate margin; e: Inflorescence; f: Bud; g: Flower; h: Petals; i: Dissected flowers; j: Anthers; k: Gynoecium; l: Axile placentation; m: Disc; n: Young fruit; o: Ripened fruit; p: Seed; q: L.S of seed

communications were made via emails to which the curator of the Herbarium of the University of Copenhagen informed that all the type specimens that are preserved against the name have been digitized and clarified that there are only two sheets; no other extra specimens are available for the same. The two specimens found at C bear the barcodes C10002922 and C10002923. Both specimens are duplicates of each other and are preserved well, with all the diagnostic characters intact. These two specimens were collected in February 1763 from Wadi Sordud, Arabia (present-day Yemen) by Forsskal. Though there is an illustration cited by Lamarck while making the new combination, the same illustration does not have any evidence of the location and the date of Forsskal's collection. However, the original material has hand annotations of names with citations of protologue. Furthermore, the location mentioned by Forsskal in the protologue is also tagged on the backside of the sheet, which Ascherson approved in 1881 (Fig. 1 b). Therefore, the original specimen has priority over illustration or colour drawing according to 'ICN Article 9.12' (Turland et al., 2018). Since only one specimen could be designated as a lectotype here, the specimen with the barcode C10002922 is selected to serve as a lectotype for the name *S. rotundifolius* Forssk. as per article 9.3 (Turland et al., 2018) (Fig. 1a). We would also like to highlight that the same new combination given by the Lamarck was again published by Vahl (1794). Hence, this is an Isonym

Table 1. Karyotype analysis of *Cissus rotundifolia* Lam.

Chromosome pair	Length of long arm (l) (µm)	Length of short arm (s) (µm)	Total length (c) (µm)	d = l – s (µm)	Arm ratio = l/s	Centromeric index i = s/c × 100	Centromeric position according to "i"
I	1.25±0.54	1.09±0.38	2.34±0.62	0.16	1.14	46.67	m
II	1.25±0.54	1.00±0.27	2.25±0.48	0.25	1.25	44.44	m
III	1.13±0.38	1.00±0.13	2.13±0.47	0.13	1.13	47.06	m
IV	0.88±0.36	0.81±0.22	1.69±0.42	0.06	1.08	48.15	M
V	0.88±0.40	0.75±0.12	1.63±0.45	0.13	1.17	46.15	m
VI	1.00±0.29	0.63±0.28	1.63±0.43	0.38	1.60	38.46	m
VII	0.88±0.23	0.69±0.21	1.56±0.41	0.19	1.27	44.00	m
VIII	0.88±0.22	0.63±0.22	1.50±0.38	0.25	1.40	41.67	m
IX	0.88±0.23	0.63±0.23	1.50±0.43	0.25	1.40	41.67	m
X	0.81±0.24	0.63±0.13	1.44±0.36	0.19	1.30	43.48	m
XI	0.69±0.21	0.54±0.13	1.31±0.32	0.15	1.27	41.27	m
XII	0.66±0.23	0.47±0.12	1.13±0.34	0.19	1.40	41.67	m

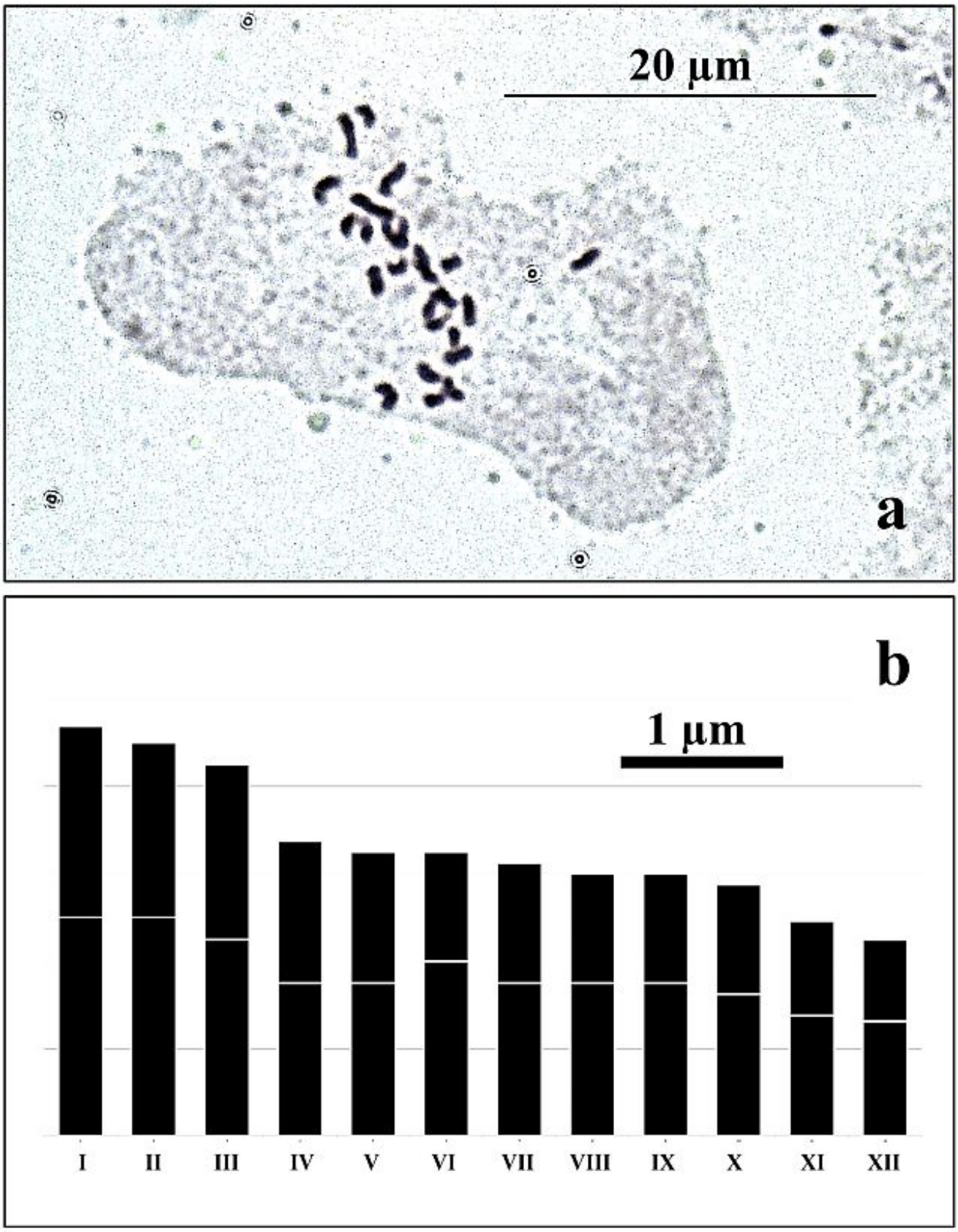

Fig. 3. a: Mitotic metaphase of *Cissus rotundifolia* Lam.; b: Ideograph of *Cissus rotundifolia* (scale bar: 1 µm)

of Lamarck's name following Article 6.3 of ICN; therefore, according to the principle of priority Article 11.1 of ICN (Turland et al., 2018), the authority of the name should be given to Lamarck over the binomial proposed by Vahl. It was observed that most floristic studies have erroneously cited the name with the authority mentioned as Vahl. Therefore, all such citations can be corrected through this communication as *C. rotundifolia* Lam.

Cytology

Somatic chromosome number in *C. rotundifolia* was observed as $2n = 24$ (Fig. 3a). Length of the chromosomes ranged from 2.34 to 1.13 µm, and centromeric position was observed i.e median region (m) in 22 and 2 are with median point (M) chromosomes as per Levan (1964) classification (Table 1). The total chromosome length of haploid compliment (TCLH) was 20.09 µm (Table 2). The total form percentage was 44.06, with the Symmetric Index (SI) at 79.36 and the Gradient Index (GI) at 48.00. The Coefficient of variation of chromosome lengths (CVcl) and Coefficient of variation of the centromeric index (CVci) were 22.41 and 6.62, respectively. The intrachromosomal asymmetry index (A1) and Interchromosomal asymmetry index (A2) were 0.74 and 0.22, respectively. The karyotype formula was $2n = 24 = 22m+2M$ Karyotype fell in the 1B category as per Stebbins (1971) asymmetry classes (Fig. 3b).

According to Karkamkar et al. (2010), Grape cultivars and *Vitis* species show chromosome number $2n = 38$, while wild types like *Ampelopsis* Michx., *Ampelocissus* Planch., and *Parthenocissus* Planch. possess $2n = 40$ and 80. Chromosome numbers in *Cissus* L., *Cayratia* Juss., and *Tetrastigma* (Miq.) Planch. range widely from $2n = 22$ to 120,

Table 2. Comparative karyotypic indices *Cissus rotundifolia* Lam.

Indice	Value
TF%	44.064
SI	79.3651
GI	48.0000
CVcl	22.416
Cvci	6.626
Ai	1.485
A1	0.744
A2	0.224
Range of TCL %	5.60- 11.66
TCLH	20.09
Karyotypic formula	$2n$=24=22m+2M
Classification as per Stebbins (1971)	1B

leading to their reclassification based on these variations. Due to distinct morphological traits, *Leea* D. Royen species, with $2n = 24$ and 48 chromosomes, are classified separately as Leeaceae.

In India, *Cissus* L. is represented by 18 species and 2 varieties, of which 11 taxa have been explored for cytological studies by different workers (Table 3). The cytology of the Indian *Cissus* species explains their wide range of variability in chromosome numbers. The gametophytic chromosome number (n) typically ranges from 12 to 28, whereas sporophytic numbers ($2n$) show greater variability, found in multiples of 12, 24, and 48. $2n$=24 is the most common sporophytic count. *C. discolor, C. elongata, C. quadrangularis*, and *C. repanda*. *C. repens*, shows higher chromosome numbers, i.e., 96. *C. quadrangularis* has a wide range (44–53) of chromosomes. According to Rice et al. (2015), 17 species of *Cissus* were studied for cytological studies, of which 11 species showed $2n$= 24, the most common number

Table 3. Cytogenetics of Indian *Cissus*

S. No.	Taxon	Gametophytic (*n*)	Sporophytic (2*n*)	Reference
1.	*Cissus adnata* Roxb.		22	Sarkar et al., 1982
2.	*C. adnata* Roxb.		20	Hazra & Sharma, 1970
			48	Kumar & Subramaniam, 1987
			24	Kumar & Subramaniam, 1987
			26	Kumar & Subramaniam, 1987
			48	Fedorov, 1974
			48	Cave, 1959
		12	24	Cave, 1959
3.	*C. assamica* (M.A. Lawson) Craib		48	Hazra & Sharma, 1970
			48	Kumar & Subramaniam, 1987
4.	*C. discolor* Blume	24	48	Agarwal, 1983
		24II	48	Agarwal,1983
			24	Kumar & Subramaniam, 1987
		12	24	Kumar & Subramaniam, 1987
5.	*C. elongata* Roxb.	12	24	Patil et al., 1980
6.	*C. elongata* Roxb.		24	Kumar & Subramaniam, 1987
7.	*C. heyneana* Planch.		24	Kumar & Subramaniam, 1987
8.	*C. heyneana* Planch.		28	Kumar & Subramaniam, 1987
9.	*C. heyneana* Planch.		28	Darlington & Wylie, 1956
10.	*C. heyneana* Planch.		24	Cave, 1959
11.	*C. quadrangularis* L.		24	Kumar & Subramaniam, 1987
12.	*C. quadrangularis* L.		44-53	Kumar & Subramaniam, 1987
13.	*C. quadrangularis* L.		44-53	Kumar & Subramaniam, 1987
14.	*C. quadrangularis* L.		c.45	Darlington & Wylie, 1956
15.	*C. quadrangularis* L.		24	Cave, 1959
16.	*C. quadrangularis* L.		24	Cave, 1959
17.	*C. repanda* (Wight & Arn.) Vahl		24	Kumar & Subramaniam, 1987
18.	*C. repanda* (Wight & Arn.) Vahl		26	Darlington & Wylie, 1956
19.	*C. repens* Lam.		24	Petria, 1973
20.	*C. repens* Lam.		24	Kumar & Subramaniam, 1987
21.	*C. repens* Lam.		50	Kumar & Subramaniam, 1987
22.	*C. repens* Lam.		96	Kumar & Subramaniam, 1987
23.	*C. repens* Lam.		24	Darlington & Wylie, 1956
24.	*C. repens* Lam.		24	Cave,1959
25.	*C. vitiginea* L.	13	26	Cave,1959
26.	*C. woodrowii* (Stapf) Santapau	12	24	Patil et al., 1980
27.	*C. woodrowii* (Stapf) Santapau		24	Kumar & Subramaniam, 1987

in the genus. This chromosomal variability plays a vital role in the evolution and differentiation of the family. Vitaceae exhibits a basic chromosome number of $x = 6$, which can shift to $x = 5$ or $x = 7$ through chromosomal fusion or duplication, contributing to the evolutionary differentiation among genera (Karkamkar et al., 2010). Chu et al. (2018) also observed $2n=24$ in *C. rotundifolia* grown as a garden ornamental plant. Karkamkar et al. (2010) stated that the diploid population of *Vitaceae* usually grow as succulent shrubs or climbers, and *C. rotundifolia* also falls under the same morphology. They also stated that the genetically diploid species are either deciduous or evergreen, with ellipsoidal to globose berries having a single seed per fruit, which is a very prompt characterization. Cytological data strongly supports the present classification of Indian taxa based on morphological characters.

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