RESEARCH ARTICLE



Cyphostemma calcarium, a new species of Vitaceae from the Ankarana Special Reserve, Madagascar

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Abstract

Cyphostemma calcarium Rabarij & L.M.Lu, **sp. nov.**, is herein described as a new species found on limestone outcrops in northern Madagascar. Its diagnostic morphological characteristics were compared to the species occurring in the Ankarana Special Reserve. We present detailed descriptions, illustrations, distribution map, and a preliminary conservation assessment of the species. An identification key to all known species of *Cyphostemma* from the Ankarana Special Reserve is also provided.

Keywords

Ankarana, Cyphostemma, Cyphostemma calcarium, Madagascar, Vitaceae

Introduction

The genus *Cyphostemma* (Planch.) Alston contains ca. 200 species, representing the second largest genus following *Cissus* within the grape family, Vitaceae (Wen et al. 2018; Rabarijaona et al. 2020). Species of *Cyphostemma* are distributed mainly in Africa with a few species occurring in southern India, Thailand and southwest China (Dang et al. 2017; Wen et al. 2018). The genus is distinguished by several unique morphological characters: floral buds constricted at the middle, a floral disc of 4-large free glands, conspicuous stipules, and seeds with extra layers of endotestal sclereids covering the ventral infolds in cross-section (Wen 2007; Chen and Manchester 2007, 2011).

In Madagascar, *Cyphostemma* consists of ca. 25 species and displays substantial morphological diversity (Baker 1887; Descoings 1967; The Madagascar Catalogue Project 2019). Species are found in a diversity of habitats, including rainforests, savannas, dry thickets, dunes, and seasonal arid habitats such as the vegetation on limestones or an area within the reserve referred to as "Tsingy". Several of the species of Vitaceae from Madagascar exhibit features that are very unusual in the family, such as succulent shrubs or trees, rather than lianas, and the lack of leaf-opposed tendrils (Hearn et al. 2018). Northern Madagascar possesses the highest species diversity for Vitaceae across the island, with ca. 68% at the family level and ca. 56% for *Cyphostemma* (The Madagascar Catalogue Project 2019).

Since *Cyphostemma* species were reported to exhibit distinct morphotypes during their vegetative and flowering stages, we conducted an in-depth morphological investigation of all 25 described *Cyphostemma* species from Madagascar. Of the eight species of *Cyphostemma* in the Ankarana Special Reserve (Fig. 1), *Cyphostemma ankaranense* Desc., *C. caerulans* Desc., *C. rutilans* Desc., and the newly described species in this paper, all lack tendrils. However, the new species can be distinguished from the other three species based on several traits such as habit, stipule shape and size, leaf architecture, flower color, style length, and fruit shape (Table 1). We herein describe and illustrate this new species, assess its conservation status, and provide an identification key to all the species found in the Ankarana Special Reserve.

Materials and methods

The morphological description is based on measurements of dried specimens, supplemented by photos of mature living plants collected from the field. Herbarium specimens and digital images of the most closely-related species to the new described species were examined from the following herbaria: K, P, PE, and TAN. Protologues of type specimens were gathered from Descoings (1967) and JSTOR Global Plants (http:// plants.jstor.org). Flowers, fruits, and seeds were dissected after briefly soaking in hot water. Images of floral parts and seeds were captured using a stereomicroscope (Leica DVM6 camera, Wetzlar, Germany). Terminologies describing seed morphology followed Chen and Manchester (2011).



Figure 1. Distribution map of *Cyphostemma calcarium* sp. nov. with the black dot showing the locality of the type specimens. Map on the right shows the position of Ankarana Special Reserve in Madagascar.

Table 1. Morphological comparison of four shrubby tendril-less species of *Cyphostemma* in the Ankarana Special Reserve, Madagascar.

Taxon	Habit	Stipule	Branch	Leaf architecture	Flower	Style length	Fruit
			and leaf		colour	(mm)	
C. ankaranense Desc.	suberect or	ovate to widely	glabrous	bi-ternate to bi-	reddish	± 0.7	ellipsoid; 5–7 ×
	prostrate	triangular, 12–25		pinnate			4–5.5 mm
		× 6–10 mm					
C. caerulans Desc.	prostrate	\pm falcate, 10–15	glabrous	bi-pinnate	yellowish	± 1.5	globose or subglobose;
		× 2.5–3.5 mm					6.5–9 mm in diameter
C. rutilans Desc.	erect	triangular; up to	glabrous	3-foliolate	reddish	± 1.5	ellipsoid; 6–8 ×
		ca. 5 × 3 mm					5–6 mm
C. calcarium Rabarij &	erect	triangular to \pm	pubescent	3-foliolate, central	reddish	± 2.5	ellipsoid; 9–12 ×
L.M.Lu		falcate; 4–5 ×		leaflet often			5–7 mm
		1.5–2.5 mm		dropped			

Taxonomic treatment

Cyphostemma calcarium Rabarij & L.M.Lu, sp. nov. urn:lsid:ipni.org:names:77218853-1 Figs 2, 3

Diagnosis. Cyphostemma calcarium is most closely comparable to C. rutilans Desc. in morphology. It differs from the latter in having distinct pubescent branches (vs.



Figure 2. Comparison of two shrubby *Cyphostemma* species with 3-foliolate leaves in the Ankarana Special Reserve **A–E** *Cyphostemma calcarium* sp. nov. **A** branches showing puberulent leaves and infructescence **B–E** seed morphology from *Rakotovao C. et al.* 6376 (Dorsal, ventral, lateral, and cross-section presented from left to right) **F–J** *Cyphostemma rutilans* Desc. **F** branches with glabrous leaves and inflorescence **G–J** seed morphology from Bardot-Vaucoulon M. 817 (Dorsal, ventral, lateral, and cross-section presented from left to right). Photos by Rakotovao Charles, Missouri Botanical Garden (**A**); Billiet Frieda, Meise Botanic Garden (**F**). The red arrow indicates an extra layer of endotestal sclereids covering the ventral infolds. Scale bars: 1 mm

branches entirely glabrous in *C. rutilans*); leaves minutely puberulous and shiny on the adaxial surface, abaxial surface densely whitish pubescent to velvety particularly on the veins (vs. leaves entirely glabrous and shiny on both sides in *C. rutilans*); and leaflets broadly oblong or elliptic, base cuneate (vs. leaflets narrowly ovate, base subcordate in *C. rutilans*). Seeds of *C. calcarium* are ellipsoid in outline, $7-7.5 \times 3-3.5$ mm, surface rugose to \pm muricate (vs. seeds globose, 5-6.5 mm in diameter, surface strongly rugose in *C. rutilans*).

Type. MADAGASCAR. Antsiranana: Diana, Ankarana Special Reserve, Tsingy Rary, 12°56'24.00"S, 49°07'04"E, 97 m, 16 May 2013, *Rakotovao C. et al.* 6376 (holo-type: TAN!).

Description. Succulent erect shrub, up to 2 m tall. Old stems swollen, succulent; bark smooth, lenticellate; branches brown to reddish, shortly pubescent. Tendrils absent. Stipules triangular to \pm falcate, $4-5 \times 1.5-2.5$ mm, soon caducous. Leaves 3-foliolate, central leaflet often drooping, somewhat thick and fleshy when fresh, becoming coriaceous when dry, usually folded upwards along the midrib; leaflets $3-5 \times 1.5-2.5$ cm, broadly oblong or elliptic, base cuneate, rounded to obtuse at the apex, margin shallowly denticulate; minutely puberulous and shiny on the adaxial surface, abaxial surface densely whitish pubescent to velvety particularly on the veins; venation closely reticulate, prominent. Petioles 1.5-2.5 cm long. Petiolules equal, up to 1 cm long. Inflorescence a compound dichasium, terminal, ca. 7.5 cm long, very shortly



Figure 3. *Cyphostemma calcarium* sp. nov. **A** branches showing the inflorescence and infructescence and the bark with distinct lenticels **B** trichomes on the abaxial leaflet surface **C** flower bud constricted at the middle **D** flower with petals and stamens removed to show the floral disc of 4-large free glands **E** fruit with a persistent stigma (Illustration by Ai-Li Li; based on *Rakotovao C. et al.* 6376, TAN).

pubescent; bracts inconspicuous; pedicels 2–4 mm. Floral buds \pm 2.5 mm long, minutely puberulous or glabrescent; sepals \pm 0.5 mm long; petals reddish; stamens 4, filaments cylindrical, ca. 2.2 mm long, anthers ca. 0.8 mm long; ovary glabrous, styles \pm 2.5 mm long. Fruits ellipsoid, 9–12 × 5–7 mm, glabrous. Seeds broadly ellipsoid, 7–7.5 × 3–3.5 mm, rugose; base rostrate; beak conspicuous; apex revolute; rugae apex shallowly conspicuous on both surfaces; chalaza linear, sinuate, up to 6 mm long (ca. 6/7 of seed length); ventral ridge raised, elongate but widened in the middle, extending up to 6/7 of seed length; endosperm m-shaped in cross-section.

Phenology. Flowering and fruiting around May.

Etymology. The epithet of the species refers to the habitats on limestone outcrops. **Distribution and habitat.** It grows on limestone outcrops in northern Madagascar at an altitude of 90–300 m. (Fig. 1)

Provisional conservation assessment. The new species is endemic to Madagascar with distribution restricted to its type locality. It is assessed here as Critically Endangered (CR) according to the IUCN Categories and Criteria (IUCN 2019). Even though the species occurs within a protected area, succulent plants are still highly sought after by collectors for their horticultural values. Seeds of *Cyphostemma calcarium* should therefore be collected, banked, and propagated to ensure its longterm conservation.

Taxonomic notes. This species is described from materials collected by *Ra-kotovao C. et al.* in 2013. It was initially identified as *Cissus pileata* Desc., but it clearly belongs to *Cyphostemma* in having constricted flower buds and floral disks with four free glands. These characters, together with its M-shaped endosperm as viewed in cross sections of the seeds and the presence of extra layers of endotestal sclereids covering the ventral infolds in cross-section, clearly distinguish the new species from *Cissus* L. A summary of some diagnostic characters that differentiate this new species from other shrubby species of *Cyphostemma* in Ankarana Special Reserve is provided in Table 1.

Key to the species of Cyphostemma in Ankarana Special Reserve, Madagascar

1a	Shrubby succulent plants; tendrils absent
1b	Climbers to woody vines, sometimes tree-like; tendrils usually present5
2a	Stems erect or suberect; leaves usually 3-foliolate; flowers reddish
2b	Stems rather prostrate; leaves pinnately arranged; flowers green to yellowish4
3a	Young stems, branches, and petioles glabrous; leaves entirely glabrous and
	shiny on both sides; leaflets narrowly ovate, base subcordate C. rutilans
3b	Young stems, branches, and petioles puberulent; leaves minutely puberulous
	and shiny on the adaxial surface, abaxial surface densely whitish pubescent to
	velvety particularly on the veins; leaflets elliptic, base cuneate C. calcarium
4a	Leaflets narrowly oblong-elliptic, overall with a reddish tone; stipules ovate
	to widely triangular, 12–25 × 6–10 mm; flowers pale green; fruits ovoid or
	elongate-ellipsoid, apiculate C. ankaranense
4b	Leaflets rhomboid, ovate or suborbicular, rather green; stipules \pm falcate, lan-
	ceolate-acuminate, $10-15 \times 2.5-3.5$ mm; flowers yellowish; fruits globose or
	subglobose, not apiculate
5a	Leaves digitately arranged, 3–5-foliolate C. glanduloso-pilosum
5b	Leaves pinnately arranged6
6a	Trunk sub-spherical, 0.50-0.70 m diameter; bark flaking, corky to reticu-
	lately fissured; inflorescences and flowers reddish
6b	Trunk tree-like, up to 5 m tall or even taller; bark smooth, peeling, papery;
	inflorescences and flowers green to yellowish7

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References

- Baker JG (1887) Further contributions to the Flora of Madagascar. Botanical Journal of the Linnean Society 22(148): 441–472. https://doi.org/10.1111/j.1095-8339.1887.tb00472.x
- Chen I, Manchester SR (2007) Seed morphology of modern and fossil *Ampelocissus* (Vitaceae) and implications for phytogeography. American Journal of Botany 94(9): 1534–1553. https://doi.org/10.3732/ajb.94.9.1534
- Chen I, Manchester SR (2011) Seed morphology of Vitaceae. International Journal of Plant Sciences 172(1): 1–35. https://doi.org/10.1086/657283
- Dang VC, Nguyen VH, Dao BH, Yang WL, Li FL, Yang XK, Wen J, Chen ZD, Lu LM (2017) A new species and new records of *Cyphostemma* (Vitaceae) from China and Vietnam based on morphological and molecular evidence. Systematic Botany 42(3): 449–457. https:// doi.org/10.1600/036364417X696032
- Descoings B (1967) Vitacées. In: Humbert H (Ed.) Flore de Madagascar et des Comores (124e famille). Muséum national d'histoire naturelle, Paris, 156 pp.
- Hearn DJ, Evans M, Wolf B, McGinty M, Wen J (2018) Dispersal is associated with morphological innovation, but not increased diversification, in *Cyphostemma* (Vitaceae). Journal of Systematics and Evolution 56(4): 340–359. https://doi.org/10.1111/jse.12417

- IUCN (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. [accessed 20.01.2020]
- Rabarijaona RN, Dang VC, Parmar G, Liu B, Wen J, Chen ZD, Lu LM (2020) Phylogeny and taxonomy of *Afrocayratia*, a new genus of Vitaceae from continental Africa and Madagascar. Journal of Systematics and Evolution 58(6): 1090–1107. https://doi.org/10.1111/jse.12697
- The Madagascar Catalogue Project (2019) Catalogue of the Vascular Plants of Madagascar. Missouri Botanical Garden, St. Louis, U.S.A. & Antananarivo, Madagascar. http://legacy. tropicos.org/Project/Madagascar
- Wen J (2007) Vitaceae. In: Kubitzki K (Ed.) The Families and Genera of Vascular Plants (Vol. 9). Springer-Verlag, Berlin, 466–478. https://doi.org/10.1139/B07-071
- Wen J, Lu LM, Nie ZL, Liu XQ, Zhang N, Ickert-Bond S, Gerrath J, Manchester SR, Boggan J, Chen ZD (2018) A new phylogenetic tribal classification of the grape family (Vitaceae). Journal of Systematics and Evolution 56(4): 262–272. https://doi.org/10.1111/jse.12427