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***Cyphostemma calcarium* (Vitaceae), a new species from Ankarana Special Reserve, Madagascar**

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Running head: *Cyphostemma calcarium*, a new species in Vitaceae

Cyphostemma calcarium (Vitaceae), a new species from Ankarana Special Reserve, Madagascar

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Cyphostemma calcarium (Vitaceae), a new species from Ankarana Special Reserve, Madagascar

Abstract

Cyphostemma calcarium Rabarijaona & L.M.Lu, **sp. nov.**, is herein described as a new species on the limestone outcrops in northern Madagascar. Its diagnostic morphological characteristics were compared to the species occurring in 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 from 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 mostly in Africa with a few species in southern India, Thailand and southwest China (Dang et al. 2017; Wen et al. 2018). The genus has several unique morphological characters including their 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 2011).

In Madagascar, *Cyphostemma* consists of ca. 25 species and displays substantial morphological diversity (Baker 1887; the Madagascar Catalogue Project 2019; Descoings 1967). Species of the genus adapt to diverse habitats, including rainforests, savannas, dry thickets, dunes, and seasonal arid habitats such as the vegetation on limestones or “Tsingy”. Several unusual forms in the whole Vitaceae family have been discovered in Madagascar, notably shrubs with no leaf-opposed tendrils (Hearn et al. 2018). Northern Madagascar possesses the highest species diversity for Vitaceae across the island, with 68% at the family level and 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 described *Cyphostemma* species. Of the eight species of *Cyphostemma* in Ankarana Special Reserve (Fig. 1), *Cyphostemma ankaranense* Desc., *C. caeruleans* Desc., *C. rutilans* Desc., and the newly described species in this paper, are all tendril-less. However, the new species can be distinguished from the other three species based on several traits such as habits, stipule shape and size, leaf architecture, flower colour, 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 from Ankarana Special Reserve.

Materials and methods

The morphological description is mainly based on measurements of dried specimens, supplemented by photos of mature living plants 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: TAN, P, PE, and K. Protologues of type specimens were gathered from Descouings (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 (2011).

Taxonomic treatment

Cyphostemma calcarium Rabarijaona & L.M.Lu, sp. nov.

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. branches entirely glabrous in *C. rutilans*); leaves \pm discolorous (vs. concolorous leaves in *C. rutilans*); and leaflets broadly oblong or elliptic, base cuneate, minutely puberulous above, densely pubescent to velvety particularly on the nerves beneath (vs. leaflets narrowly ovate, base subcordate, entirely glabrous in *C. rutilans*). Seeds of *C. calcarium* are ellipsoid in outline, $7\text{--}7.5 \times 3\text{--}3.5$ mm, surface rugose to \pm muricate (vs. seeds globose, $11\text{--}13.5$ mm in diameter, surface \pm smooth in *C. rutilans*).

Type. MADAGASCAR. Antsiranana: Diana, Ankarana Special Reserve, Tsingy Rary, $12^{\circ} 56' 24.00'' S$, $49^{\circ} 07' 04'' E$, 97 m, 16 May 2013, Rakotovo *C. et al.* 6376 (holotype: TAN!; isotype: P!).

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 facate, $4\text{--}5 \times 1.5\text{--}2.5$ mm, soon caduceous. Leaves 3-foliolate, central leaflet often dropped, somewhat thick and fleshy when fresh, becoming coriaceous when dry, usually folded along the midrib; leaflets $3\text{--}5 \times 1.5\text{--}2.5$ cm, broadly oblong or elliptic, base cuneate, rounded to obtuse at the apex, margin shallowly denticulate; \pm discolorous, minutely puberulous above, densely pubescent to velvety particularly on the nerves beneath; venations closely reticulate, prominent. Petioles $1.5\text{--}2.5$ cm long. Petiolules up to 1 cm long. Inflorescences a compound dichasium, terminal, ca. 7.5 cm long, very shortly pubescent; bracts inconspicuous; pedicels 2–4 mm. Floral buds ± 2.5 mm long, minutely puberulous or glabrescent; sepals ± 0.5 mm long; petals reddish; styles ± 2.5 mm long. Fruits ellipsoid, $9\text{--}12 \times 5\text{--}7$ mm, glabrous. Seeds broadly ellipsoid, $7\text{--}7.5 \times 3\text{--}3.5$ mm, rugose; base rostrate; beak conspicuous; apex revolute; ruga 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 a restricted distribution to its type locality, in the north. 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 subject of illegal trading for horticulture. Seeds of *Cyphostemma calcarium* should therefore be collected, banked, and propagated to ensure its long-term conservation.

Taxonomic notes. This species is described from materials collected by *Rakotovo 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 4-free glands. These characters, together with its M-shaped endosperm 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 species of *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.....2
- 1b. Climbers to woody vines; tendrils always present.....3
- 2a. Stems erect or suberect; leaves usually 3-foliolate; flowers reddish.....4
- 2b. Stems rather prostrate; leaves pinnately arranged; flowers green to yellowish.....5
- 3a. Leaves digitately arranged, 3–5-foliolate*C. glanduloso-pilosum*
- 3b. Leaves pinnately arranged6
- 4a. Young stems, branches, and petioles glabrous; leaflets narrowly ovate, base subcordate, concolorous, glabrous.....*C. rutilans*
- 4b. Young stems, branches, and petioles puberulent; leaflets elliptic, base cuneate, ± discoloured, minutely puberulous above, densely pubescent to velvety particularly on the nerves beneath.....*C. calcarium*
- 5a. 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*

- 5b. Leaflets rhomboid, ovate or suborbicular, rather green; stipules \pm falcate, lanceolate-acuminate, $10\text{--}15 \times 2.5\text{--}3.5$ mm; flowers yellowish; fruits globose or subglobose, not apiculate.....*C. caerulans*
- 6a Trunk sub-spherical, 0.50–0.70 m diameter; bark flaking, corky to reticulately fissured; inflorescences and flowers reddish.....*C. pachypus*
- 6b. Trunk tree-like, up to 5 m tall or even taller; bark smooth, peeling, papery; inflorescences and flowers green to yellowish.....7
- 7a. Leaflets 1-pinate, densely pubescent; petioles 4–6 cm long; inflorescences 6–10 cm, usually shorter, densely pubescent; fruits subglobose, 12–13 mm in diameter.....*C. macrocarpum*
- 7b. Leaflets 2-pinate, glabrous; petioles 6–12 cm long; inflorescences 8–15 cm long, glabrous above, with scattered white-pubescent at the base of the nerves beneath, like domatia; fruits ellipsoid, $10\text{--}12 \times 6\text{--}7$ mm..... *C. greveanum*

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

Figure 2. Comparisons of two shrubby *Cyphostemma* species with 3-foliolate leaves in Ankarana Special Reserve. **A–E:** *Cyphostemma calcarium*. **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*. **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) of pubescent branches and leaves. 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

Figure 3. *Cyphostemma calcarium* **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).

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

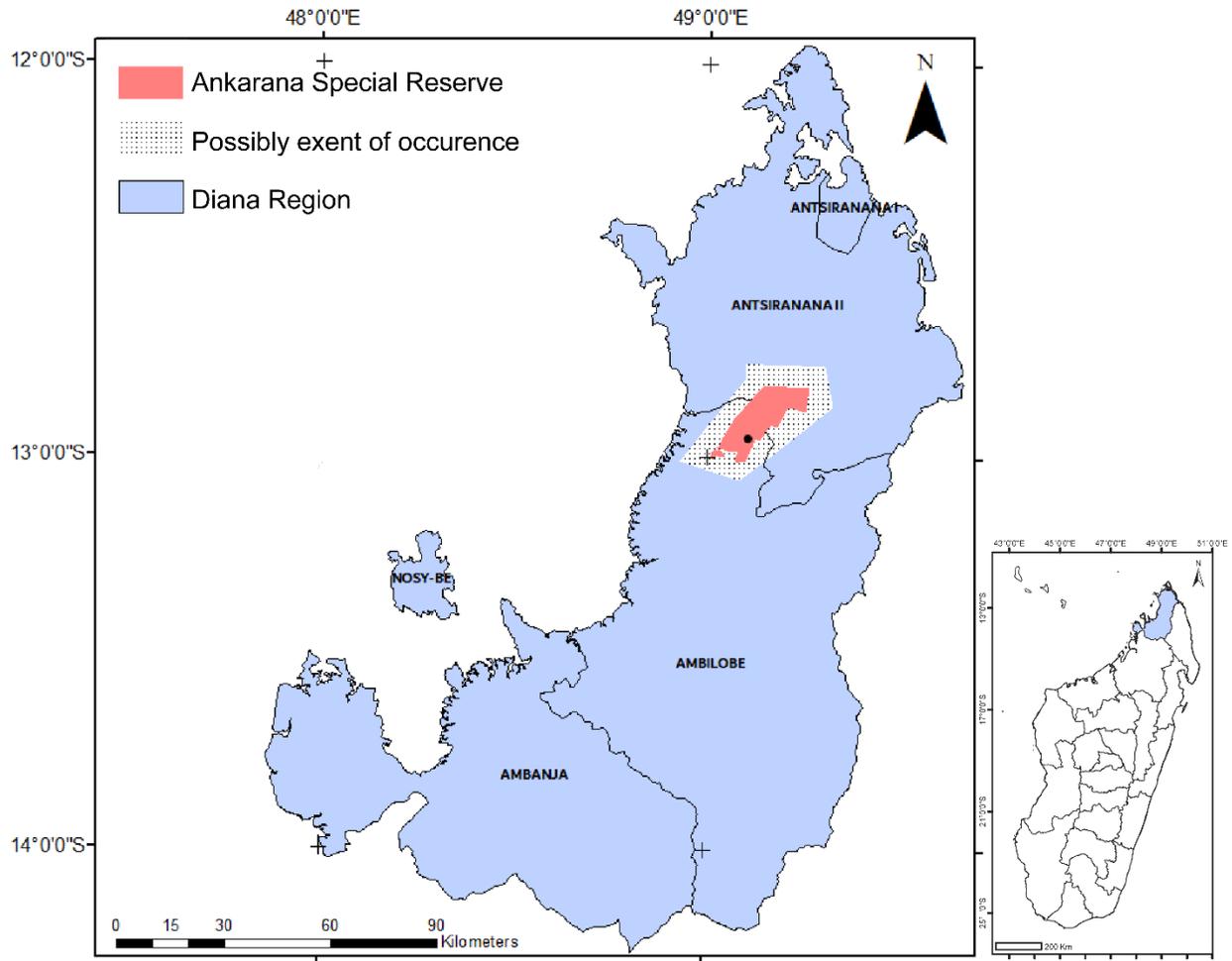


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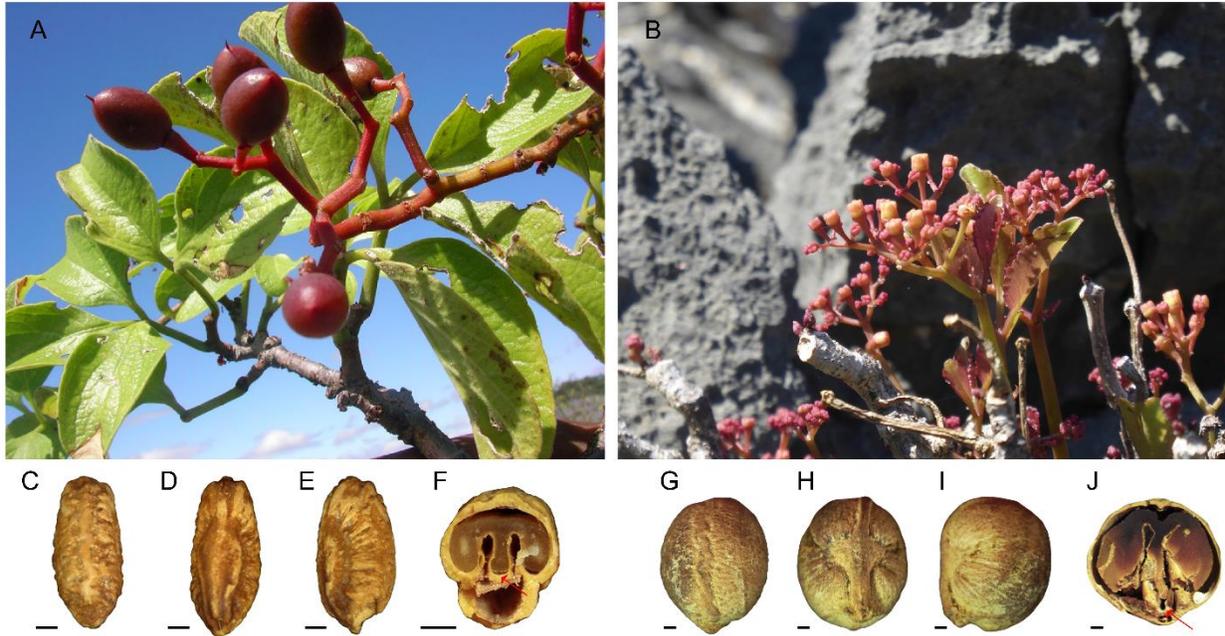


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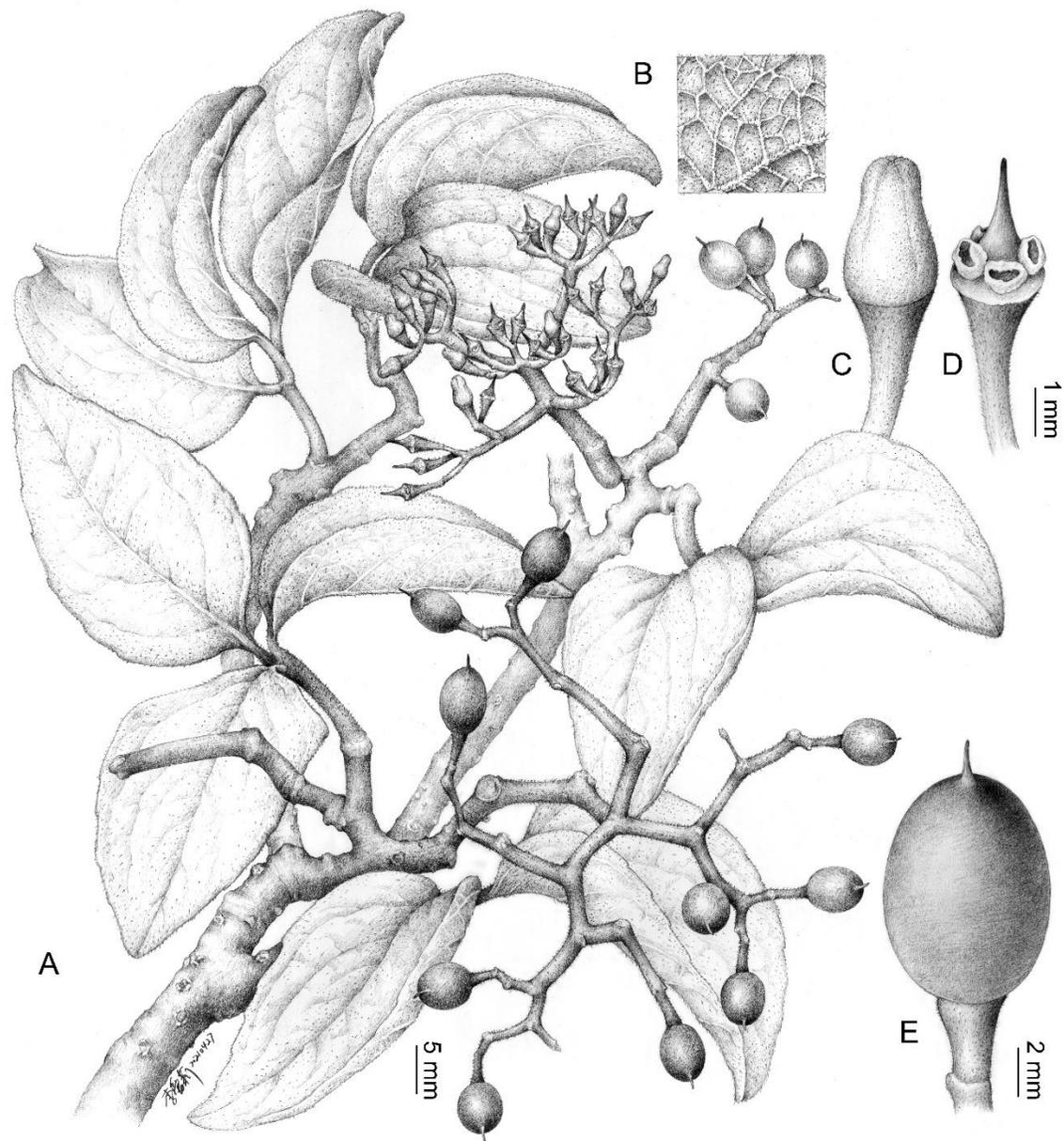


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Table 1. Morphological comparisons of four shrubby tendril-less species of *Cyphostemma* in Ankarana Special Reserve, Madagascar

Taxon	Habit	Stipule	Branch and leaf	Leaf architecture	Flower colour	Style length (mm)	Fruit
<i>C. ankaranense</i> Desc.	suberect or prostrate	ovate to widely triangular, 12– 25 × 6–10 mm	glabrous	bi-ternate to bi-pinnate	reddish	± 0.7	ellipsoid; 5–7 × 4–5.5 mm
<i>C. caeruleans</i> Desc.	Prostrate	± falcate, 10– 15 × 2.5–3.5 mm	glabrous	bi-pinnate	yellowish	± 1.5	globose or subglobose; 6.5– 9 mm in diameter
<i>C. rutilans</i> Desc.	Erect	triangular; up to ca. 5 × 3 mm	glabrous	3-foliolate	reddish	± 1.5	ellipsoid; 10–11 × 6–7 mm
<i>C. calcarium</i> Rabarijaona & L.M.Lu	Erect	triangular to ± falcate; 4–5 × 1.5–2.5 mm	pubescent	3-foliolate, lateral leaflets often dropped	reddish	± 2.5	ellipsoid; 9–12 × 5–7 mm