



## A complete description of *Mitracarpus diversifolius* (Rubiaceae) reveals new characters for the genus

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### ABSTRACT

A complete description of *Mitracarpus diversifolius* (Rubiaceae, Spermaceae) based on morphological features not previously studied and now available from two recent collections, is here presented. Details of fruits and seeds of this species are now known, and some of them are unusual for the genus. The corolla is persistent on mature fruit, and the seeds have a reticulate-foveate exotesta with thickened anticlinal walls and concave periclinal walls. Illustrations and SEM micrographs of these and other characters are also provided.

**Keywords:** conservation, Neotropics, seeds, Spermaceae, taxonomy

### INTRODUCTION

*Mitracarpus* Zuccarini ex J.A. Schultes & J.H. Schultes (1827: 210) is a Neotropical genus of the tribe Spermaceae (Rubiaceae), and belongs to the *Spermaceae* clade (Kårehed *et al.* 2008). *Mitracarpus* comprises about 50 species (Souza *et al.* 2010) distributed throughout tropical and subtropical regions of the New World, from southern United States to central Argentina (Andersson 1992; Souza & Sales 2001; Borhidi & Lozada 2007). In addition, *M. hirtus* (Linnaeus 1762:148) Candolle (1830: 572–573) is introduced and naturalized in Africa, Asia and Oceania (Dessein 2003; Nicolson 1977).

The diagnostic characters of *Mitracarpus* are calyx 4-lobed, lobes two large and two small or rarely subequal; corolla hypocrateriform or sub-infundibuliform; fruit capsular, with circumscissile dehiscence; seeds smooth or reticulate-foveate, ventral groove distinct, quadrangular or rectangular, or X-shaped or inverted Y-shaped (Souza *et al.* 2010). Nevertheless, due to the similarity in morphological characters of herbarium specimens, most genera of the *Spermaceae* clade, such as *Borreria* Meyer (1818: 79), *Diodia* Linnaeus (1753: 104), *Hexasepalum* Bartling ex Candolle (1830: 561), *Richardia* Linnaeus (1753: 330), *Spermaceae* Linnaeus (1753: 102), *Staelia* Chamisso & Schlechtendal (1828: 364) and *Mitracarpus*, are frequently confused. Recent molecular analyses using *rps16* and ITS markers confirmed the monophyly of *Mitracarpus*, although its relationships with other genera of the *Spermaceae* clade are still uncertain (Dessein 2003; Salas *et al.* 2015).

Terrel & Wunderlin (2002), taking into account the morphology of the seed ventral groove and the peculiar dehiscence of the fruit, unique characters within *Spermaceae* clade, suggested that *Mitracarpus* should be positioned in its own subtribe. However the fruit characters have never been critically studied as support of infratribal categories. Dessein (2003) based on the molecular phylogenies obtained, questioned the extensive use of the fruit characters within the *Spermaceae* clade to delimit genera.

There are several regional contributions to the taxonomy of *Mitracarpus* (Schumann 1888; Steyermark 1972, 1974; Bacigalupo 1974, 1993; Borhidi 2006; Borhidi & Lozada 2007; Souza *et al.* 2010). In addition, Borhidi & Lozada (2007) and Souza *et al.* (2010) contributed the revisions of Mexican and Brazilian species, respectively; however, a revision of the entire genus is still needed.

*Mitracarpus diversifolius* Souza & Cabral (in Souza *et al.* 2010: 328) is endemic to Bahia state, northeastern Brazil. It is distinguished from the other species of the genus by its creeping habit, rooting at nodes, anisophyllous leaves, and pseudoaxillary pauciflorous inflorescences. The authors positioned this species in *Mitracarpus* due to the corolla shape and calyx morphology (lobes two longer and two smaller), because fruits and seeds were unknown when it was described.

*Mitracarpus diversifolius* was described based on a single gathering, *W.W. Thomas & S. Sant'Ana 12485* (CEPEC, NY), collected in the municipality of Boa Nova, Bahia, Brazil. According to label data, Souza *et al.* (2010) stated that this species is shade-loving with a preference for moist soils. The authors considered it as Critically Endangered, due to its rarity of collection and ongoing habitat loss due to deforestation.

Two collections of *Mitracarpus diversifolius*, *J.H. Hage & H.S. Brito 1826* (CEPEC, NY) and *T.S. Santos 3651* (CEPEC) have recently been located, and have mature fruits and seeds. Based on the study of these specimens, we present an expanded description of this species and a re-evaluation of its conservation status.

## MATERIAL AND METHODS

Seeds were sputter-coated with gold-palladium, examined and imaged with a scanning electron microscope Jeol 5800 LV SEM, at the Universidad Nacional del Nordeste, Corrientes, Argentina. The conservation status of the species was assessed by range size (criterion B), following IUCN (2014) methodology. AOO (Area of Occupancy) was estimated using GeoCAT (Bachman *et al.* 2011).

## RESULTS

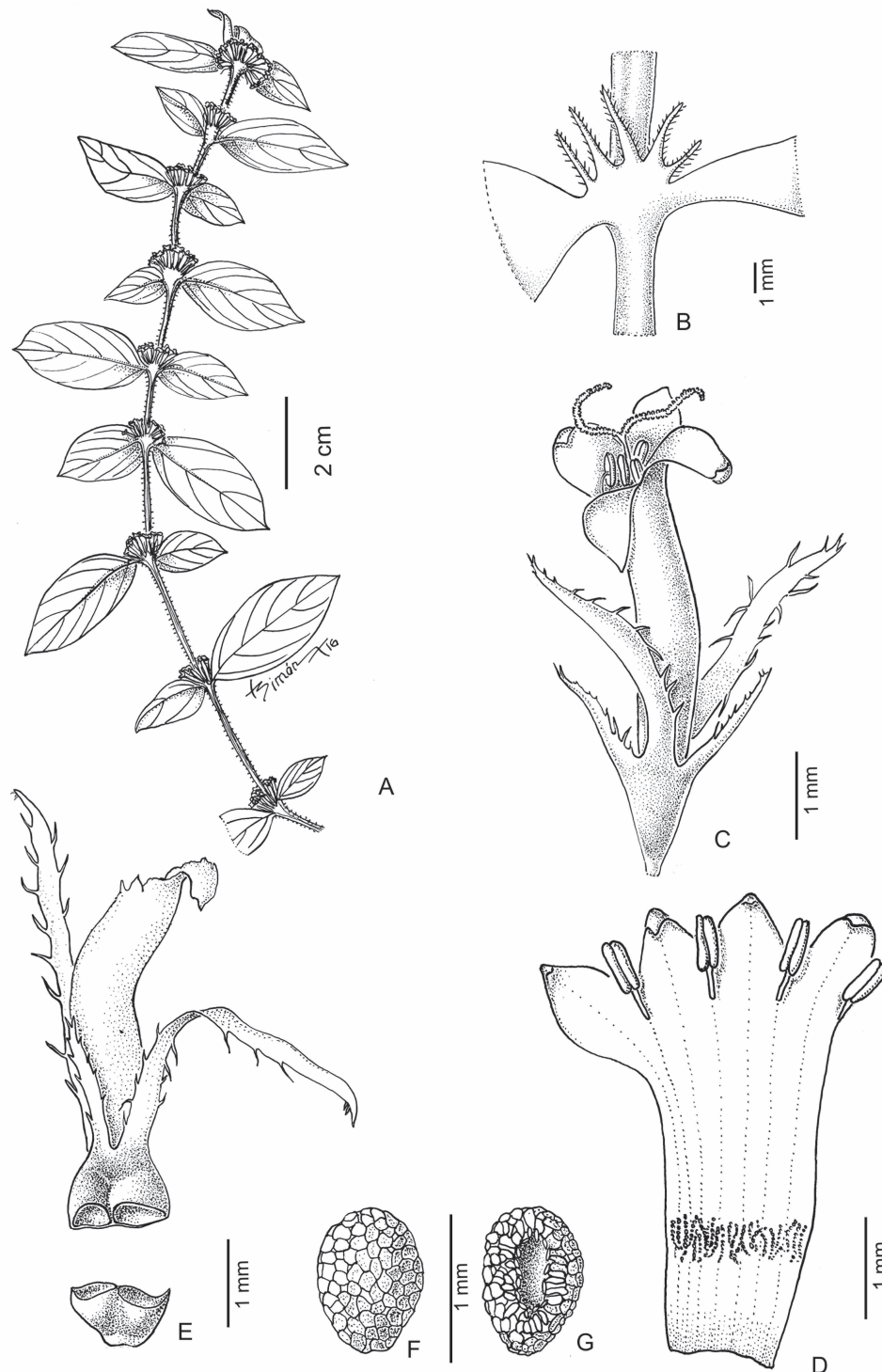
Our new observations of the morphology of *Mitracarpus diversifolius* complement the original description. Newly documented for this species and for the genus are the fruits with a persistent corolla and seeds with a reticulate-foveate exotesta with thickened anticlinal walls and concave periclinal walls.

## TAXONOMY

*Mitracarpus diversifolius* E.B. Souza & E.L. Cabral, *Rodriguésia* 61(2): 328. 2010.

**Type:**—BRAZIL. BAHIA: Boa Nova, Fazenda Cotemaia, entrance 1.2 km E of Boa Nova on road to Dario Meira, 14°22.419'S, 40°11.305'W, 810 m, 18 May 2001 (fl.), *W.W. Thomas & S. Sant'Ana 12485* (holotype CEPEC!; isotypes MO!, NY!, SP!) Fig. 1

Creeping herb, rooting at nodes. Stems 15–50 cm long, tetragonal, winged at angles, densely pubescent along margins. Stipular sheath 3–5 mm long, glabrous, with 6–7 fimbriae, 2–5 mm long, pubescent. Leaves opposite, without axillary brachyblasts, pseudopetiolate, anisophyllous at each node, with large and small leaves alternating along the branches, the large ones lanceolate, 2.5–4 × 1.2–2.6 cm, the small ones ovate, 1.2–2.1 cm acute at apex, cuneate or slightly oblique at the base, chartaceous, scabrous at margins, adaxial surface scabrous, abaxial surface glabrous; 2–4 secondary veins on each side of midrib. Inflorescences with 2–15 fascicles per branch; fascicles pseudoaxillary, pauciflorous, 3–7 mm diam., subtended by 2 leaf-like, anisophyllous bracts. Flowers sessile. Hypanthium obconic, glabrous. Calyx 4-lobed, one pair of lobes longer than the other, the two longer ones lanceolate, 2.2–2.5 mm long, acuminate, ciliate; the two smaller ones narrowly triangular, 1–1.2 mm long, acuminate, ciliate. Corolla hypocrateriform, 4–5 mm long; tube 3–4 mm long, glabrous externally, with a ring of moniliform trichomes in the lower third of the corolla throat; lobes ovate, 1 mm long, glabrous on both surfaces or papillate at the apex on both surfaces. Stamens subsessile, inserted at corolla throat; anthers oblong, 0.8–1 × 0.2–0.4 mm, partially included. Style filiform, 4–5 mm long; stigmatic branches ca. 1 mm long. Capsules globose or oblong-ellipsoid, 0.9–1.1 × 0.9–1.2 mm, glabrous; corolla tube and a portion of corolla lobes persistent. Seeds oblong-obovate, 0.3–0.7 × 0.7–0.9 mm, brown or dark brown, dorsal surface without depressions, exotesta reticulate-foveate, with polygonal cells, anticlinal walls thickened, periclinal walls concave, ventral surface with a longitudinal medial groove, narrowly oblong, without the projections of the ventral groove, covered by the strophiole.



**FIGURE 1.** *Mitracarpus diversifolius*. **A**, Branch, showing anisophyllous leaves; **B**, Stipular sheath; **C**, Flower; **D**, Open corolla; **E**, Fruit, with persistent corolla; **F**, Seed, dorsal side; **G**, Seed, ventral side. All from *J.H. Hage & H.S. Brito 1826*. Drawn by Laura Simón.

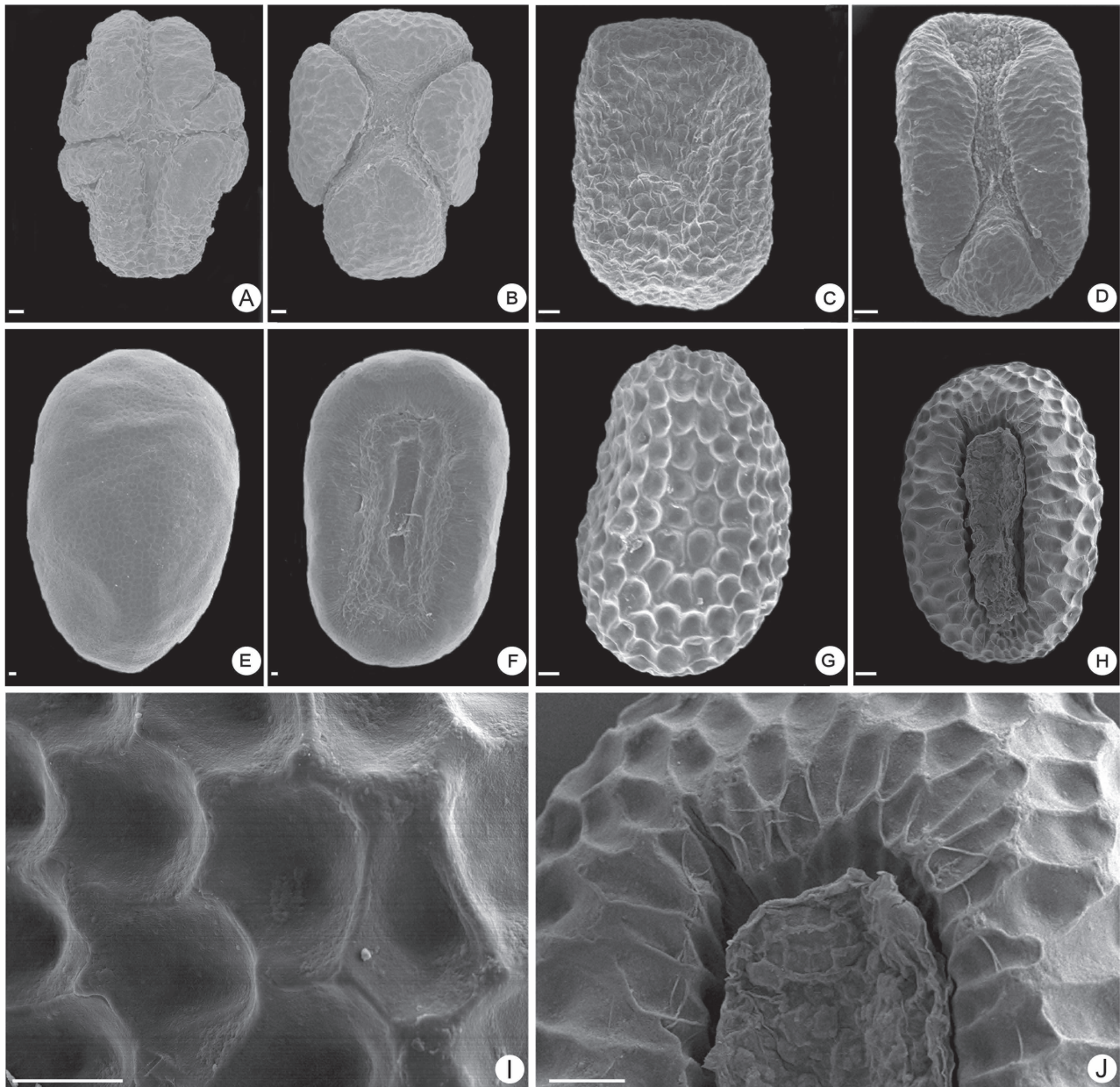
**Distribution and ecology:**—*Mitracarpus diversifolius* is only known from two collections from two localities in Bahia state, in the municipalities of Boa Nova and Ilhéus. Unlike most *Mitracarpus* species, which are heliophilous, this species inhabits humid areas and forest interiors.

**Conservation status:**—We calculate the AOO for *Mitracarpus diversifolius* as 50 km<sup>2</sup> (cell width 5 km), based on the three collections that represent two subpopulations, both in Bahia state; because there are only two localities, EOO cannot be calculated. IUCN (2014) guidelines consider this AOO to indicated an Endangered (EN) status, under the B2 ab (ii, iii) criteria: number of subpopulations less than 5, and continuing decline in the area, extent and quality of habitat, due to deforestation and land use for agriculture. The type collection was gathered in a transitional area



between semideciduous and humid forest, at Boa Nova municipality. The last two collections were made in areas of Cocoa cultivation [*Theobroma cacao* Linnaeus (1753: 782)], at the CEPEC institute, which since many years have agricultural practices as the extraction of herbaceous weeds and application of herbicides. Originally, this area was a humid forest; hence the actual vegetation is strongly modified. The collections date from 1981 and 1986 and until now no new registers are known at the CEPEC herbarium. To arrive to a better conclusion on its conservation assessment, new studies at population level and intensive herbaria consultation covering all *Mitracarpus* species and related genera are needed.

**Examined material:**—BRAZIL. **Bahia:** Mun. Ilhéus, Área do CEPEC (Centro de Pesquisas do Cacau), km 22 da Rodovia Ilhéus/Itabuna (BR 415), região de Mata Higrófila Sul Baiana, Quadra A, entre o Setor de Treinamento e a residência do diretor do CEPEC, 50 m, 28 January 1986, *J.H. Hage & H.S. Brito 1826* (CEPEC, NY); same locality, 27 August 1981 *T.S. Santos 3651* (CEPEC).



**FIGURE 2.** SEM micrographs of *Mitracarpus* seeds. **A–B**, *M. bacigalupoe*, X-shaped ventral groove, **A**, Dorsal view; **B**, Ventral view; **C–D**, *M. strigosus*, inverted Y-shaped ventral groove; **C**, Dorsal view; **D**, Ventral view; **E–F**, *M. megapotamicus*, rectangular-quadrangular ventral groove; **E**, Dorsal view; **F**, Ventral view; **G–J**, *M. diversifolius*, rectangular ventral groove; **G**, Dorsal view; **H**, Ventral view, with strophiole dehydrated; **I**, Detail of the dorsal surface; **J**, Detail of the ventral surface. **A–B** from *A. Schinini et al.* 22976 (CTES), **C–D** from *G. Hatschbach & A.C. Cervi 51422* (HUEFS), **E–F** from *Wood J.R.I.* 24586 (CTES), **G–J** from *J.H. Hage & H.S. Brito 1826* (CEPEC). Scale bar: 50  $\mu$ m.

## DISCUSSION

In their generic description of *Mitracarpus*, Souza *et al.* (2010) distinguished three groups of species based on the shape of the seed ventral groove and seed surface features. The first group (Fig. 2A–B) is characterized by X-shaped ventral groove, with 4 arms so that the seed has four ventral lobes; with a reticulate-foveate, reticulate or papillate exotesta, e.g. *M. bacigalupoe* Cabral, Medina & Souza (2009: 152–153). The second group (Fig. 2C–D) includes species with seeds with an inverted Y-shaped ventral groove, but the distal arms are short, so that 3 lobes are observed on the ventral surface; with a reticulate-foveate exotesta, e.g. *M. strigosus* P.L.R. Moraes, De Smedt & Hjertson (2014: 130). The third group (Fig. 2E–F) is characterized by a quadrangular or rectangular ventral groove, but the arms of the ventral groove are short so that four inconspicuous lobes are observed at the corners; with a reticulate-areolate exotesta, e.g. *M. megapotamicus* (Sprengel 1827: 40) Kuntze (1931: 331).

Fruits and seeds of *Mitracarpus diversifolius* are here documented and discussed for the first time, and extend the morphological variation within the genus. The seeds of *M. diversifolius* (Fig. 2G–J) are characterized by a narrowly oblong ventral groove, without arms so that no lobes are present on the ventral surface, and reticulate-foveate exotesta with thickened anticlinal walls and notably concave periclinal walls. The combination of these two characters makes this species peculiar within *Mitracarpus*. The seed observed in *M. diversifolius* probably represents a new seed type for *Mitracarpus*.

At fruit maturity the corolla tube and a basal portion of the corolla lobes are persistent. In this character *Mitracarpus diversifolius* is unique in the genus and the *Spermacoce* clade. To our knowledge, in the entire tribe Spermacoceae, persistent corolla has been previously reported only in *Houstonia teretifolia* Terrell (1979: 166–168) of western Coahuila, Mexico (Terrell 1979). Regarding this feature, *H. teretifolia* differs from *M. diversifolius* in having lax inflorescences, short-funnelform corolla, fruit with loculicidal dehiscence, and many-seeded carpels. In an extensive bibliographic search no other examples of Rubiaceae species with persistent corollas were found. In Table 1 are compared the main characters of *M. diversifolius* with remainder of the genus, the *Spermacoce* clade and the tribe Spermacoceae.

**TABLE 1.** Comparison of floral and vegetative morphological features of *Mitracarpus diversifolius*, with the remaining species of *Mitracarpus*, the *Spermacoce* clade, and tribe Spermacoceae in America.

	<i>Mitracarpus diversifolius</i>	Remaining species of <i>Mitracarpus</i>	<i>Spermacoce</i> clade	Tribe Spermacoceae in America
Leaves and bracts	Opposite and anisophyllous, with large and small leaves alternating along the branches	Opposite or pseudovercillate, pairs equal	Opposite or pseudovercillate, anisophyllous ( <i>Astiella latifolia</i> Groeninckx <i>et al.</i> 2017), rarely with large and small leaves alternating along the branches (e.g. <i>Spermacoce rubescens</i> )	
Inflorescences	Congested, pseudoaxillary	Congested, axillary and terminal, bilateral	Lax and multiflorous, congested, axillary, pseudoaxillary and/or terminal, ( <i>Spermacoce</i> sensu Florentín, 2016).	Mostly lax, pauciflorous and multiflorous, congested, axillary, pseudoaxillary and/or terminal, ( <i>Spermacoce</i> sensu Florentín, 2016).
Corolla	Persistent on mature fruit	Caducous		Mostly caducous, persistent in mature fruit only in <i>Houstonia teretifolia</i>
Seed	Brown or dark brown; exotesta reticulate-foveate, anticlinal walls thickened, periclinal walls concave; ventral surface with a narrowly oblong groove	Brown to light brown; exotesta reticulate, reticulate-areolate, reticulate-papillate or reticulate-foveate	Highly variable; exotesta reticulate, reticulate-areolate, reticulate-papillate or reticulate-foveate	

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